

# MORTALITY EFFECTS OF SIX DIFFERENT ENTOMOPATHOGENIC FUNGI STRAINS ON RICE WEEVIL, *SITOPHILUS ORYZAE* (L.) (COLEOPTERA: CURCULIONIDAE)

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## ABSTRACT

The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), is one of the most destructive pests on the stored rice throughout the world. In present study, the mortality effects of six entomopathogenic isolates of *Beauveria bassiana* (BB-4984), *Paecilomyces farinosus* (PAF-2538), *Isaria fumosorosea* (ISFUM-4501), *Isaria farinosa* (IFA-3580), *Lecanicillium muscarium* (LECMUS-972) and *Lecanicillium muscarium* (LECMUS-5128), were tested against the adults of *S. oryzae* under 85±5% R.H. and with 1x10<sup>5</sup> and 1x10<sup>7</sup> conidial concentrations (ml<sup>-1</sup>). Treatments were carried out in a climate chamber with 27±1°C and 16 h. light and 8 h. dark photoperiods. Each concentration was replicated three times, and the mortality percentages were monitored on the 2nd, 4th, 6th, 8th and 10th days of incubation. *Lecanicillium muscarium* isolate extracted from Mycotal (Koppert, NL) was used as the commercial (positive) control, while Tween20+sterile water was used as control in this study. Results revealed that *I. farinosa* (IFA-3580) yielded the greatest mortality rates (between 62.60 - 90.60%) for *S. oryzae* adults under 85±5% R.H. and 1x10<sup>7</sup> conidial concentrations (ml<sup>-1</sup>) during incubation, but *L. muscarium* (LECMUS-5128) yielded the least mortality rates (between 26.60 - 73.30%, except *B. bassiana* (BB-4984) and *L. muscarium* (LECMUS-972) on the 8<sup>th</sup> day) the same conditions and with 1x10<sup>5</sup> dose on 10<sup>th</sup> days. As compared to control treatments, six entomopathogenic fungi isolates led to a significant amount of mortality on *S. oryzae* adults at all doses and periods. Therefore, they might have a potential effect in biological control of *S. oryzae* adults due to their strong entomopathogenic activity.

## KEYWORDS:

Entomopathogenic fungi, mortality effects, stored rice pest, *Sitophilus oryzae*

## INTRODUCTION

Recent studies mostly focus on increasing production levels to meet the food demand of ever-increasing world population. However, significant crop losses still exist due to pests, diseases, pathogens, fungi, bacteria and viruses [1]. The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), is one of the most important pests with serious impacts on stored raw cereals throughout the world [2]. The pest is usually found in grain storage facilities or processing plants, infesting wheat, rice, oats, rye, barley and corn; and also feeds on these foods, especially on rice grains. Females oviposit directly into the seeds and the larvae complete development by feeding inside the seeds and emerge as adults [3]. This pest can cause both quantitative and qualitative damages on rice grains. Quantitative damages are commonly observed as seed weight loss caused by feeding of larvae and adults. Qualitative damages are usually experienced as loss of nutritional and aesthetic values, increased levels of rejects in the grain mass and loss of industrial characteristics (for preparation of breads and other products) [4]. In order to control this pest, different synthetic chemicals (insecticides) have been used in grain storages [5]. However, some synthetic chemicals may leave toxic residues over the treated product surfaces [6, 7]. Such residues then have negative impacts on environment and human health [8, 9]. Therefore, health authorities are reluctant to allow chemical insecticides and their residues on grains [10]. Furthermore, *S. oryzae* has been reported to develop resistance to synthetic chemicals [4]. The growing need for research on biological protectants has revealed the positive role played by microbial [2]. For this purpose, the use of entomopathogenic fungi in biological control could be a viable alternative method to control this pest.

Entomopathogenic bacteria, fungi, protozoans or viruses can cause disease by infecting insects or

other arthropods and subsequently are capable of causing rapid declines in large populations of their arthropod hosts. Among these entomopathogens, fungi have garnered the most interest for research and used as biological insecticides. There are more than 100 genera containing insect pathogens, which are environmentally safe with low mammalian toxicity [11, 12]. It has known that at least 10 entomopathogenic species of fungi have been widely used as bio-control agents [13]. The use of entomopathogenic fungi in biological control against pests is an attractive alternative to conventional pesticides, because these fungi are quite friendly control agents for plants, animals, other living organisms and the environment [14, 15].

There are many studies with the use of entomopathogenic fungi for control of stored product pests and related product pests [16, 17, 18, 19, 4, 2, 20, 21, 22, 23, 24, 25, 26, 15, 27, 28].

The aim of this study was to investigate the potential use of six entomopathogenic fungi isolates [*P. farinosus* (PAF-2538), *I. farinosa* (IFA-3580), *I. fumosorosea* (ISFUM-4501), *B. bassiana* (BB-4984), *L. muscarium* (LECMUS-972) and *L. muscarium* (LECMUS -5128)] in controlling *S. oryzae* adults under laboratory conditions.

## MATERIALS AND METHODS

**Stored pest.** *Sitophilus oryzae* adults were obtained as infested stored wheat grains from the Laboratory of Diyarbakır Plant Protection Research Institute, Turkey. They were reared on healthy wheat grains in glass jars under laboratory conditions ( $27\pm 1^\circ\text{C}$  temperature and  $70\pm 5\%$  relative humidity) with 16 h. light and 8 h. dark photoperiods. The pest adults were kept in wheat seeds under laboratory conditions in cloth mesh covered plastic pots (15 cm in diameter and 20 cm high) until the initiation of experiments. Newly emerged adults (mixed males and females) were used for subsequent experiments. The experiments were carried out in three replicates with 25 adults of the pest in each Petri dishes. The adult insects were fed with sufficient amount of wheat seeds during the entomopathogenic tests.

### Entomopathogenic isolates and preparation.

Six entomopathogenic fungi strains were obtained from an entomopathogenic fungi collection (ARSEF, USA). These are *B. bassiana* (BB-4984), *P. farinosus* (PAF-2538), *I. fumosorosea* (ISFUM-4501), *I. farinosa* (IFA-3580), *L. muscarium* (LECMUS-972) and *L. muscarium* (LECMUS-5128). Another *L. muscarium* isolate, used as positive control, was obtained from a commercial product (Mycotal, Koppert, NL). Fungal isolates were cultivated in Potato Dextrose Agar (PDA, Oxoid, CM0139) medium at  $25\pm 2^\circ\text{C}$  in dark for two weeks and they were used

as spray source on the storage pests. Conidia harvested from 14-day old cultures grown on PDA plates were thoroughly mixed with the carrier in screw capped bottles containing 3 ml distilled sterile water. Spore solutions of entomopathogenic fungi isolates were prepared at  $1\times 10^5$  spore  $\text{ml}^{-1}$  and  $1\times 10^7$  spore  $\text{ml}^{-1}$  concentrations and mixed with Tween 20 (0.04%). The suspension was sieved through and 1 ml suspension was sprayed onto each replicate of 25 beetles as storage pests in each Petri dishes. The sprayed Petri dishes were then incubated at  $25\pm 2^\circ\text{C}$  and the dead beetles were counted in every 48 h. For evaluating the conidial viability, the spores of different isolates were saved in the suspension of distilled sterile water and Tween 20, checked by light microscopy (Olympus BH2) on the 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup>, 8<sup>th</sup> and 10<sup>th</sup> days of the treatment.

**Bioassays.** The application of fungal entomopathogen treatments was carried by adding  $1\times 10^5$  and  $1\times 10^7$  conidia to 15 g wheat seeds in 9 cm diameter plastic Petri dishes with sterile paper. Twenty-five newly emerged *S. oryzae* adults were fed with wheat seeds in each Petri dishes and were sprayed with 1 ml of the entomopathogenic fungal suspension, and incubated at  $25\pm 2^\circ\text{C}$ . After these treatments, the mortality of *S. oryzae* adults was evaluated for 10 days in every 48 h (Table 1).

**Statistical analyses.** Resultant findings were subjected to one-way ANOVA with SPSS 17.0 software package. Means were separated with Duncan's multiple range test at  $p < 0.01$ .

## RESULTS

Percent mortality rates of *S. oryzae* adults treated with six entomopathogenic fungi isolates of *B. bassiana* (BB-4984), *P. farinosus* (PAF-2538), *I. fumosorosea* (ISFUM-4501), *I. farinosa* (IFA-3580), *L. muscarium* (LECMUS-972) and *L. muscarium* (LECMUS-5128) are given in Table 1. The results show that these entomopathogenic fungi isolates had insecticidal effects on *S. oryzae* adults as compared to control treatments. In all experiments, the mortality rates generally increased with increasing exposure times. The greatest mortality was observed at 10-day exposure. The mortality of *S. oryzae* adults varied between 77.3% - 90.6% 10 days after treatments. In general, treatments with  $1\times 10^7$  doses of all entomopathogenic fungi isolates caused significantly higher mortalities than the treatments with  $1\times 10^5$  doses (Table 1). The mortalities of *S. oryzae* adults for commercial (positive) control (Mycotal extract of *L. muscarium*) and negative control (distilled sterile water with Tween 20) was respectively recorded as 70.6% and 2.66% 10 days after treatments. There were not significant differences in

mortality of *S. oryzae* adults 8 and 10 days after treatments.

According to these results, *I. farinosa* (IFA-3580) yielded the greatest mortality rates (min. 62.60% and max. 90.60%) for *S. oryzae* adults under 85±5% R.H. and 1×10<sup>7</sup> conidia ml<sup>-1</sup> on the 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup>, 8<sup>th</sup> and 10<sup>th</sup> days, but *L. muscarium* (LECMUS-5128) yielded the least mortality rates (between 46.6% and 81.3%). Accordingly, the greatest mortality rates at 1×10<sup>5</sup> conidial concentrations (ml<sup>-1</sup>) 10 days after treatments ranged from 50.6% to 90.6% for *I. fumosorosea* (ISFUM-4501) fungi isolate. However, *L. muscarium* (LECMUS-5128) yielded the least mortality (between 26.6% and 77.3%, except *B. bassiana* (BB-4984) and *L. muscarium* (LECMUS-972) on the 8<sup>th</sup> day) under the same laboratory conditions and with 1×10<sup>5</sup> conidial concentrations (ml<sup>-1</sup>) 10 days after the treatments.

Two days after the treatments, although the lowest mortality rate at 1×10<sup>5</sup> dose was recorded as 26.6% for *L. muscarium* (LECMUS-5128) isolate, the greatest mortality rate was found as 50.6% for *I. fumosorosea* (ISFUM-4501) and *P. farinosus* (PAF-2538) isolates. Similarly, the lowest mortality rate two days after the treatments and at 1×10<sup>7</sup> dose was recorded as 46.6% for *L. muscarium* (LECMUS-5128) isolate, but the greatest mortality rate was recorded as 62.6% for *I. farinosa* (IFA-3580) isolate (Table 1).

On the 4<sup>th</sup> day of the treatments, it was observed that among six entomopathogenic fungi isolates, *I. farinosa* (IFA-3580) caused significant mortalities at 1×10<sup>7</sup> dose with the greatest mortality rate of 64.0%. However, the lowest mortality rate at 1×10<sup>5</sup> dose was recorded as 33.3% for *L. muscarium* (LECMUS-5128) isolate. The highest mortality at the same dose was recorded as 62.6% for *I. fumosorosea* (ISFUM-4501).

On the 6<sup>th</sup> day of the treatments, the highest mortality of *S. oryzae* adults was observed for *I. farinosa* (IFA-3580) isolate with 80.0% mortality at 1×10<sup>7</sup> dose. But, the minimum mortality rate was recorded as 61.3% for *L. muscarium* (LECMUS-5128) isolate at the same dose. Similarly, although the highest mortality rate of *S. oryzae* adults at 1×10<sup>5</sup> dose of the treatment was recorded as 74.6% for *I. fumosorosea* (ISFUM-4501) isolate, the lowest mortality was noted for *L. muscarium* (LECMUS-5128) isolate as 58.6% (Table 1).

Furthermore, on the 8<sup>th</sup> day of the treatments, the highest mortality rates of *S. oryzae* adults were found for *L. muscarium* (LECMUS-972) and *I. farinosa* (IFA-3580) isolates with 86.6% and 84.0% mortalities at 1×10<sup>7</sup> dose, respectively. But, the minimum mortality rate was recorded as 68.0% for *L. muscarium* (LECMUS-5128) isolate at the same dose of the treatment. Similarly, although the highest mortality rate of *S. oryzae* adults at 1×10<sup>5</sup> dose of the

**TABLE 1**  
Percent mortalities of *Sitophilus oryzae* adults inoculated with two different conidial concentrations (1×10<sup>5</sup> and 1×10<sup>7</sup>) of six entomopathogenic fungi isolates, *B. bassiana* (BB-4984), *P. farinosus* (PAF-2538), *I. fumosorosea* (ISFUM-4501), *I. farinosa* (IFA-3580), *L. muscarium* (LECMUS-972) and *L. muscarium* (LECMUS-5128). Positive control; *Mycotal-Lecanicillium muscarium*

		<i>Sitophilus oryzae</i> (L.)				
Treatments Entomopathogenic fungi	Dose	Mortality (%) <sup>a</sup>				
		Days After Treatment <sup>b</sup>				
		2 <sup>nd</sup> day <sup>a</sup>	4 <sup>th</sup> day <sup>a</sup>	6 <sup>th</sup> day <sup>a</sup>	8 <sup>th</sup> day <sup>a</sup>	10 <sup>th</sup> day <sup>a</sup>
<i>Paecilomyces farinosus</i> (PAF-2538)	1×10 <sup>7</sup>	52.0 ± 2.30 de	58.6 ± 2.66 de	64.0 ± 4.0 bcdef	73.3 ± 7.05 bcde	88.0 ± 6.11 c
	1×10 <sup>5</sup>	50.6 ± 1.33 d	57.3 ± 1.33 de	69.3 ± 5.81 cdefg	76.0 ± 4.61bcde	86.6 ± 8.11 c
<i>Isaria fumosorosea</i> (ISFUM-4501)	1×10 <sup>7</sup>	52.0 ± 2.30 de	53.3 ± 2.66 cde	65.3 ± 7.05 bcdef	70.6 ± 10.4 bcd	82.6 ± 6.66 bc
	1×10 <sup>5</sup>	50.6 ± 4.80 d	62.6 ± 4.80 e	74.6 ± 2.66 efg	78.6 ± 1.33 cde	90.6 ± 2.66 c
<i>Beauveria bassiana</i> (BB-4984)	1×10 <sup>7</sup>	53.3 ± 3.52 de	60.0 ± 2.30 de	69.3 ± 8.11 cdefg	78.6 ± 8.11cde	88.0 ± 8.32 c
	1×10 <sup>5</sup>	48.0 ± 2.30 cd	52.0 ± 2.30 cde	62.6 ± 4.80 bcdef	70.6 ± 5.81 bcd	85.3 ± 4.80 c
<i>Lecanicillium muscarium</i> (LECMUS-972)	1×10 <sup>7</sup>	50.6 ± 5.81 d	53.3 ± 4.80 cde	76.0 ± 2.30 fg	86.6 ± 2.66 e	90.6 ± 4.80 c
	1×10 <sup>5</sup>	38.6 ± 2.66 c	44.0 ± 2.30 bc	60.0 ± 6.11 bcd	70.6 ± 4.80 bcd	77.3 ± 5.33 bc
<i>Isaria farinosa</i> (IFA-3580)	1×10 <sup>7</sup>	62.6 ± 7.42 e	64.0 ± 8.32 e	80.0 ± 6.11 g	84.0 ± 4.0 de	90.6 ± 1.33 c
	1×10 <sup>5</sup>	45.3 ± 3.52 cd	49.3 ± 4.80 cd	72.0 ± 2.30 cdefg	77.3 ± 3.52 bcde	90.6 ± 4.80 c
<i>Lecanicillium muscarium</i> (LECMUS-5128)	1×10 <sup>7</sup>	46.6 ± 10.9 cd	52.0 ± 10.0 cde	61.3 ± 5.81 bcde	68.0 ± 4.0 bc	81.3 ± 3.52 bc
	1×10 <sup>5</sup>	26.6 ± 1.33 b	33.3 ± 1.33 b	58.6 ± 7.05 bc	73.3 ± 7.05 bcde	77.3 ± 8.74 bc
Commercial Control ( <i>Lecanicillium muscarium</i> - Mycotal)	1×10 <sup>7</sup>	54.6 ± 6.66 de	62.6 ± 8.11 e	73.3 ± 8.11 defg	70.6 ± 9.33 bcd	88.0 ± 6.92 c
	1×10 <sup>5</sup>	48.0 ± 2.30 cd	49.3 ± 2.66 cd	54.6 ± 3.52 b	62.6 ± 4.80 b	70.6 ± 3.52 b
Negative Control (Tween20+steril water)	-	0.0 ± 0.0 a	2.66 ± 1.11 a	2.66 ± 1.11 a	2.66 ± 1.11 a	2.66 ± 1.11 a

<sup>a</sup>Mean ± SE of three replicates, each set-up with 25 adults;

<sup>b</sup>Exposure day

Values followed by different letters in the same column differ significantly at  $p < 0.05$

treatment was found as 78.6% for *I. fumosorosea* (ISFUM-4501) isolate, the lowest mortality was recorded as 70.6% for *B. bassiana* (BB-4984) and *L. muscarium* (LECMUS-972) isolates (Table 1).

However, on the 10<sup>th</sup> day of the treatment, the lowest mortality rate at 1×10<sup>7</sup> dose was recorded as 81.3% for *L. muscarium* (LECMUS-5128) isolate. But, the highest mortality rate (90.6%) was recorded for *L. muscarium* (LECMUS-972) and *I. farinosa* (IFA-3580) isolates. Similarly, the highest mortality rate 10 days after treatments and at 1×10<sup>5</sup> dose was found as 90.6% for *I. fumosorosea* (ISFUM-4501) and *I. farinosa* (IFA-3580) isolates. The lowest mortality rate was determined for *L. muscarium* (LECMUS-972 and 5128) isolates as 77.3% within the same period and the same dose of the treatments.

More than 85% mortality of *S. oryzae* adults was observed with 1×10<sup>5</sup> dose of *B. bassiana* (BB-4984), *P. farinosus* (PAF-2538), *I. fumosorosea* (ISFUM-4501) and *I. farinosa* (IFA-3580), while *I. farinosa* (IFA-3580) and *L. muscarium* (LECMUS-972) at 1×10<sup>7</sup> dose caused more than 90% mortality of *S. oryzae* adults (Table 1).

All fungi isolates displayed different mortality values ( $P < 0.05$ ) and there was no significant difference between then and the control (Tween20+steril water). But, there were significant differences for Mycotal (*Lecanicillium muscarium*), used as positive control. According to present values, it was found that *I. farinosa* (IFA-3580) isolate had the most toxic effect on *S. oryzae* adults with 90.6% mortality rate at 1×10<sup>5</sup> and 1×10<sup>7</sup> doses and 10 days after the treatments (Table 1).

TABLE 2

The LC values of two different conidial concentrations (1×10<sup>5</sup> and 1×10<sup>7</sup>) of six entomopathogenic fungi isolates, *B. bassiana* (BB-4984), *P. farinosus* (PAF-2538), *I. fumosorosea* (ISFUM-4501), *I. farinosa* (IFA-3580), *L. muscarium* (LECMUS-972) and *L. muscarium* (LECMUS-5128) against *Sitophilus oryzae* adults.

Entomopathogenic fungi	Day	LC <sub>50</sub> <sup>a</sup>	LC <sub>90</sub> <sup>b</sup>	( $\lambda^2$ ) <sup>c</sup>	Slope ± S.E. <sup>d</sup>
<i>Paecilomyces farinosus</i> (PAF-2538)	2 <sup>nd</sup>	8.282	0.000	0.427	0.229 ± 1.401
	4 <sup>th</sup>	43.354	0.000	0.549	0.233 ± 1.411
	6 <sup>th</sup>	2.199	41.438	3.425	1.005 ± 1.451
	8 <sup>th</sup>	0.405	71.333	5.573	0.571 ± 1.520
	10 <sup>th</sup>	2359.204	2.861	13.841	0.439 ± 1.790
<i>Isaria fumosorosea</i> (ISFUM-4501)	2 <sup>nd</sup>	8.282	0.000	1.707	0.229 ± 1.401
	4 <sup>th</sup>	4.445	26.928	1.910	1.638 ± 1.415
	6 <sup>th</sup>	3.057	15.124	3.861	1.846 ± 1.480
	8 <sup>th</sup>	2.414	13.432	8.006	1.719 ± 1.527
	10 <sup>th</sup>	2.170	6.762	5.913	2.597 ± 1.806
<i>Beauveria bassiana</i> (BB-4984)	2 <sup>nd</sup>	5.530	72.689	3.011	1.146 ± 1.403
	4 <sup>th</sup>	5.083	18.087	4.644	2.325 ± 1.414
	6 <sup>th</sup>	4.625	10.850	5.799	3.461 ± 1.455
	8 <sup>th</sup>	3.055	21.567	7.785	1.510 ± 1.453
	10 <sup>th</sup>	3.742	7.409	7.719	4.320 ± 1.656
<i>Lecanicillium muscarium</i> (LECMUS-972)	2 <sup>nd</sup>	6.171	0.246	1.071	0.916 ± 1.402
	4 <sup>th</sup>	7.606	0.911	0.654	1.391 ± 1.409
	6 <sup>th</sup>	12.708	1.193	6.122	1.247 ± 1.448
	8 <sup>th</sup>	14.498	2.606	8.323	1.719 ± 1.527
	10 <sup>th</sup>	120.772	3.746	12.618	0.850 ± 1.769
<i>Isaria farinosa</i> (IFA-3580)	2 <sup>nd</sup>	5.093	1.237	2.477	2.085 ± 1.411
	4 <sup>th</sup>	5.637	0.897	1.718	1.606 ± 1.404
	6 <sup>th</sup>	8.449	3.261	2.772	3.100 ± 1.477
	8 <sup>th</sup>	9.665	4.518	2.595	3.881 ± 1.628
	10 <sup>th</sup>	10.892	5.116	6.531	3.905 ± 1.762
<i>Lecanicillium muscarium</i> (LECMUS-5128)	2 <sup>nd</sup>	5.469	14.562	4.287	3.013 ± 1.416
	4 <sup>th</sup>	5.076	16.019	5.901	2.567 ± 1.417
	6 <sup>th</sup>	2.343	12.403	3.897	1.771 ± 1.545
	8 <sup>th</sup>	1.782	10.390	2.851	1.674 ± 1.620
	10 <sup>th</sup>	*	*	4.412	0.000 ± 1.949
(Commercial Control) Mycotal- <i>Lecanicillium muscarium</i>	2 <sup>nd</sup>	4.746	2.133	7.315	3.690 ± 1.454
	4 <sup>th</sup>	5.179	2.112	6.210	3.291 ± 1.425
	6 <sup>th</sup>	20.351	0.039	5.216	0.472 ± 1.417
	8 <sup>th</sup>	1.814	29.182	4.921	1.062 ± 1.481
	10 <sup>th</sup>	42.236	1.957	7.771	0.961 ± 1.588

<sup>a</sup> The lethal concentration causing 50% mortality

<sup>b</sup> The lethal concentration causing 90% mortality

<sup>c</sup> Chi square value

<sup>d</sup> Slope of the concentration-mortality regression line ± standard error.

\*LC values were not calculated due to very high levels

On the other hand, according to LC values (LC<sub>50</sub> and LC<sub>90</sub>), the lowest toxic effects on the adults of *S. oryzae* were found for *P. farinosus* (PAF-2538) and *L. muscarium* (LECMUS-972) isolates. The highest LC<sub>50</sub> figures were noted as 2359.204 and 120.772 for this pest 10 days after the treatments, respectively, but the lowest LC<sub>50</sub> figure was recorded as 0.405 for *P. farinosus* (PAF-2538) isolate 8 days after the treatments. In addition, the highest LC<sub>90</sub> figures were noted for *B. bassiana* (BB-4984) and *P. farinosus* (PAF-2538) isolates as 72.689 and 71.333 for this pest 2 and 8 days after the treatments, respectively. But, the lowest LC<sub>90</sub> figures were recorded for *P. farinosus* (PAF-2538) and *I. fumosorosea* (ISFUM-4501) isolates as 0.000 two days after the treatments. According to these values, it was found that *P. farinosus* (PAF-2538) and *I. fumosorosea* (ISFUM-4501) isolates had the most toxic effects on *S. oryzae* adults two days after the treatments (Table 2).

## DISCUSSION

In this study, six entomopathogenic fungi isolates of *B. bassiana* (BB-4984), *P. farinosus* (PAF-2538), *I. fumosorosea* (ISFUM-4501), *I. farinosa* (IFA-3580), *L. muscarium* (LECMUS-972) and *L. muscarium* (LECMUS-5128) were found to be pathogenic on *S. oryzae* adults. The mortality rates of all entomopathogens for *S. oryzae* adults at both doses ( $1 \times 10^5$  and  $1 \times 10^7$ ) increased gradually with time. In other words, when the effects of the exposure times on the mortality were compared, six entomopathogenic fungi isolates displayed higher efficiency in longer exposures (Table 1).

There are many studies focused on the mortality effects of entomopathogenic fungi against *S. oryzae*. Recently *B. bassiana* was declared as a common entomopathogenic fungus. Use of *B. bassiana* to control *S. oryzae* has been studied by various researchers worldwide [29, 5, 2]. [5] reported 17.0%, 59.0% and 94.1% mortality rates at  $1 \times 10^5$ ,  $1 \times 10^6$  and  $5 \times 10^6$  doses of a conidial powder of *B. bassiana* 7 days after the treatments, respectively. The authors also noted 32.0%, 93.0% and 100.0% mortality rates at the same doses 14 days after the treatments. It was found that *B. bassiana* isolate KTU – 24 had 100% mortality rate for *Corythucha ciliata* (Say) adults (Hemiptera: Tingidae) within 14 days of treatments with conidial concentration of  $1 \times 10^8$  conidia mL<sup>-1</sup> [15]. On the other hand, *B. bassiana* was investigated to be an effective controlling agent for rice weevil in storage houses and research records showed an increase in mortality rates for stored wheat pests at higher doses [19, 5, 2] stated 75.8. % mortality rate for *S. oryzae* adults at  $28 \pm 2$  °C and RH  $70 \pm 5\%$  under the laboratory conditions by using *B. bassiana* 25 days after the treatments. In present

study, *B. bassiana* (4984) yielded the greatest mortality rates for *S. oryzae* adults at  $1 \times 10^5$  and  $1 \times 10^7$  doses 10 days after the treatments respectively as 85.3% and 88.0%. But, the lowest mortality rates were recorded as 48.0% and 53.3% at the same dose 2 days after the treatments (Table 1).

*I. fumosorosea* is known as a common entomopathogenic fungus all over the world especially in tropical and subtropical countries [20]. Many studies were recorded by different authors about this fungus to control many pests [31, 32, 33]. [15] reported that *I. fumosorosea* KTU-42 yielded 63% and 50% mortality rates for *C. ciliata* adults and nymphs, respectively. The authors also advised that this isolate might be a good biological control agent against *C. ciliata*. In another study, it was revealed that virulent strain of *P. fumosorosea* had considerable potential effects to control the whitefly [35]. [34] used *I. fumosorosea* as entomopathogenic isolate against an important pest of pear, the pear psylla and reported mortality rates up to 40%. Also, [36] established that *I. fumosorosea* isolate had mortality effect on *Diphorina citri* nymphs and adults; 35% in nymphs; and 22% in adults, respectively. In this study, *I. fumosorosea* (ISFUM-4501) isolate yielded different mortality rates for *S. oryzae* adults at two doses ( $1 \times 10^7$  and  $1 \times 10^5$ ) and 2, 4, 6, 8 and 10 days after the treatments (respectively as 52.0%, 53.3%, 65.3%, 70.6%, 82.6%; and as 50.6%, 62.6%, 74.6%, 78.6%, 90.6%) (Table 1).

There are about 700 species of entomopathogenic fungi in approximately 90 genera. Among these, *I. farinosa* is one of the most commercially produced entomopathogenic fungi. This entomopathogenic fungus can infect a quite wide range of insects including lepidopterous larvae, aphids and thrips species, which are of great concern in agriculture worldwide [37]. In a previous study, it was found that *I. farinosa* yielded 22.5% and 45% mortality rates for *A. rostrata* at  $1 \times 10^6$  and  $1 \times 10^8$  conidial concentrations (mL<sup>-1</sup>) after 9 days of incubation, respectively [38]. The same authors also established that this fungus had 52.5% and 70% mortality rates for this pest adults at  $1 \times 10^6$  and  $1 \times 10^8$  conidial concentrations (mL<sup>-1</sup>) after 12 days of incubation, respectively. In another study, the lowest mortality rate for larvae of pine defoliator *Bupalus piniaria* (L.) was reported as 56.67% for *I. farinosa* isolate after the second spray (at the 7<sup>th</sup> day) [39]. On the other hand, [40] recorded that *I. farinosa* fungus triggered at  $1 \times 10^8$ ,  $1 \times 10^7$ ,  $1 \times 10^6$  and  $1 \times 10^5$  doses and 95% RH with different mortality rates for the eggs (89.39%, 52.53%, 26.87%, 24.77%); 1<sup>st</sup> instar larvae (78.71%, 60.69%, 49.75%, 20.45%) and adults of *Planococcus citri* (Risso) (84.53%, 32.29%, 19.24%, 20.54%), respectively. [27] recorded that *I. farinosa* isolate had significant mortality effect on the sunn pest adults (*Eurygaster austriaca* (Schrk.)). The authors found that *I. farinosa* yielded 0.00%, 5.00%, 10.00%; and 33.75%, 63.75%, 86.25% mortality

rates for *E. Austriaca* adults at  $1 \times 10^6$  and  $1 \times 10^8$  conidia concentration doses  $\text{ml}^{-1}$  6, 9 and 12 days after the treatments, respectively. In present study, *I. farinosa* (IFA-3580) fungus isolate yielded different mortality rates for *S. oryzae* adults at  $1 \times 10^5$  and  $1 \times 10^7$  doses 2, 4, 6 and 10 days after the treatments (respectively as 45.3%, 49.3%, 72.0%, 77.3%, 90.6%; and as 62.6%, 64.0%, 80.0%, 84.0%, 90.6%). These percentages were significantly higher than the control ( $P < 0.05$ ) (Table 1).

[41] recorded that 29 isolates of *P. farinosus* had highly significant mortality on the *Bemisia argentifolii* (Homoptera: Aleyrodidae), from 68 to 94% with no significant difference between these isolates. [42] recorded that *P. farinosus* had a mortality effect on two spotted spider, *Tetranychus urticae* K. (Tetranychidae, Acarina). In another study, *P. farinosus* isolates (290 and 290re) showed lethal effect at  $1 \times 10^8$  conidia  $\text{ml}^{-1}$  on *Ips sexdentatus* and *I. acuminatus* adults (Coleoptera: Scolytidae), 45% and 66.67%, respectively [43]. In present study, *P. farinosus* isolate (PAF-2538) yielded different mortality rates for *S. oryzae* adults at  $1 \times 10^7$  and  $1 \times 10^5$  doses 2, 4, 6 and 10 days after the treatment (respectively as 52.0%, 58.6%, 64.0%, 73.3%, 88.0%; and as 50.6%, 57.3%, 69.3%, 76.0%, 86.6%) (Table 1).

*L. muscarium* is known as an important natural enemy of *Scolytopa australis* (Walker) (Hemiptera: Ricaniidae) in kiwi orchards [44]. This fungus is an important commercially produced entomopathogenic fungi and has been commercialized worldwide under different brand names like Mycotal (Koppert, NL) against whiteflies and thrips and Verticillin against whiteflies, aphids and mites [45]. In a recent study, it was stated that *L. muscarium* isolates had mortality effects on the adults and nymphs of *Bactericera cockerelli* (Sulc) (Hemiptera: Trioizidae) (up to 100% for adults and 70% for nymphs) at  $1 \times 10^7$  dose 7 days after the treatments [46]. In present study, *L. muscarium* isolate (LECMUS-972) yielded different mortality rates ranging from 38.6% to 77.3% at  $1 \times 10^5$  dose (38.6%, 44.0%, 60.0%, 70.6%, 77.3%); and ranging from 50.6 to 90.6% at  $1 \times 10^7$  dose (50.6%, 53.3%, 76.6%, 86.6%, 90.6%) respectively 2, 4, 6 and 10 days after the treatments (Table 1).

Furthermore, *L. muscarium* (LECMUS-5128) isolate had mortality effect on *S. oryzae* adults (46.6%, 52.0%, 61.3%, 68.0% and 81.3%) at  $1 \times 10^7$  dose 2, 4, 6, 8 and 10 days after the treatments, respectively. But, the same fungus isolate had mortality rates of 26.6%, 33.3%, 58.6%, 73.3% and 77.3% at  $1 \times 10^5$  dose. These percentages were significantly higher than the control ( $P < 0.01$ ) (Table 1).

In brief, six entomopathogenic fungi isolates of *B. bassiana* (BB-4984), *P. farinosus* (PAF-2538), *I. fumosorosea* (ISFUM-4501), *I. farinosa* (IFA-3580), *L. muscarium* (LECMUS-972) and *L. muscarium* (LECMUS-5128) were tested against *S. oryzae*

adults under laboratory conditions in this study. Present findings demonstrated that the fungal isolates could be used as potential bio-control agents against this pest. Among the tested fungi isolates, *I. farinosa* (IFA-3580) isolate with the greatest mortality rates was identified as the most promising one. Further studies should be carried out to evaluate the effectiveness of this isolate in the field. Additionally, use of *I. farinosa* (IFA-3580) isolate as a biological control agent in an integrated pest management (IPM) program may help to reduce the dependence on chemical control in the future.

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