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Original article (Orijinal araştırma)

The UV protectant properties of tea extracts on entomopathogenic fungus spores and their lethal effect on *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae)

Çay ekstraktlarının entomopatojen fungus sporları üzerindeki UV koruyucu özellikleri ve *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae) üzerindeki öldürücü etkileri

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Abstract

The present study aims to investigate the effectiveness of various tea extracts in providing ultraviolet (UV) protection for entomopathogenic fungi. UV radiation could have detrimental effects on viability of these fungi, which are important biocontrol agents against insect pests. This study was carried out in the Microbiology Laboratory of the Department of Biology Karadeniz Technical University in 2023. We evaluated the UV protective properties of various tea extracts in entomopathogenic fungi [*Beauveria bassiana* (Bals.) Vuil. (Hypocreales: Cordycipitaceae) and *Metharizium flavoviride* (Gams and Rozsypal 1956) (Hypocreales: Clavicipitaceae)] and tea extracts effectiveness against *Galleria mellonella* (L.,1758) (Lepidoptera: Pyralidae) larvae. Our findings demonstrate that certain tea extracts exhibit significant UV protection for entomopathogenic fungi, suggesting their potential application in improving the performance of biocontrol agents in outdoor environments. The highest UV-B protection was observed by adding black and green tea extracts to fungal spores, resulting in a radial growth measurement of 14.6 mm and 14.3 mm, respectively, at the end of 10 days of exposure for 120 minutes. These results contribute to the development of eco-friendly strategies for pest management in agriculture.

Keywords: *Beauveria bassiana*, biological activity, *Metharizium flavoviride*, tea extracts, UV protection

Öz

Bu çalışma, entomopatojenik funguslar için çeşitli çay ekstraktlarının ultraviyole (UV) koruması etkinliğini araştırmayı amaçlamaktadır. UV radyasyonu, böcek zararlılarına karşı önemli biyokontrol etmenleri olan bu fungusların canlılığı üzerinde olumsuz etkilere sahip olabilir. Bu çalışma 2023 yılında Karadeniz Teknik Üniversitesi Biyoloji Bölümü Mikrobiyoloji Laboratuvarında yürütülmüştür. Çay ekstraktlarının entomopatojen funguslar [*Beauveria bassiana* (Bals.) Vuil. (Hypocreales: Cordycipitaceae) ve *Metharizium flavoviride* (Gams and Rozsypal 1956) (Hypocreales: Clavicipitaceae)] üzerindeki UV koruyucu özelliklerini ve *Galleria mellonella* (L.,1758) (Lepidoptera: Pyralidae) larvalarına karşı çay ekstraktlarının etkinliklerini değerlendirilmiştir. Bulgularımız, belirli çay özlerinin entomopatojen funguslar için önemli bir UV koruması sergilediğini göstermekte ve bu ekstraktların açık hava ortamlarında biyokontrol ajanlarının performansını artırmada potansiyel uygulamalarına işaret etmektedir. En yüksek UV-B koruması, fungus sporlarına siyah ve yeşil çay ekstraktları eklenerek elde edilmiş ve 10 günlük 120 dakikalık maruziyet sonunda sırasıyla 14.6 mm ve 14.3 mm'lik bir radyal büyüme ölçümü elde edilmiştir. Bu sonuçlar, tarımda çevre dostu zararlı yönetimi stratejilerinin geliştirilmesine katkıda bulunacaktır.

Anahtar sözcükler: *Beauveria bassiana*, biyolojik aktivite, *Metharizium flavoviride*, çay ekstraktları, UV koruma

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Introduction

Entomopathogenic fungi have been widely utilized for controlling insect pests, playing an incredibly significant role in pest management (Ansari et al., 2011). As a result, they are seen as alternatives to chemical insecticides (Zimmerman, 2007). Fungal pesticides play a crucial part in the biological control of pathogens and insect pests in agriculture and horticulture (Maqbool et al., 2016). *Beauveria bassiana* (Bals.) Vuil. (Hypocreales: Cordycipitaceae), *Metarhizium anisopliae* (Metschn.) (Hypocreales: Clavicipitaceae), and *M. flavoviride* (Gams & Rozsypal 1956) have emerged as key players in the control against many pests, and they are found on various insects worldwide (Feng et al., 1994; Bridge et al., 2005; Faria & Wraight, 2007). Several bioinsecticide products based on entomopathogenic fungi, such as *M. anisopliae, B. bassiana*, and *Isaria fumosorosea* (Wize) Brown y Smith (Hypocreales: Clavicipitaceae) have been effectively employed for insect pest control (Faria & Wraight, 2007).

Applications made in the field have shown negative effects on pests exposed to direct sunlight (Faria & Wraight, 2007; Jackson et al., 2010). The spores of the *B. bassiana* fungus can lose their effectiveness when exposed to ultraviolet radiation (UV) (Fargues et al., 1996; Fernandes et al., 2007). This is considered one of the most significant factors that bound the permanence and effectiveness of entomopathogenic fungi in outdoor environments (Moore et al., 1993). Consequently, it is essential to develop formulations that can extend the survival time of these fungi, which are utilized as microbial control agents. These formulations should incorporate substances capable of providing UV protection.

Various abiotic factors in nature can impede the capability of fungal agents to biocontrol insect pests. Among these factors, UV, including UV-A and UV-B, was considered the primary factors in the environment that influenced the survival of fungi in pest control (Ignoffo & Garcia, 1992; Moore et al., 1993). While certain strains of fungi that are UV-tolerant can be capable of enduring extended periods of direct exposure to solar UV radiation, lasting for several hours, strains that are susceptible to UV could not withstand it. Furthermore, the effects of UV-B exposure on fungi (Fargues et al., 1996; Braga et al., 2001a; Fernandes et al., 2007; Nascimento et al., 2010) or UV-A (Fargues et al., 1997; Braga et al., 2001b) could prolong the germination of surviving conidia and hinder fungal growth. This decrease in fungal resistance and the impact of infective field propagules undermines the control of arthropod pests (Jaronski, 2009). To address these challenges, efforts have been made to select strains with inherent UV tolerance and to develop formulations that incorporate adjuvants capable of absorbing or blocking solar radiation. These strategies aim to protect fungi from the harmful impacts of UV radiation.

Comprehensive studies have been conducted on the UV tolerance of various species of entomopathogenic fungi, including *B. bassiana* (Inglis et al., 1995; Fargues et al., 1996; Morley Davies et al., 1996; Huang & Feng 2009; Posadas et al., 2012), *I. fumosorosea* (Fargues et al., 1996), *Metharizium acridum* (Fargues et al., 1996; Morley Davies et al., 1996; Braga et al., 2001a), and *M. anisopliae* (Fargues et al., 1996; Braga et al., 2001a). These fungi are generally highly sensitive to UV radiation.

Tea is among the most widely consumed beverages globally, second only to water. The different types of tea produced and consumed worldwide include black, green, oolong, and white teas. These teas vary in their polyphenolic content due to the fermentation process during tea manufacturing. Numerous studies have investigated the effects of plant material on pest control, long-term preservation, and protection from sunlight (Abudulai et al., 2001; Shapiro et al., 2007a, b; Shapiro et al., 2008; El Salamouny et al., 2009a, b). Among these studies, the UV protective effects of green and black tea, particularly against nucleopolyhedroviruses, were demonstrated (Shapiro et al., 2008; El Salamouny et al., 2009a, b; El-Husseini et al., 2012; Gifani et al., 2021). Additionally, some research have examined the UV protective impact of tea extracts on entomopathogenic fungi (Kaiser et al., 2018).

In this study, our objective was to evaluate the efficacy of extracts obtained from four different tea extracts in offering protection against ultraviolet (UV) radiation, with the goal of improving the persistence of *B. bassiana* and *M. flavoviride* spores under laboratory condition.

Materials and Methods

Fungal isolates and conidia production

The fungi used in this study, namely *Beauveria bassiana* and *Metharizium flavoviride*, were procured from the entomopathogenic culture collection at Karadeniz Technical University, Department of Biology, Microbiology Laboratory. In previous studies, *B. bassiana* (Pa4) and *M. flavoviride* (As-2) were isolated from *Pristiphora abietina* (Christ, 1791) (Hymenoptera: Tenthredinidae) and *Amphimallon solstitialis* (Linnaeus, 1758) (Coleoptera: Scarabaeidae) in Türkiye, respectively. These fungi exhibited significant insecticidal activity on insect pests (*P. abietina* and *A. solstitialis*) (Biryol et al., 2020, 2021).

Each isolate was inoculated with Sabouraud CAF Agar medium (Liofilchem s.r.l., Italy) in flasks and put in incubator at 25 °C for approximately 2 weeks to supply new conidia from the fungal isolates. The growing fungal spores were harvested using 0.1% Tween 80 (AppliChem) and stock concentrations were calculated with a Neubauer hemocytometer. A concentration of 2×10^7 conidia/ml was used in the trials. We monitored the germination of conidia by introducing them onto Sabouraud CAF Agar medium and then quantifying the count of conidia that had undergone germination following a 24-hour incubation period at a temperature of 25°C. We defined conidia as germinated when their germ tubes reached a length equal to or greater than the width of the conidium.

Preparation of additives

The UV protectant properties of four natural additives, namely the green tea, black tea, oolong tea, and white tea (*Cammellia sinensis* L. Kuntze, Theaceae) extracts, were tested. The tea leaves were infused in sterile distilled water at 70°C for 30 min (2 g of tea leaves with 20 ml of water). The stock solutions were supplemented to the spore suspensions to achieve last UV protectant concentration of 10% v/v in the assays.

Evaluation of UV protection of tea extracts on fungal isolates on agar plates

In the experiment aimed at assessing the resistance of two isolates (*B. bassiana* and *M. flavoviride*) and their combinations with tea extracts to UV-B radiation, the following procedure was followed:

A spore suspension containing 2×10^7 conidia/ml was obtained. Ten microliters (µl) of this suspension were inoculated at three different points on Sabouraud CAF agar plates. Plastic petri dishes with open lids were placed in the sterile cabinet, 20 cm away from the UV-B lamp (15 W, 312 nm). A UV-B fluorescent lamp (Philips, Eindhoven, Holland) with a wavelength of 260-400 nm was used as the radiation source. Petri dishes were exposed to UV-B for 0, 30, 60 and 120 min. This exposure was applied to all Petri dishes containing pure conidia (unformulated control) as well as those with conidia-tea extract mixtures. The radial growth of the fungi was examined on the 5th, 7th, and 10th days after incubation at 28 °C. To produce spores, 100 µl of the fungal spores (2×10^7 conidia/ml) was plated on Sabouraud CAF agar plates. These plates were then exposed to UV-B radiation for 0, 30, 60, and 120 min and incubated at 28 °C. Spores were harvested from the Petri dishes 12 days after incubation, and the concentration of spores was calculated. Trials were carried out in triplicate, with the 0-min exposure serving as the control group (Couceiro et al., 2021). The obtained measurements were recorded and compared using graphs. To assessment the effect of ultraviolet radiation, both the radial growth of the colonies on the agar plates and the count of conidia were measured after exposure to UV-B radiation.

Lethal effect of fungal spores on *Galleria mellonella* after UV exposure

The efficacy of fungal spores harvested after UV exposure was tested on *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) larvae. Laboratory-cultured *G. mellonella* larvae were used in the efficacy experiments. A mixture of beeswax, bran, honey, glycerin, and distilled water was used to feeding *G. mellonella* larvae. Then, the selected larvae were transferred into plastic box (15 cm wide × 8 cm deep) with diet pieces. The fungal spore concentration was calculated as 1×10^7 conidia/ml and applied to the larvae using a sterile sprayer. In the experiments, 30 healthy third instar *G. mellonella* larvae were used in triplicate. All boxes were stored at 25 ± 1°C, 65 ± 5% RH under 12/12 photoperiod for 7 days. The larvae in the control group were only treated with 0.01% aqueous Tween 80. After application, the experiments were monitored for 10 days, and dead larvae were collected. In the control group of *G. mellonella* larvae, only sterile water was used. The dead larvae were transferred to moistened petri dishes and their mycosis was observed. The Schneider-Orelli formula (Püntener, 1981) was utilized to rectify the mortality and mycosis data.

Schneider-Orelli formula:

$$\text{Corrected \%} = \left(\frac{\text{Mortality \% in treated plot} - \text{Mortality \% in control plot}}{100 - \text{Mortality \% in control plot}} \right) * 100$$

Statistical analyses

Data were analyzed using SPSS 25.0 software (IBM Corp., Armonk, NY). The effects of tea extracts on radial growth and spore production tests for two fungal isolates and lethal effect of fungal spores on *G. mellonella* were determined using one-way analysis of variance (ANOVA) and differences between groups by the least significant difference (LSD) tests. Trials with no growth in radial growth and spore production trials were not included in the statistical analysis.

Results

According to the results of the UV-B exposure experiment, it was observed that fungal growth decreased with increasing exposure time. *Beauveria bassiana* isolate of unformulated control (pure conidia) did not show any growth after incubation of 10 days at 120 min of exposure (Figure 1). Fungal spores formulated with black, green, and oolong teas exhibited growth after 120 min of exposure, while fungal spores formulated with white tea did not grow, like the unformulated control. The unformulated *Metharizium flavoviride* isolate showed a radial growth of 4.2 mm at 120 min UV-B exposure, unlike the other isolate. Likewise, the *M. flavoviride* isolate formulated with white tea extract had a radial growth of 4.5 mm at 120 min UV-B exposure (Figure 1). All tested tea extracts showed good compatibility and did not significantly inhibit the growth of *B. bassiana* and *M. flavoviride* spores. The trials without UV-B exposure (0 min) showed the best growth. The two isolates exhibited slightly different responses to UV-B exposure (Table 1).

In the radial growth study, no statistically significant difference was observed in all groups in both isolates at 0 and 30 min exposure times ($F=1.565$; $df=4, 23$; $p=0.265$) (Table 1). It was determined that there was a statistically significant difference between black and green tea and the control group in *B. bassiana* and *M. flavoviride* after 60 min of exposure ($F=77.136$; $df=4, 23$; $p<0.001$). The highest UV-B protection rate was observed with black and green tea extract supplementation to *B. bassiana* spores, resulting in radial growth of 14.6 mm and 14.3 mm, respectively, at the end of 10 days of exposure for 120 min. Treatments containing oolong and white tea also showed significantly increased radial growth compared to the unformulated control after 60 min of exposure ($F=165.985$; $df=4, 23$; $p<0.001$), with measurements of 12.1 mm and 10.9 mm, respectively. Unlike the control, oolong, and white tea formulations of the *M. anisopliae* isolate at 120 min of exposure, black and green tea formulations were statistically in different groups ($F=213.365$; $df=4, 23$; $p<0.001$). After exposure to UV-B radiation, the conidia treated with oolong and white tea displayed noticeably reduced radial growth compared to the control group of unformulated isolates (Table 1).

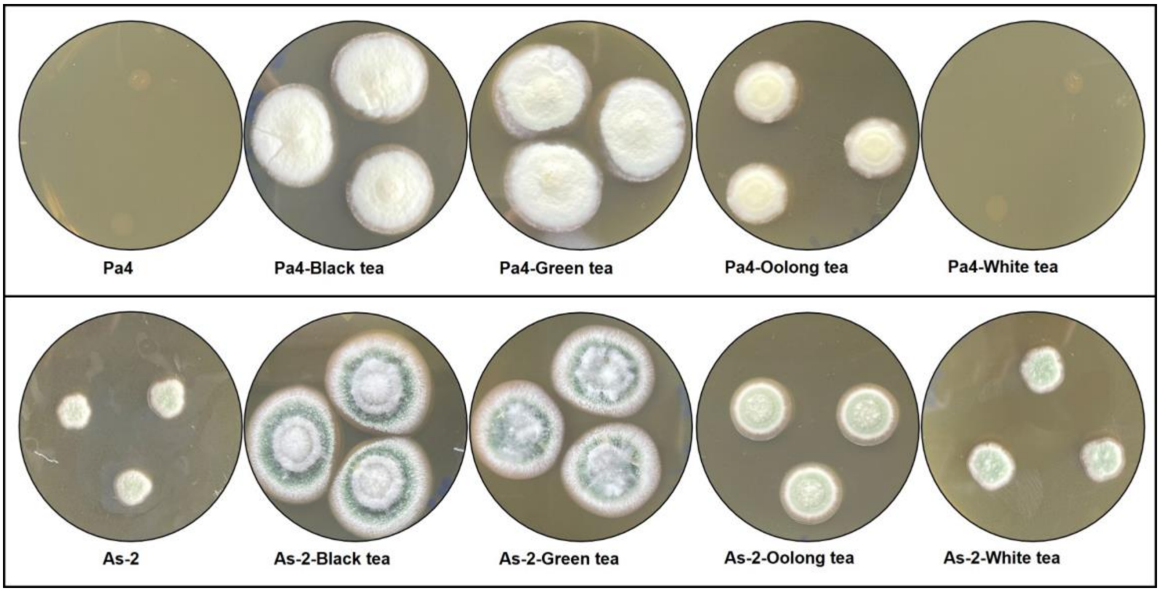

Figure 1. Radial growth of *Beauveria bassiana* Pa4 and *Metharizium flavoviride* As-2 isolates at 120 min UV-B exposure.

Table 1. Effect of UV-B on radial growth diameters (mm±SD) at various exposure times at 10th day

Fungal Isolate	Exposure time (minute)	Unformulated control (pure conidia)		Black tea		Green tea		Oolong tea		White tea	
B. bassiana	0' UV-B	23.6±1.25	aA	23.0±1.36	aA	24.3±2.34	aA	21.6±1.49	aA	24.1±2.46	aA
	30' UV-B	15.2±1.36	bB	20.7±1.65	abA	18.3±0.98	bcA	16.1±1.81	bcAB	15.9±1.03	abB
	60' UV-B	6.3±0.06	bcC	18.2±1.38	cA	16.7±1.03	caB	12.1±0.38	cB	10.9±0.94	cBC
	120' UV-B	NG		14.6±1.26	cA	14.3±1.25	cdA	6.4±0.32	dB	NG	
M. flavoviride	0' UV-B	25.6±0.86	aA	24.5±2.06	aA	23.4±1.94	aA	23.8±1.32	aA	25.1±1.76	aA
	30' UV-B	19.1±1.25	bA	18.6±1.86	bA	16.1±1.05	bA	19.65±1.77	bA	19.5±1.07	bA
	60' UV-B	10.6±1.36	cC	17.3±0.98	bcA	15.8±1.11	bA	12.7±1.68	cBC	12.4±1.09	cBC
	120' UV-B	4.2±0.85	dB	16.12±1.84	bcA	15.6±2.64	bA	5.7±0.85	dB	4.5±0.64	dB

Note: In radial growth, the averages of three replications are presented. The lowercase letters in the columns indicate significant differences between the means according to LSD analysis of UV exposure times ($p<0.05$). Capital letters in the lines indicate significant differences between the means according to the LSD analysis of the unformulated control and fungal spores formulated with tea extracts ($p<0.05$). NG: no growth, SD: standard deviation. 0' UV-B exposure time was considered as the control group.

The effect of UV-B exposure on spore production of strains appears to be highly parallel to the effect of UV-B on radial growth. It was determined that the strains produced the highest number of spores in the application without exposure, and the spore production decreased in antiparallel to the increase in exposure time. *B. bassiana* revealed a more UV-B resistant effect than *M. flavoviride* (Table 2).

The unformulated *M. flavoviride* exhibited higher UV-B resistance compared to *B. bassiana* (Figure 2). Furthermore, the extracts of black and green tea from all the samples tested showed a remarkable protective effect against UV radiation, as evidenced by significantly higher conidial counts compared to the control samples that were not formulated with the extracts.

Table 2. Effect of UV-B on conidia production (conidia/ml×10^7±SD) at various exposure times at 12th day

Fungal Isolate	Exposure time (minute)	Unformulated control (pure conidia)		Black tea		Green tea		Oolong tea		White tea	
B. bassiana	0' UV-B	2.78±1.23	aA	2.75±1.23	aA	2.76±1.52	aA	2.66±1.09	aA	2.76±1.52	aA
	30' UV-B	1.80±0.62	bAB	2.16±0.84	aA	2.19±1.27	aA	1.73±0.25	bAB	1.79±0.61	bAB
	60' UV-B	0.75±0.02	cC	1.47±0.86	bA	1.12±0.34	bA	0.98±0.26	cAB	0.91±0.18	cB
	120' UV-B	0		0.55±0.03	cA	0.46±0.11	cA	0.37±0.01	dB	0	
M. flavoviride	0' UV-B	2.35±1.54	aA	2.37±2.01	aA	2.30±2.12	aA	2.43±0.95	aA	2.50±1.91	aA
	30' UV-B	1.58±0.58	bA	1.56±1.01	aA	1.68±0.69	bA	1.63±0.37	bA	1.62±0.84	bA
	60' UV-B	0.75±0.11	cA	0.80±0.26	cA	0.79±0.11	cA	0.71±0.21	cAB	0.72±0.13	cAB
	120' UV-B	0.09±0.01	dC	0.51±0.09	dA	0.48±0.04	dA	0.29±0.01	dB	0.11±0.02	dBC

Note: The conidia count the averages of three replications are presented. The lowercase letters in the columns indicate significant differences between the means according to LSD analysis of UV exposure times (p<0.05). Capital letters in the rows indicate significant differences between the means according to the LSD analysis of tea extracts (p<0.05). SD: standard deviation. 0' UV-B exposure time was considered as the control group.

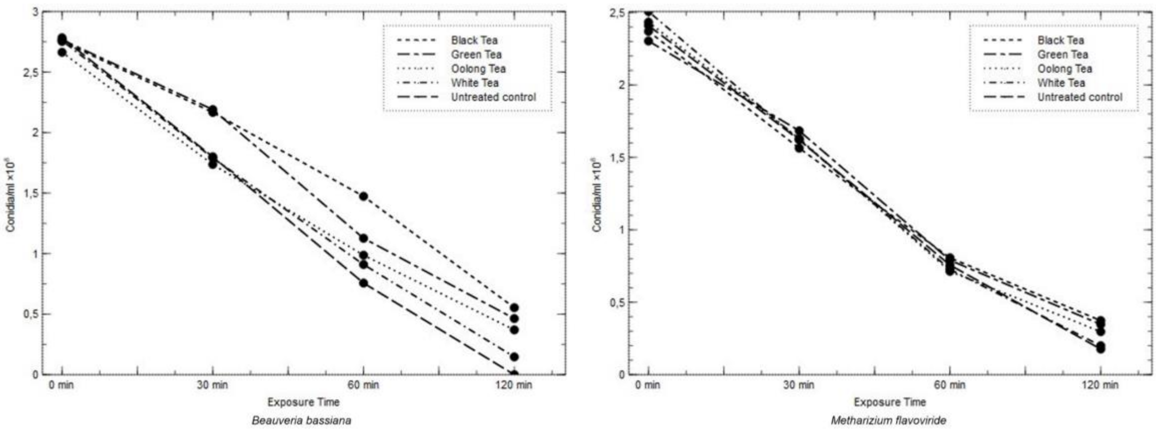

Figure 2. Conidia number of untreated control group (pure spores), black, green, oolong and white tea formulated groups of *Beauveria bassiana* and *Metharizium flavoviride* after UV-B exposure.

Insecticidal activity experiments were conducted using third-stage *G. mellonella* larvae to assess the infectivity of spores from isolates harvested after exposure to UV-B radiation. The mortality percentages of *G. mellonella* larvae infected with fungal spore suspensions (both formulated and non-formulated) following UV-B exposure durations of 0, 30, 60, and 120 minutes are presented in Figure 3.

The study's results revealed that with increasing UV-B exposure duration, lower mortality rates were observed in *G. mellonella* larvae inoculated with non-formulated fungal spores (Figure 3). This corresponded to a decrease in spore germination and infectivity when exposed to UV radiation. In contrast, fungal spores formulated with tea extracts exhibited higher rates of germination and infectivity on *G. mellonella* larvae. This suggests that tea extracts may provide a degree of protection or enhance the survival of fungal spores when exposed to UV-B radiation. No statistically significant difference in mortality rates was observed in fungal spores harvested after 0 and 30 min of exposure to *G. mellonella* (F=2.105; df=4, 23; p=0.145). Regarding the insecticidal activity of the *B. bassiana* isolate after 60 minutes of exposure, formulations containing black tea (68.9%) and green tea (65.2%) were statistically separated from the control group (44.3%) (F=283.990; df=4, 23; p<0.001). In contrast, formulations with oolong tea (51.3%) and white tea (48.2%) were closer to the control group. After 60 minutes of exposure to the *M. flavoviride* isolate, a statistically significant difference was observed between the black tea (62.1%) and green tea (59.2%) formulations and the control group (38.2%) (F=183.427; df=4, 23; p=0.618). Following 120 min of exposure to the *M. flavoviride* isolate, the control group exhibited a mortality rate of 11.2%, while the black tea, green tea, oolong tea, and white tea formulations recorded mortality rates of 37.3%, 34.6%, 29.1%, and 20.1%, respectively. No death was observed in the control group of *G. mellonella* larvae (Figure 3).

These findings indicate that black tea and green tea extracts have the potential to provide UV protection for fungal spores. In contrast, oolong tea and white tea extracts demonstrated lower UV-protection efficacy, as evidenced by their relatively higher larval mortality rates. Additionally, it was observed that with increased exposure to UV radiation, the radial growth and spore production of the *M. flavoviride* isolate were higher compared to the *B. bassiana* isolate. However, the *B. bassiana* isolate exhibited a higher mortality rate against *G. mellonella* larvae.

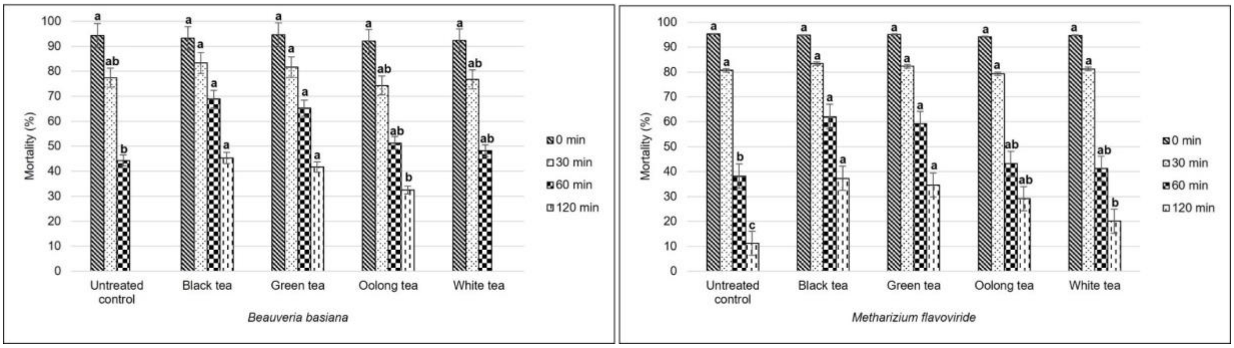

Figure 3. For each isolate, different lower-case letters in each graph column for the same exposure times represent statistically significant differences amongst mortalities according to the least significant difference (LSD) multiple comparison test (P<0.05). Mortality indicates the mean of three replications. The bars show the standard deviation of the mean values.

Discussion

Exposure of fungal spores to UV-B radiation, without UV-protective additives, leads to a notable decrease in viability (Kaiser et al., 2018). In the current study, we examined the effectiveness of four tea extracts as UV protectants using *B. bassiana* Pa4 and *M. flavoviride* As-2 isolates and their mortality on *G. mellonella* larvae. These isolates have demonstrated significant potential in previous studies focusing on biological control (Biryol et al., 2020, 2021).

UV-B solar radiation is highly destructive to fungal development and has a considerable impact on their survival and effectiveness in combating environmental insects (Ignoffo & Garcia, 1992; Inglis et al., 2001; Fernandes et al., 2015; Acheampong et al., 2020). Previous research (Braga et al., 2001b, c; Fernández-Bravo et al., 2017) has indicated that a 2-hour exposure to UV-B light significantly reduces the culturability of *Metarhizium* spp. spores, which supports the findings of our study.

The tea plant is widely recognized for its abundant supply of antioxidants and polyphenols, which play a significant role in neutralizing free radicals and reactive oxygen species (Katiyar et al., 2001; Henning et al., 2003; Caffin et al., 2004). Previous studies have demonstrated that green and black tea provide significant UV protection against entomopathogenic viruses (Shapiro et al., 2008; El Salamouny et al., 2009a, b). However, Kaiser et al. (2018) did not detect a similarly potent efficiency for *B. bassiana* spores in their study. Tea extracts are rich in polyphenols, which act as antioxidants against radicals generated by UV radiation. However, they exhibit minimal absorption of UV-A or UV-B (Yusuf et al., 2007). Research demonstrated that the UV protection of viruses and bacteria is partially achieved due to the antioxidants and antioxidative enzymes that counteract the DNA damage induced by radicals (Ignoffo & Garcia, 1978). In the research directed by Kaiser et al. (2018), it was determined that black tea, combined with *B. bassiana*, significantly increased the exposure of the fungus to UV-B in terms of colony-forming units (CFU). However, no effect was observed with green tea. Nevertheless, in the current study, both black tea and green tea demonstrated a significant UV-protective effect on both fungal isolates. In another study, the UV tolerance of *Metharizium* species was investigated, and it was observed that fungal spores in most strains were inactivated after a few hours of UV exposure (Braga et al., 2001b). In the present investigation, there was a noticeable delay in the development of fungal spores exposed to UV-B compared to the control group (0 min). Additionally, Biryol et al. (2020) examined the UV toleration of *M. flavoviride* As-2 by exposing fungal spores to UV for 30 and 60 min, but significant effects were not observed. Another study result showed that black tea and green tea extracts supply potential UV protectant for the baculovirus, however black tea has the most protectant effect (Ibrahim et al., 2019). Similarly, in this study revealed that black and green tea provided more UV protection in fungal spores. The fact that tea extracts had a higher mortality rate against *G. mellonella* larvae compared to unformulated fungal spores after UV exposure also revealed that they showed a significant protective potential. Ortucu & Algur (2017) reported that there were sudden changes in both radial and spore production, especially at 30 and 60 min of UV-B application. However, in the current study, the radial growth and spore production of fungal spores formulated with black and green tea were higher than the control at 60 and 120 min of exposure. In this study, it was observed that both UV-affected fungal isolates were able to grow and sporulate and maintain their infectivity on *G. mellonella* better than the unformulated control group. An appropriate formulation benefits the application and processing of the bioagent and increases its effectiveness by protecting the active ingredient from adverse environmental factors (Nian et al., 2015).

Although numerous studies have examined the UV tolerance of *B. bassiana* and *M. flavoviride* isolates, there is a scarcity of research on the protective effects of tea extracts as UV protectors for fungal spores. This study presents crucial data by investigating the potential of tea extracts in enhancing the UV protection of fungal spores. The findings of this study demonstrate that the addition of tea extracts as UV-protective additives in formulations can effectively prolong the lifespan of *B. bassiana* and *M. flavoviride* spores when exposed to UV-B radiation.

This study represents the first analysis of the UV-B protection of four distinct tea extracts in laboratory, specifically focusing on their effects on the entomopathogenic fungi *B. bassiana* and M. flavoviride. The present study demonstrated the advantages of black, green, oolong and white tea extracts in UV-B protection of fungal spores and black and green tea have a potential for use as UV protectors under laboratory conditions. The observed decrease in pest mortalities following exposure was attributed to the loss of viable spores due to UV. Hence, the development of formulations to increase persistence of EPF spores under exposure to UV is important in the successful use of EPFs as biological control agents in the fields. Tea extracts have proven UV protection potential in our study and may be an important additive to such a formulation. The developed formulation could be a further valuable tool for fostering the sustainable control of pest insects in the fields.

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