1 *Original Research Article*

2 # Assessment of Entomopathogenic Fungi for 3 the Biocontrol of Sucking Insect Pests: 4 Pertaining to Red Spider Mites (*Tetranychus* 5 *urticae*)

6

7 **ABSTRACT**

Aim: The study was undertaken to assess the efficiency and select the virulent entomopathogenic fungal isolates for the biological control of red spider mites (*Tetranychus urticae*) under *in vitro* conditions.

Place and duration of work: The study was carried out in the Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bangalore, India.

Methodology: A leaf disc bioassay was employed to assess the virulence of entomopathogenic fungal isolates isolated from 2 agro-climatic zones of Karnataka, India under *in vitro* conditions. The lethal concentration and lethal time were determined by obtaining the mortality data from various conidial concentrations and the data was analysed using Probit analysis. For molecular identification the fungal DNA was isolated through Cetyl trimethyl ammonium bromide (CTAB) extraction buffer, followed by purification through phenol/chloroform extraction and precipitation with isopropanol or ethanol, and the ITS regions of the 18S rDNA were amplificated using universal primers.

Results: Out of 81 fungal isolates, 16 (19.25%) showed insecticidal activity under *in vitro* conditions. The results of the *in vitro* studies revealed that two isolates, ENPF-8 and ENPF-58 had significantly higher mortality rates (93-95 %) against mites at a concentration of $1x10^8$ conidia/ml after 9 days after treatment (DAT). The virulent fungal isolates were identified as *Isaria fumosorosea* (with accession no: MT997932) and *Hirsutella thompsonii* (MT997936) using the sequencing of the ITS region of the 18S rDNA gene. *H. thompsonii* (ENPF-58) was found to be more virulent against mites, with lower LC_{50} (7.6×10^5 conidia/ml) and LT_{50} (5.7, 5.89, and 5.99 days) values compared to the other EPF.

Conclusion: Based on results, it is evidenced that though all isolates caused mortality in the mite population but the percent mortality was varied. Based on these results, *H. thompsonii* (MT997935) and *Isaria fumosorosea* (MT997932) could be a promising biocontrol agent due to their high virulence against mites.

8 *Keywords:* Biological control, Sucking pest, Leaf disc bioassay, $LC_{50,}$ *Hirsutella* sp.

9 **1. INTRODUCTION**

10 Sucking pests, also known as sap-sucking insects, include aphids, thrips, whiteflies,
11 mites, and leafhoppers. These pests are called "sucking" pests because of their piercing
12 and sucking mouth parts, which they use to extract sap from host plants. As a result of
13 this sap removal, the affected plants become stunted in growth, distorted in appearance,
14 and lose chlorophyll content, leading to reduced strength and premature leaf loss. In
15 addition to transmitting diseases, some of these pests also inject toxic substances into
16 the host plants while feeding, resulting in significant agricultural losses (15-45 %) and
17 increased management costs.

18 In recent times, the need for alternative, sustainable, and eco-friendly pest management
19 techniques in agriculture has become increasingly important due to the decline in global
20 crop losses. Despite the use of approximately 2.5 million tonnes of pesticides annually,
21 crop losses due to pests have only decreased from 41.1% during 1988-90 to 32.1 %
22 during 2001-03 (Dhaliwal *et al*., 2015). The use of pesticides has led to various
23 problems, including the development of resistance and resurgence of sucking pests
24 (Sharma *et al*., 20l9) and residual toxic effects on humans, animals, insect parasites, and
25 predators. Additionally, the use of pesticides increases the cost of production. To
26 address these challenges, finding a sustainable and environmentally friendly pest
27 management solution is essential. Microbial biocontrol agents (MBCAs) have gained
28 popularity as an alternative to chemical pesticides in controlling insect pests. These
29 natural enemies are effective in reducing pest populations without posing a risk to human
30 and environmental health. This method of biological control has been adopted globally
31 for controlling both field and forest insect pests due to its persistence, mode of action,
32 cost-effectiveness, non-polluting characteristics, and compatibility with chemical
33 pesticides (Ramanujam *et al*., 2014). Fungi, viruses, and bacteria are the most
34 commonly used microbial agents in field trials, while Rickettsia, protozoa, and
35 nematodes have seen limited use due to their dependence on environmental conditions
36 or difficulty in the application (Shahid *et al*., 2012).

37 Entomopathogenic fungi are potentially the most diverse and versatile biological control
38 agents due to their wide host range, whichoften resulting in natural epizootics. An
39 attractive feature of these fungi is that they infect by contact and act through penetration
40 (Nadeau and Boisvert, 1996). They have certain advantages in pest control programs
41 over other insect pathogens as they infect all stages of insects and directly infect through
42 the cuticle, while other agents need to be ingested. Mass production techniques of these
43 fungi are simpler, easier, and cheaper, and they have a persistent nature, making them a
44 potential candidate in pest control programs over other insect pathogens. The mode of
45 entry and action of these fungi make them a promising option for combating sucking and
46 piercing insects (Ramanujam *et al*., 2014). Among the numerous types of fungi, the fungi
47 that can invade dead insects are called saprophagous and fungi that infect living insects
48 are called entomophagous (Butt *et al*., 2006). Of the estimated 1.5 to 5.1 million species
49 of fungi in the world, approximately 750 to 1,000 are considered entomopathogens
50 placed in over 100 genera many of these have great significance in insect pest
51 management (Charnley and Leger, 2010). The most common and extensively studied
52 entomopathogens are *Metarhizium* spp. *Beauveria* spp. *Nomuraea rileyi*, *Lecanicillium*
53 spp. *Paecilomyces* and *Hirsutella* spp. (Kachhawa, 2017, Wraight *et al*., 2007 and Lacey
54 *et al*., 2008). The *Metarhizium* spp. and *Beauveria* spp. have been extensively used to
55 combat different sucking pests under both greenhouse and field conditions. However,
56 the success of biological control depends on environmental conditions, such as high

relative humidity, moderate temperatures, and soil organic matter (Fargues *et al*., 1997; Vu *et al.,* 2008; Sabbahi *et al*., 2008).

Several researchers studied and evaluated the different entomopathogenic fungi for the control of different sap-sucking insect pests of agriculture (Lacey *et al*., 2008) providing the most satisfying results and evidence in many crops. The present study aimed to isolate and identify the virulent native entomopathogenic fungal strains from soil and insect cadaver samples collected from various regions of South Karnataka, India. This was done to assess their potential for biocontrol against the sap-sucking insects like mites in flower crops. Despite extensive research on the topic in India, limited information exists on the biocontrol of these pests. Screening of local fungal isolates for their virulence characteristics is crucial for the success of biocontrol strategies. Conservation and periodic improvement of the efficacy of these biological control agents will significantly aid in crop protection and help produce pesticide residue-free agricultural commodities, reducing the usage of pesticides in agriculture.

2. MATERIALS AND METHODS

2.1 Entomopathogenic fungal isolates

The fungal isolates were isolated from two agro-climatic zones (eastern dry zoon and southern dry zone) of Karnataka, India. The spore suspension of isolates was prepared by adding 10ml 0.5 % sterile tween 80 to 10 days old cultures and various concentrations of conidial suspension were prepared by serial dilution. The conidial count was determined using an improved Neubauer Hemocytometer.

2.2 Virulence of entomopathogenic fungal isolates

The leaf disc bioassay method was performed following the protocol by Nazir *et al*. (2018). Healthy gerbera leaves were used to obtain 8 cm diameter leaf discs, which were later surface sterilized with 70 percent alcohol. The leaf discs were separately immersed in fungal spore suspension of concentrations $1×10^8$ conidia per mL for 10 seconds, and control was maintained by dipping the leaf discs in sterile distilled water. All the leaf discs were air dried to remove excess moisture and transferred onto sterile Petri plates containing filter paper to maintain humidity during incubation. Three replications for each treatment were maintained throughout the experiment. Twenty laboratory-reared red spider mites were placed onto the treated and control leaf discs using a sterile camel brush. The complete setup was then incubated at 25±1 ºC. The plates were observed at two-day intervals from 3 days after treatment (DAT) up to 9 DAT for mortality of test insect pests. Dead insects were collected from leaf discs and transferred onto PDA plates, which were then incubated at 25±1 ºC with 90 percent relative humidity to promote fungal development and sporulation to confirm that the death of test insects was caused by infection from individual fungal isolates.

2.3 Determination of lethal concentration (LC_{50}) and Lethal time (LT_{50})

The screened fungal isolates which were showed high virulence against the mites were subjected to further testing to determine their lethal concentration and lethal time. This was done at six different conidia concentrations ($1×10^4$, $1×10^5$, $1×10^6$, $1×10^7$, $1×10^8$ and $1×10^9$ conidia per mL) using the leaf disc bioassay method (Trinh *et al*., 2020).

2.4 Molecular identification of virulent entomopathogenic fungal isolates.

The molecular identification of the virulent entomopathogenic fungal isolates was an important step in the study, as it allowed us to accurately identify the fungal species and determine their relationships with other known fungal species. To identify the efficient entomopathogenic fungal isolates at the species and strain level, the 18S rRNA gene was isolated, amplified, and sequenced from all six isolates that exhibited higher mortality of test insects under *in-vitro* studies.

2.4.1 DNA extraction

The mycelia of entomopathogenic fungal cultures were inoculated onto potato dextrose agar and incubated for 10-15 days, depending on the growth of the organisms. After incubation, the mycelia were collected by scraping the fungal mat using a sterile glass slide. The DNA extraction method was standardized and certain steps were optimized to obtain a good concentration of DNA using the cetyl trimethyl ammonium bromide (CTAB) extraction buffer (Doyle & Doyle, 1987), followed by purification through phenol/chloroform extraction and precipitation with isopropanol or ethanol (Ashktorab and Cohen, 1992).

The ITS1 and ITS2 rDNA regions were amplified using universal primers, ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'- TCCTCCGCTTATTGATATGC-3'), each at a concentration of 0.5 µl. The amplification reaction included 5 µl of sample DNA as a template, 1 µl of 1X Buffer, 1 µl of dNTP mix, 0.25 µl of DNA polymerase, and 13.25 µl of ultrapure water (Saito *et al*., 2012). The thermocycler was used to perform the reaction. The PCR conditions were: initial denaturation for 5 minutes at 94°C, followed by 35 cycles of denaturation (30 seconds at 94°C), annealing (30 seconds at 58°C), and extension (1 minute at 72°C). The final extension step was 7 minutes at 72°C. The amplified DNA products were sequenced through outsourcing. The initial identification of the strain was performed using BLASTN against the EzTaxon-e database, which contains all 18s rRNA gene sequences of type strains with validly published eukaryotic names. Further, detailed phylogenetic analyses were conducted in MEGA 6.06 based on partial 18S rRNA gene sequences. The partial 18S rRNA gene sequence of ENPF isolates was aligned with the type strain sequences of the respective genera. Sequence similarities were calculated using the neighbour-joining tool without applying an evolutionary model. Phylogenetic trees were reconstructed using the maximum-parsimony method, the neighbour-joining method, and the Jukes-Cantor correction. All trees were based on partial 18S rRNA gene sequences and were resampled 100 times using bootstrap analysis.

2.5 Statistical analysis

The data obtained from the results were analysed using ANOVA (Analysis of Variance) with the help of the software Web Agri Stat Package 2.0 (https://ccari.icar.gov.in/wasp2.0/index.php, accessed on October 22, 2022), and the means were compared using a post-hoc test (Duncan's multiple range test) at a 5 percent level. The lethal concentration ($LC_{50}/_{90}$) and median lethal time (LT_{50}) were used as relative measures of the susceptibility of the host population and are convenient and commonly used indices for evaluating the efficacy of biological control agents. The LC_{50} values were calculated using the Probit analysis (Finney, 1971) function in the IBM SPSS Statistics v 20 software (IBM Corp., Armonk, NY, USA). The LC_{50} values for

145 entomopathogenic fungal isolates were considered significantly different if the 95 percent
146 confidence intervals (CIs) did not overlap with the CIs of other isolates. LT_{50} values for
147 mortality were estimated by survivorship analysis (Kaplan-Meier survival curves) using
148 IBM SPSS v 20.0 with censored data for insects surviving more than 8 days of
149 incubation period in bioassay studies and survival curves were compared using the log-
150 rank test $\chi 2$ (chi-square) value at $P \leq 0.05$.

151 **3. RESULTS AND DISCUSSION**

152

153 3.1 Virulence of entomopathogenic fungal isolates

154 The results of the virulence assay on mites exhibited diverse outcomes in terms of the
155 mortality caused by different isolates over time. All the entomopathogenic isolates
156 successfully caused mycosis, starting from the third day after treatment (3 DAT) to the
157 ninth day after treatment (9 DAT). The results of virulence studies on mites revealed that
158 only *Hirsutella* and *Isaria* fungal isolates showed significantly higher mortality, starting
159 from 3 DAT to 9 DAT. *Hirsutella* isolates (ENPF-58) caused significantly higher percent
160 mortality of mites, 17.78, 46.67, 73.33, and 95.53 percent at 3, 5, 7, and 9 days after
161 treatment, followed by *Isaria* (ENPF-8) isolate, which caused 15.56, 40, 68.89 and 93.33
162 percent mortality at 3, 5, 7 and 9 DAT respectively (Table 1).

163 On the third day after treatment, many of the fungal isolates failed to cause mortality in
164 mites, among sixteen isolates the significantly the highest mortality of mites is caused by
165 *Hirsutella* sp. (17.78 %) followed by *Isaria* sp. (15.56 %). On the fifth and seventh days
166 after treatment, the mortality rate of mites was significantly increased in all isolates.
167 *Hirsutella sp.* and *Isaria* sp. caused pronounced mortality ranging from 65-85 percent on
168 the fifth to seventh days after treatment. Among the *Beauveria* isolates, ENPF-16 caused
169 the highest percent mortality (10 and 33.33 percent) on the 5th and 7th days after
170 treatment respectively. Whereas, among 4 *Metarhizium* isolates, 13.33 and 31.11
171 percent mortality was caused by ENPF-68. Out of 4 *Aspergillus* isolates, ENPF-26
172 caused 3.33 and 17.78 percent mortality of mites on the 5th and 7th day after treatment
173 respectively. The statistically highest (95.33 and 93.33 %) mortality was observed in the
174 leaf discs dipped in the spore suspension of *Hirsutella* isolate ENPF-58 and *Isaria* isolate
175 ENPF-8 on the ninth day after treatment respectively (Table 1). All *Metarhizium* and
176 *Aspergillus* isolates were not effective in causing mortality (30-60 % on the ninth day
177 after treatment).

178 Although all the fungal isolates were able to cause mortality of mites *in vitro* the percent
179 mortality varied significantly among the isolates. This might be due to various factors
180 such as the higher efficiency or virulence of strains isolated from closely related hosts
181 against the same insects (Vu *et al.*, 2008), variations in the production of extracellular
182 enzymes such as protease, chitinase, lipase, endoprotease, esterase, and
183 carboxypeptidase, and the role of mycotoxins like beuvericin, bassianolidae, aphidiocolin
184 (specific to aphids), and beauverolide (anti-immune activity) in causing mycosis (Kim *et*
185 *al*. in 2013; Sayed *et al*. in 2019). Other factors may include genetic virulence, conidia
186 production, and germination (Sani *et al*., 2020). The only two isolates caused
187 significantly high mortality, which may be because mites, although arthropods, do not
188 belong to the insect class and specific secondary metabolites such as Hirsutellin-A
189 produced by *Hirsutella* spp. inhibit ribosomal activity and fumosorinone produced by *I.*
190 *fumosorosea* inhibits calmodulins activity.

191 The varying mortality rate among the fungal isolates belonging to the same genera is
192 intriguing. This variation could be due to the host specificity of the species, even though
193 all four *Beauveria* isolates belong to the same genus. The host preference of the isolates
194 might vary based on the species, or it could be attributed to differences in the production
195 of extracellular enzymes (Sayed *et al*. in 2019; Márquez-Gutiérrez *et al*., 2022). Rachana
196 *et al*. (2009) reported that the treatment with *H. thompsonii* at 4.6x10^8 spores /mL
197 resulted in the highest recorded mortality of 78.20 percent, followed by treatment with *H.*
198 *thompsonii* at 4.6x10^8 spores /mL + dicofol at 0.025 %. This treatment caused 81.21
199 percent mortality. *F. semitectum* at 2.1x10^9 spores /mL + *H. thompsonii* at 4.6x10^8
200 spores /mL also resulted in high mortality, with a recorded rate of 81.48 percent at 15
201 days after spraying in greenhouse conditions against the red spider mite *T.*
202 *neocaledonicus* on okra in Shimoga. Tamai *et al*. (2002), also confirmed similar results.

203 3.2 Mortality responses of sucking pests to lethal concentration (LC_{50}) and 204 lethal time (LT_{50})

205 The results of the virulence studies under *in vitro* conditions showed that *Isaria* sp.
206 (ENPF-8) and *Hirsutella* sp. (ENPF-58) showed significant mortality of mites in virulence
207 studies under *in vitro* conditions. The LC_{50} and LC_{90} values for *Isaria* sp. were 3.5×10^6
208 and 8.3×10^9, and for *Hirsutella* sp. were 7.6×10^5 and 1.3×10^9, respectively, on the 7th
209 day after treatment. The LC_{50} and LC_{90} values were lower on the 9th day after treatment.
210 The LC_{50} and LC_{90} for Isaria and *Hirsutella* isolates were 2.4×10^5, 8.5×10^4 and 3.6×10^8,
211 9.1×10^7 respectively (Table 2). The results of lethal time (50 and 90 percent mortality)
212 showed that the LT_{50} and LT_{90} values for *Isaria* isolate were higher (6.19 days and 11.03
213 days, respectively) compared with *Hirsutella* isolate (5.99 days and 11.12 days,
214 respectively) (Table 3).

215 Overall, the *Hirsutella* isolate was found to be more effective than the *Isaria* isolate in
216 causing mite mortality. A higher concentration of conidial spores (10^{10} spores/mL) was
217 required to cause mortality of mites at early stages compared to later stages (more than
218 9 days). This is because as the spore concentration increases, the number of conidia per
219 square area also increases, increasing the likelihood of causing mycosis in the insect
220 pest. Table 2 and 3 present the results of lethal concentration and lethal time (LC and
221 LT). Variation in lethal concentration and time by isolates may be because of specificity,
222 growth rate, enzyme production rate, and even conidial germination. Similar results were
223 obtained by Vu *et al*., 2008, Nazir *et al.,* 2018 and Trinh *et al.,* 2020. In 2015, El-
224 Sharabasy conducted a laboratory study to assess the effectiveness of
225 entomopathogenic fungi *Hirsutella thompsonii* (Fisher) and *Paecilomyces fumosoroseus*
226 against all stages of citrus mites. Leaf discs containing larvae, nymphs, adults, and eggs
227 were sprayed with different concentrations of conidia. The results showed that all stages
228 were susceptible to both fungal pathogens, but *H. thompsonii* exhibited greater virulence,
229 with LC_{50} values of 3.5×10^8, 2.9×10^7, and 1.4×10^6 conidia/mL and LT_{50} values of 7.78,
230 7.11, and 6.92 days for larvae, nymphs, and adults, respectively. *P. fumosoroseus* had
231 LC_{50} values of 5.9×10^7, 5.8×10^9, and 3.3×10^9 conidia/mL and LT_{50} values of 7.49, 7.14,
232 and 4.31 days for the same stages. *H. thompsonii* was more effective against eggs at its
233 LC_{50} value. Similar results were obtained by Fiedler *et al*. (2002), Tamai *et al*. (2002),
234 and Rachana *et al*. (2009) during their studies on mites.

235 3.3 Molecular identification of selected entomopathogenic fungal isolates

236 The molecular identification of the virulent entomopathogenic fungal isolates was an
237 important step in the study, as it allowed us to accurately identify the fungal species and
238 determine their relationships with other known fungal species. The results of partially
239 sequencing the 18S rRNA gene of fungal isolates contained more than 530 nucleotide
240 bases, which is sufficient to identify the organisms by blasting the sequence in the NCBI
241 database. The results of the phylogenetic analysis indicate the presence of two major
242 distinct clusters of isolates. The relationships obtained through pairwise sequence
243 similarities were confirmed by the phylogenetic trees generated using different treeing
244 methods.

245 The results of the phylogenetic tree analysis showed two broad distinct clusters for
246 *Beauveria bassiana* isolates. The relationships obtained through pairwise sequence
247 similarities were confirmed by the phylogenetic trees generated using different treeing
248 methods. The partial 18S rRNA gene sequences of entomopathogenic fungal isolates
249 ENPF- 8 were identical to those of the species *Isaria fumosorosea* with accession
250 numbers MT997932. On the other hand, ENPF-58 was identified as *Hirsutella*
251 *thompsonii* with accession numbers MT997936. The ENPF-8 and ENPF-58 isolates
252 showed a similarity of nearly 93% and 97% to the *I. fumosorosea* strain_AMEP094 and
253 *H. thompsonii* strain MTCC_6686 respectively (Figure 1)**.**

254 4 CONCLUSION

255 It can be inferred from the results that the different entomopathogenic fungal isolates
256 caused varied levels of mortality in red spider mites (*Tetranychus urticae*). *Hirsutella*
257 *thompsonii* and *Isaria fumosorosea* were found to be the most virulent and were
258 successful in causing significant mortality in insect pests. *Beauveria* and *Lecanicillium*
259 isolates showed moderate mortality, while *Aspergillus* and *Metarhizium* isolates were not
260 as effective in causing mortality. The concentration of conidial spores per millilitre has a
261 significant impact on the lethal concentration and time of the fungi, with higher
262 concentrations resulting in higher mortality rates in a shorter time frame. The results of
263 these studies are consistent with previous research and highlight the potential of
264 *Hirsutella* and *Isaria* as biological control agents for mites. However, more research is
265 needed to fully understand the factors that contribute to the variation in lethal
266 concentration and time among different fungal isolates.

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269 study, performed the statistical analysis, and wrote the protocol and first draft of the
270 manuscript. Authors KN, MR, and VKR edited the whole draft. All authors read and
271 approved the final manuscript.

272 COMPETING INTERESTS

273 Authors have declared that no competing interests exist.

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Table 1: Virulence of entomopathogenic fungal isolates on percent cumulative mortality of red spider mites (*Tetranychus urticae*) under *in vitro* conditions.

Treat -ments	Isolates	Percent cumulative Mortality (DAT*)			
		3 DAT	5 DAT	7 DAT	9 DAT
T_1	*Beauveria* sp. (ENPF-3)	0.00 $(0.74)^{c}$	6.67 $(14.66)^{e}$	15.56 $(22.67)^{ef}$	33.33 $(35.21)^{e}$
T_2	*Metarhizium* sp. (ENPF-6)	0.00 $(0.74)^{c}$	10.00 $(18.00)^{d}$	24.44 $(29.33)^{cd}$	43.33 $(41.23)^{d}$
T_3	*Isaria* sp. (ENPF-8)	15.56 $(22.94)^{a}$	40.00 $(38.33)^{b}$	68.89 $(55.21)^{ab}$	93.33 $(75.23)^{a}$
T_4	*Metarhizium* sp. (ENPF-9)	2.22 $(5.60)^{bc}$	13.33 $(20.67)^{c}$	26.67 $(30.67)^{cd}$	40.00 $(39.54)^{d}$
T_5	*Beauveria* sp. (ENPF-16)	0.00 $(0.74)^{c}$	10.00 $(18.00)^{d}$	33.33 $(33.67)^{c}$	63.67 $(49.23)^{d}$
T_6	*Lecanicillium* sp. (ENPF-24)	4.44 $(10.47)^{b}$	13.33 $(20.67)^{c}$	26.67 $(30.67)^{cd}$	43.33 $(41.23)^{d}$
T_7	*Aspergillus* sp. (ENPF-26)	0.00 $(0.74)^{c}$	3.33 $(10.33)^{f}$	17.78 $(24.33)^{ef}$	40.00 $(39.54)^{d}$
T_8	*Aspergillus* sp. (ENPF-33)	0.00 $(0.74)^{c}$	3.33 $(10.33)^{f}$	15.56 $(22.67)^{ef}$	33.33 $(35.21)^{e}$
T_9	*Lecanicillium* sp. (ENPF-41)	6.67 $(12.40)^{b}$	10.00 $(18.00)^{d}$	46.67 $(42.33)^{b}$	68.33 $(54.54)^{b}$
T_{10}	*Beauveria* sp. (ENPF-48)	2.22 $(5.60)^{bc}$	6.67 $(14.66)^{e}$	15.56 $(22.67)^{ef}$	43.33 $(41.23)^{b}$
T_{11}	*Aspergillus* sp. (ENPF-53)	0.00 $(0.74)^{c}$	0.00 $(0.74)^{g}$	13.33 $(20.67)^{f}$	26.67 $(31.27)^{f}$
T_{12}	*Hirsutella* sp. (ENPF-58)	17.78 $(24.75)^{a}$	46.67 $(42.33)^{a}$	73.33 $(58.00)^{a}$	95.53 $(78.00)^{a}$
T_{13}	*Beauveria* sp. (ENPF-60)	2.22 $(0.74)^{d}$	6.67 $(14.66)^{e}$	15.56 $(22.67)^{ef}$	43.33 $(41.23)^{d}$
T_{14}	*Metarhizium* sp. (ENPF-67)	0.00 $(0.74)^{c}$	11.11 $(19.32)^{cd}$	28.89 $(32.45)^{cd}$	40.00 $(39.54)^{d}$
T_{15}	*Metarhizium* sp. (ENPF-68)	2.22 $(5.60)^{bc}$	13.33 $(20.67)^{c}$	31.11 $(33.67)^{c}$	43.33 $(41.23)^{d}$
T_{16}	*Aspergillus* sp. (ENPF-79)	0.00 $(0.74)^{c}$	4.44 $(12.23)^{f}$	13.33 $(20.67)^{f}$	33.33 $(35.21)^{e}$

Note:
*DAT: Days After Treatment
Values in the parentheses are ARCSINE transferred values.
The values represented by same letters in each column are statistically on par with each other by DMRT mean of three replications at 95 % confidence interval (CI).

1 **Table 2: Dose mortality responses of mites (*Tetranychus urticae*) to entomopathogenic**
2 **fungal isolates under *in vitro* conditions.**

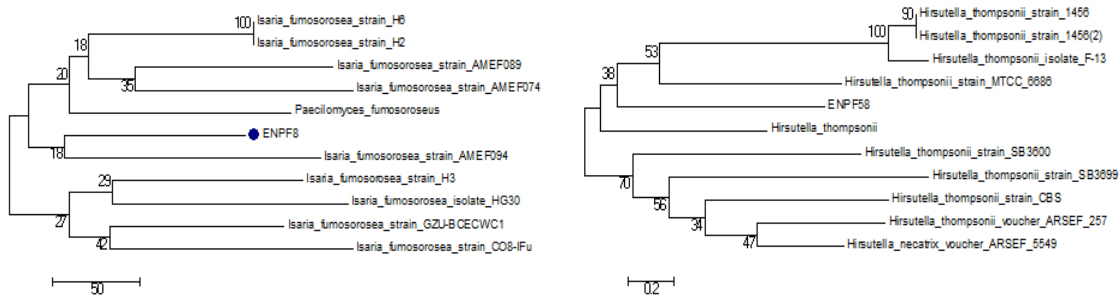
DAT	Isolates	χ^2	LC_{50}[1] (Conidia/mL)	95% CI[2]		LC_{90} (Conidia/mL)	95% CI	
				Lower	Upper		Lower	Upper
7	*Isaria* sp. (ENPF-8)	1.20	3.5×10^6	2.9×10^5	6.7×10^7	8.3×10^8	1.6×10^7	2.6×10^9
	Hirsutella sp. (ENPF-58)	1.54	7.6×10^5	6.3×10^4	1.4×10^7	1.3×10^8	9.6×10^6	9.6×10^8
9	*Isaria* sp. (ENPF-8)	0.68	2.4×10^5	1.3×10^4	3.6×10^6	3.6×10^7	5.4×10^6	6.7×10^8
	Hirsutella sp. (ENPF-58)	1.24	8.5×10^4	8.3×10^3	1.1×10^6	9.1×10^6	1.1×10^6	1.1×10^8

3 **Note**: [1]The LC_{50} values were calculated by Probit analysis using IBM SPSS v 20.0 from the
4 mortality data collected from 7 various conidia concentration at 7 days after treatment. [2]95%
5 Confidence intervals that did not overlap indicate differences between LC50 and LT50
6 values.

7 **Table 3: Estimation of lethal time mortality responses of entomopathogenic fungal**
8 **isolates against mites (*Tetranychus urticae*) under *in vitro* conditions.**

Isolates	χ^2	LT_{50}[1] (±SE) (Days)	95% CI[2]		LT_{90} (±SE) (Days)	95% CI	
			Lower	Upper		Lower	Upper
Isaria sp. (ENPF-8)	1.10	6.19±0.2	5.06	7.21	11.83±0.4	10.65	12.93
Hirsutella sp. (ENPF-58)	1.47	5.99±0.1	5.01	7.16	11.12±0.6	10.48	12.72

9 **Note**: [1]LT50 values for mortality were estimated by survivorship analysis (Kaplan-Meier
10 survival curves) using IBM SPSS v 20.0 with censored data for insects surviving >8d
11 incubation period in bioassay studies and survival curves were compared using the log-rank
12 test χ2 (chi-square) value at P=0.05. [2]95% Confidence intervals that did not overlap indicate
13 differences between LC50 and LT50 values.



14 **Figure 1:** Phylogenetic placement of *Isaria* and *Hirsutella* isolate based on nearly full-length
15 18S rRNA gene sequences. The tree was calculated with the Neighbour-joining method.
16 Bootstrap values are based on 1,000 replicates. Numbers shown above branches are
17 bootstrap percentages for clades supported above the 70% level. The bar indicates 5%
18 sequence divergence.