The efficacy of the Entomopathogenic Fungus, *Beauveria bassiana*, in Date Palm Protection against the Red Palm Weevil, *Rhynchophorus Ferrugineus*, Under Hot Dessert Climate

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Abstract

The Red Palm Weevil (RPW) is a devastating pest to palm trees. Commonly used insecticides for its control, can be harmful to the environment and humans. Moreover, some populations of the Red Palm Weevil have developed resistance to these compounds. Lab and semi-field data showed that Entomopathogenic fungi (EPF) can be a non-toxic and effective solution to reducing these pest infestations. However, the efficacy of EPF against the RPW has not been demonstrated in date palms on field trials with their variables. This study aims to examine the impact of various climate conditions the survival and efficacy of EPF *Beauveria bassiana* (*B. bassiana*) in prophylactic applications against the RPW in males and females Date palm groves cultivated using different agricultural practices. Healthy Date palms from the "Arava" region in Israel were selected and equipped with "IoTree" sensors to monitor RPW infestation. At the beginning of each season, the experimental group palms were treated with *B. bassiana* emulsions, while the control group was left untreated. Soil samples were gathered at the beginning and end of each season in both groves to quantify EPF survival rates and test their regression with EPF prophylactic treatments efficacy. Results showed significant effect for the season, on EPF treatments prophylactic efficacy, in the male and female groves. Generally, EPF treatments applied in the autumn season showed significant higher efficacy, while in the summer, the opposite results observed. Analysis of the results for males and females groves separately, displayed superiority for the spring season treatments on the males grove, and higher efficacy for autumn treatments on the females grove. Climate index significantly affects EPF treatment effectiveness. Analysis of soil tests at the end of each season revealed positive association between the number of *B. bassiana* fungus Colony-Forming units (CFUs) detected in the soil and the number of healthy palms within each group. Further research is needed to investigate the survival of EPF in various timeframes and explore diverse application schedules using larger palm samples to evaluate EPF efficacy against the RPW.

1. Introduction

1.1 The Red Palm Weevil

The Red palm weevil (RPW) *Rhynchophorus ferrugineus* (Olivier) (Dryophthorinae; Curculionidae) is an important pest of palm trees worldwide particularly the genus Phoenix. RPW life cycle is cryptic and most of its life cycle occurs inside the tree trunk (Murphy and Briscoe, 1999; Blumberg, 2008; Prabhu & Patil, 2009; El-Shafie and Faleiro, 2020). The female RPW bore holes into the palm tissue via its mouthparts. Within these holes and in natural cervices or wounds within the palm trunk, she lays eggs individually (Ince et al., 2011; Hussain, 2013; Matveev et al., 2023). A larva emerges from the egg within 4 to 7 days. The larval stage lasts between 60 to 105 days. Within the palm trunk, the larva undergoes metamorphosis, transforming into a pre-pupa stage that lasts for 3 to 6 days before becoming a pupa for 13 to 17 days. After this transformation, an adult RPW emerges and lives for a period of 100 to 120 days (Figure 1) (Murphy and Briscoe, 1999). The damage to the palm is caused by the RPW larvae feeding on the palm tissue, thereby destroying it (Giblin-Davis, 2001). The RPW is polyphagous and demonstrates a fast dispersion rate due to the capabilities of flying distance in a short period of time (Dembilio and Jaques, 2015; Hoddle et al., 2015).

1.2 Management of RPW in date palm groves

Conventional farming practices often rely on chemical insecticides, such as imidacloprid and thiamethoxam, as the primary means to control RPW infestations (Kaakeh, 2006; Chihaoui-Meridja et al., 2019; Dhouibi et al., 2017). They are being used as a preventive tool that stops the RPW establishment in healthy palms and as a curative tool in RPW-infested palms. Imidacloprid and thiamethoxam can be given to each tree's root area with irrigation water as a preventive treatment, while a mixture of imidacloprid and gamma cyhalothrin is sprayed on the palm's offshoots and trunk up to 2-meter height, serving both preventive and curative purposes. They have a common mode of action that affects the nervous system of insects, causing paralysis and death (Kurwadkar et al., 2013). Although these compounds provide good efficacy levels, there are drawbacks to such insecticides. There are reports on RPW resistance to these compounds (Wakil, 2018). Moreover, they are poisonous and even deadly to human beings (Mundhe, 2017; Hashemi-Domeneh et al., 2016). A review of the available literature indicates that imidacloprid poisoning can involve gastrointestinal, cardiorespiratory, and nervous systems, or it can be multisystem and can be life-threatening (Mohamed, 2009).

Integrated pest management (IPM) is being used by many countries, including Israel, to control the spread of RPW (Al-Dosary et al., 2016). The IPM program in Israel comprised of sanitation, use of seismic-sensors for RPW detection and preventive and curative treatments (Israeli extension service, 2022). The sanitation measures including weed removal in the trunk area, sprinklers distancing to avoid wetness of the trunk, and offshoots removal. Offshoot removal is performed in the winter when the RPW activity is low, and the wound is treated with tree paste containing asphalt emulsion. Sensors are installed on the palms trunk to detect RPW infested palms (Mendel et al., 2024a, Figure 2). Another component of the management is the use of monitoring traps based on the beetle aggregation pheromone. It assists in timing the preventive application of pesticides based on the seasonal activity of RPW adults.

In organic farming practices, beneficial nematodes are sprayed on the palm's offshoots and trunk up to 2-meter height as a curative treatment. Laboratory and field trials had proven their pathogenicity to the pest larvae, pupae, and adults (Abbas et al., 2003; Yaacobi et al., 2023). Bioveria (Rimi, Israel), a *B. bassiana-based* product, is applied onto the same palm parts as a preventive treatment. It is the only preventive treatment registered for organic management practice (Israeli Extension Service, 2022).

1.3 EPF as a Biological Control Agent

Fungi are a kingdom of Heterotrophic Eukaryote organisms divided to seven phyla. Their unique character is a cell wall made of Chitin and Glucan. They receive its energy by secreting enzymes to their outer environment and absorbing disassembled compounds into their cells (Chandler, 2017). Entomopathogenic fungi (EPF) are described mainly in main two; Ascomycota and Enthomophthoromycota. EPF can infect insects and arthropods, causing a detectable disease that can affect them in all stages of their life from egg to adult (Araujo and Hughes, 2016) (Figure 3). These characteristics make them an excellent biologically friendly substitute for chemical insecticides. *Beauveria bassiana* (*Vuliumin*) and *Metarhizium robertsii (Metchnikoff, Sorokin)* (Ascomycota: Hypocreales) infect a vast variety of arthropods of different orders and used commercially in various agricultural crops (Roberts and Hajek, 1992; Van Lenteren et al., 2018). These characteristics turn them to the most suitable EPF species in biological pest control (Faria and Wraight, 2007). There is large commercial use in EPF insecticides based on *B. bassiana* species in the USA, the European Union, Brazil, Japan, and Mexico. They are being used in various crops against numerous pests of different orders (Mascarin and Jarosnski, 2016). The main practices for using EPF are: The Classical biological control, the augmentative and the inundative practice. In the classical biological control, the fungi applied to exterminate a foreign pest, which is not in his natural habitat that contains his natural enemies. The goal is to make the fungi a long term or a permanent enemy of the pest and by that dilute his population. In the augmentative practice, the idea is to augment the pest natural enemies. The fungi usually applied in a small amount in the beginning of the crop season. The desired affect is that the fungi will go through a few pathogenic life cycles in the pest population and by that will keep it in a small amount. In the inundative practice, a large quantity of the fungi is implemented to exterminate the pest population in short time (Shah et al., 2003).

1.4 EPF development in arthropod hosts

EPF infection and penetration processes are complex and involve different compounds. First, spores of the fungus attach to the insect cuticle through hydrophobic interactions. The spores contain proteins of the hydrophobins family, which create a kind of rod shape that makes the attachment. After the initial attachment, in optimal temperature and humidity, the spores germinate and penetrate the cuticle. This process involves degrading enzymes such as proteases, lipases and chitinases that degrade the insect's cuticle and Appressoria cells that uses penetration pegs to help penetrate the body cavity (Ortiz-Urquiza and Keyhani, 2013). After the penetration, the fungus enters and occupies the insect body. In this process, it evades the insect immune system by changing its cell wall biochemistry. It also depresses the immune response by secondary metabolites. Few days after the penetration, the insect is dead due to loss of water, starvation, and loss of function of its organs. Then, in the final stage, the fungus will outgrow of the cadaver and form new conidia (Brivio and Mastori, 2020) (Figure 4).

1.5 Effect of Abiotic Factors on EPF

Different abiotic factors such as: temperature, ultraviolet radiation and humidity affect EPF's survival, growth, and virulence. It is noteworthy that different isolates have different durability according to the climate in the area from which they were isolated. Thus, there can be intraspecies variation in the tolerance to stress. Below is a background of the central abiotic stress factors relevant to the presented research.

1.5.1 Temperature

Temperature is considered a major factor affecting mostly the growth and virulence of EPF. The longevity of Ascomycete conidia is best under conditions of low temperatures, while growth and disease progression in hosts is favorable under higher temperatures (Ment et al., 2017). Luz and Fargues (1999) found that increasing the temperature from 15 to 25 degrees Celsius in turn decreased the half-life of EPF infected Sawtoothed Grain Beetles. Increasing the temperature more than 25 degrees Celsius caused the opposite effect, by lowering the fungus pathogenicity (Latifian et al., 2018). Regardless of the environmental conditions, the host body temperature is a significant factor in disease progression. Grasshoppers infected with either *B. bassiana* or *M. flavoviride* that were allowed to bask for different periods of time were less susceptible to infection as basking periods increased (Ment *et al.*, 2017).

1.5.2 Ultraviolet radiation

Ultraviolet radiation (UV) is the most influential abiotic factor on the survival of cells in general. Regarding EPF, even the most durable isolates will not survive after a few hours of direct UV exposure (Fernandes et al., 2015). UVA radiation causes Reactive Oxygen Species (ROS) accumulation in the cell, while UVB radiation changes the structure of the Pyrimidine nucleotides (Griffiths et al., 1998). In experiments conducted on *M. anisopliae,* it has been found that UVA radiation has reduced the sporulation and durability of the conidia (Braga et al., 2001). Another experiment showed the same effects in *B. bassiana* from exposure to UVB. A noteworthy fact that rose from this experiment is that distance from the Equator affects the durability of the isolates when exposed to UVB. Isolates produced near the Equator were more resistant to UVB than the isolates produced further from the Equator (Fernandes et al., 2007).

EPF Formulation contained UV protectants additives such as vegetable oils, adjuvant oils, oil-soluble sunscreens, optical brighteners, and clay increased the survival of conidia under field conditions (Ment et al., 2017).

1.5.3 Humidity

High Relative humidity (RH) is especially important for spore germination and fungus sporulation. In the laboratory experiment performed, RH of 100% ensured very fast and efficient infection of saw-toothed grain beetle by *B. bassiana*. In less than 90% RH, infection occurred sporadically and at low rates (Searle and Doberski, 1984). While high RH is crucial for conidia germination and disease progression in insects in aerial habitats, low RH is prerequisite for conidial longevity in the environment (Ment et al., 2017; Ment et al., 2020).

1.6 Effect of nitrogen fertilizers on EPF

1.6.1 Urea

The effect of urea on *B. bassiana* by itself is positive, leading to growth and reproduction. Nevertheless, different microbes in soil can cause fungistatic effect when urea is added. In an experiment performed, addition of urea to a sterile soil caused dramatic increase in conidia numbers after 21 days. When added to non-sterile soil, no conidia left after the same time frame. Isolates of *Penicillium urticae* was extracted from the soil and found to produce a water-soluble inhibitor of *B. bassiana*. (Lingg and Donaldson, 1981)

1.6.2. Ammonium nitrate

Limited research has been conducted on the potential effects of ammonium nitrate on the survival, virulence, and sporulation of *B. bassiana*. Shapiro et al. (2013), reported that the fungus did not exhibit any significant changes in survival or virulence when exposed to ammonium nitrate. Conversely, Cojocaru and Lumînare (2021) found that among various nitrogen sources, ammonium nitrate proved to be the most effective in promoting spore production of *B. bassiana* during submerged liquid fermentation.

1.7. EPF against the RPW

Several laboratory studies showed that EPF can control RPW populations by reducing their egg hatching rate and eliminating both larva and adult stages and can complete their full life cycle on RPW dead cadavers (Al-keridis et al., 2020; Gindin et al., 2006). Additional laboratory and semi-field experiments have confirmed that species such as *B. bassiana* and *M. anisopliae* can infect and eliminate all life stages of the RPW with high efficacy rates, sometimes achieving mortality rates of up to 100%. (Dembilio et al., 2010; Fong et al., 2018; Güerri-Agulló et al., 2010). Recently, Ment et al. (2023) reported that Velifer®, a *B. bassiana* product, and *M. brunneum* (Mb7) resulted in 60–88% female mortality. Mb7—as a conidial suspension or powder—resulted in 18–21% egg-hatching rates, approximately 3 times less than in the non-treated control. Treating palms under greenhouse conditions with Mb7 significantly inhibited infestation signs and results in palms' protection. Regarding fungal persistence, a previous study conducted in our research group investigated the persistence of EPF in date palm soil and trunk in Israel. The results showed that viable fungal units persisted in the soil up to 280 days and in the trunk up to 90 days. Additionally, the quantities of colony-forming units (CFUs) of EPF taken from soil and trunk samples were found to be moderately correlated, and their levels decreased over time in a similar way (Ment et al. 2020; Livne, 2024). EPF was also found to be effective against the RPW in several field trials (Sabbahi and Hock, 2023). However, the persistence and efficacy of EPF in RPW control have not been tested on large-scale field trials, nor have they been evaluated under various agricultural practices and climate conditions. Another unexplored subject is the relationship between the quantity of EPF propagules present in the soil and the level of RPW-infected palms.

This study aims to determine EPF survival and efficacy in protecting palms against the RPW under different abiotic factors and different agricultural management practices; as well as to analyze the correlation between palm protection rate and EPF persistence measure in the soil. To our knowledge, this is the first field study conducted in palm groves that test EPF persistence and efficacy against the RPW.

The hypotheses in this study are described here in two parts. First, we hypothesized that as the abiotic factors will be optimal for the fungi survival and establishment, the fungi efficacy will increase regarding palm-protection against RPW. Our second hypothesis posits that variations in palm canopy height, and the application of chemical fertilizers versus compost, will have distinct effects on fungi survival in the soil. We also predict a positive correlation between the quantity of EPF colonies sthat urvived in the soil and the amount of healthy treated trees. To test these hypotheses, we chose suitable groves, installed sensors on palm trees, treated them with EPF-based products, monitored the palms for RPW infestation, and compared the results to untreated palms. At the beginning and end of each EPF application, soil samples were collected, and EPF persistence was quantified. The experimental period spanned a little over 2 years, with 15 experiments in total.

1.8. Research questions

1.8.1. How do various seasons and climatic conditions affect the persistence and efficacy of preventative EPF treatments against RPW palm infestation?

1.8.2. How do differences in palm's morphology and farming practices effect the efficacy of EPF treatments in RPW prevention alongside fungal survival?

1.8.3. Is there a correlation between the quantity of surviving units of EPF to the rate of palms protection against RPW infestation?

1.9. Research hypotheses

1.9.1 Whereas the climate conditions in the experiment will be optimal for the fungi's survival and persistence, their efficacy in RPW prevention will increase.

1.9.2. Canopy height variations and fertilization practices will affect the survival and establishment of EPF, which in turn will impact the protection of palms toward RPW.

1.9.3 High survival of EPF in the palm grove will positively correlate with high levels of palm protection against the RPW.

1.10 Research objectives

1.10.1 Evaluating the effectiveness of EPF application in various climate conditions in reducing the prevalence of RPW-infested palms at two types of palm groves (Tall organic grove vs. short conventional grove).1.9.2. Evaluate EPF persistence and establishment under various climatic conditions in two types of palm groves: tall, organically fertilized palms versus short, chemically fertilized palms.

1.10.2 Determine the correlation between EPF persistence and palm protection in treated palms, considering both seasonal variations and morphological and agricultural practices differences between the two grove types.

2. Materials and Methods

2.1 Date palm groves properties and treatments procedure

2.2.1. Date palm groves properties

Date palm groves were picked nearby Moshav Idan, which located in the central Arava Valley in Israel which is a hot dessert area

(Figure 1A). The suitability of the groves, as well as each palm in the grove, is determined by specific criteria. The age of the palm groves were six to eight years old when starting the experiments (June 2021). The area of the groves was previously reported for RPW infestations (Dobrinin S., Personal communication). The two types of groves where experiments were carried out during the study, were 1.5 kilometer apart from each other (Figure 1B-C).

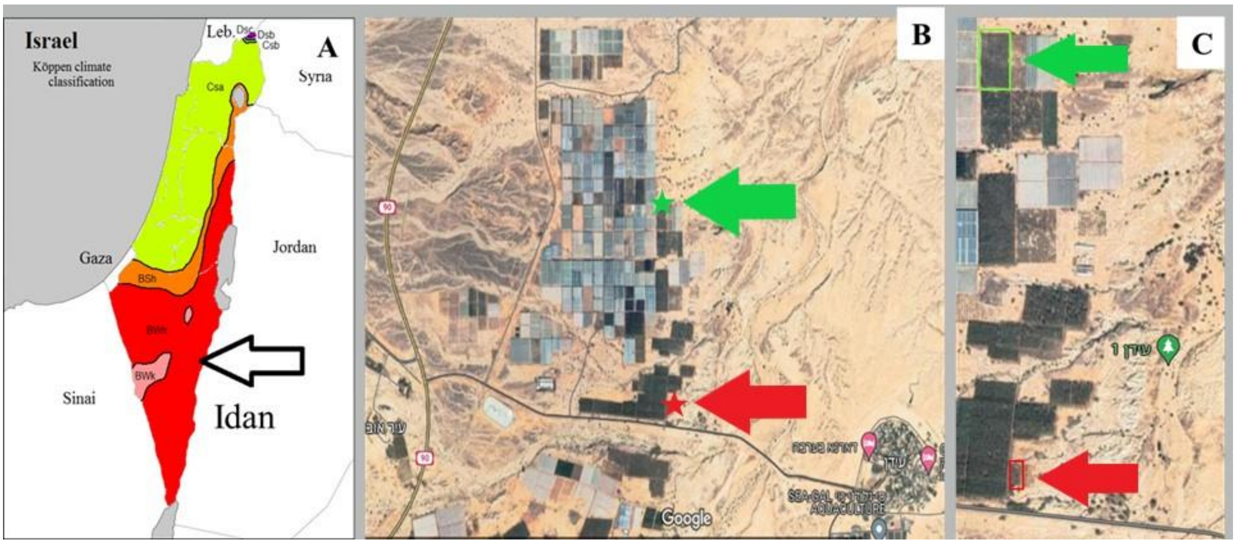

Figure 1. The study area and plantation location in Moshav Idan at the central Arava Valley. (A) Idan marked on the Koppen classified map of Israel at a climate designated as BWh, Hot desert (Source: Fleischer, 2018). (B-C) The studied plantation location is marked by green for the Medjoul variety palms and red for male palms (Source: Google Maps).

Females grove

The female date palm grove is an organic grove, planted in 2014, which comprised of 250 female palms of the Medjool variety. Palms' fruits are harvested each year in August-October. Fertilization is performed through application of compost to the palms' trunk surroundings in March. Palms were not treated by any growth retardant. Morphology is typical for medjool variety plantations relatively to their age, with a uniform trunk height of approximately 2.5 meter at the beginning of the experiment. 3.2 % of the palms in the grove were considered infested in the RPW two weeks before the experiments were started. Response treatments for RPW were performed using beneficial nematodes before and during experiments.

Males grove

Males palm grove was planted in 2015 and comprised of 56 male palms of mixed varieties. Spathes are harvested every year in February-April to produce date palm pollen. Chemical fertilization is performed every year in the months of March-July using Uran 32, which comprises of 16% Urea, 8% Ammonium, and 8% Nitrate. Palms are treated with the growth retardant uniconazole every two years to inhibit their vegetative growth. This is displayed by lower trunk heights relative to palms age, and shorter leaves which slightly leaning towards the ground. Trunk height was ranging between 1.5-2 meters in the beginning of our experiments. This grove demonstrated higher infestation rate prior to our trials, when 13.2% of its palms considered infested in the RPW by the acoustic sensors, two weeks before starting our trials. Before and during our research trials, RPW infested palms were treated with drenched imidacloprid a responsive measure. However, these treated palms were not categorized as either control or EPF treated palms in our study. EPF treated or control palms were treated with beneficial nematodes.

Table 1. Descriptive information regarding experimental grove in Idan

Category	**Males grove**	**Females grove**
Total number of palms	56	250
Variety	Mixed varieties	Medjool
Agricultural practice	Conventional	Organic
Coordinates	30° 48' 36.115" N 35° 16' 31.090" E	30° 49' 24.611" N 35° 16' 27.808" E
Planting year	2015	2014
Fertilization (Type, Period of application)	Uran 32, March- July.	Compost, March
Growth retardant application (Name, period of application)	Uniconazole, April, Every two years, since 2022.	-
Curative RPW treatment given	Imidacloprid	Beneficial nematodes

(Insecticide active compound name/species)		(*Steinernema carpocapsae*)
RPW infested palms (%) (Measured by sensors, 2021)	13.2	3.2
Average trunk height (Meters) (At the beginning of the trials at 2021)	1.5-2	2.5

2.2.2. Experiments procedure

Each tested palm was equipped with transmitting sensor (Agrint Inc.) which indicates its health index status, for at least a month prior to the onset of experiments. The palms in each grove were divided into three groups: Control (untreated), Treatment 1, Treatment 2. The main treatment applied during the study was the spray application of BotaniGard (LAM, US) distributed in Israel under the name Bioveria (Rimi, Israel). The palms of each experimental group are arranged in clusters (Figure 2). Control and experiment groups were irrigated by the same watering schedule and were treated equally pre-harvest and post-harvest according to each groves' regimen. We sprayed each experiment group with either Velifer (BASF) or Bioveria emulsion in experiments 1-4 and 10-12. Afterwards, on experiments 5-6 and 13-14, the Velifer treated groups were replaced by Bioveria + L77 emulsifier treatments. Subsequently, these treatments were also replaced on experiments 7-9 and 15, for treatments of double concentration of Bioveria emulsion (Bioveria X2). A description of all the experiments conducted is shown in Table 2 and will be further described in the next section.

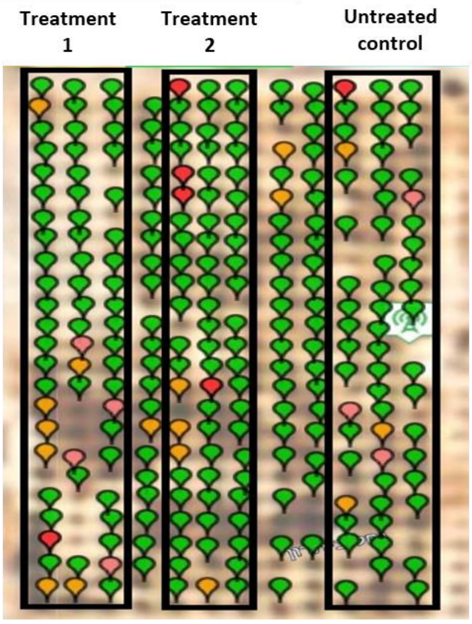

Figure 2. Satellite image of the female grove. Each map pin represents a sensor installed on a palm. Cluster arrangement is demonstrated when each group is separated by 1-2 rows of palms. (Source: Agrint website screenshot)

2.3. EPF application

Each experimental group palm was sprayed manually using a spray gun, by a tractor-mounted hydraulic sprayer with a capacity of 500 liters. To achieve 0.2% v/v Bioveria emulsion, 1 liter of Bioveria emulsion were diluted with 499 liters of fresh water. Before each application, spray gun flow rate was measured, and spraying time was calculated to apply 8-12 liters per palm. "Bioveria+L77" group sprayed with 0.2% of "Bioveria" emulsion mixed with water and addition of 0.025% or 0.1% "L77" spreader according to the trial protocol (Table 2). Application performed by spraying each palm trunk rotary from its bottom to a height of 1.5 meters includes the ground surrounding the palms in a radius of half a meter from the trunk base. The treatment is given once at the beginning of each experiment. EPF preparation specification are specified below in table 3.

Table 2. Experiments description in the Arava according to name of the site, season, and number of palms in each group.

Experimental grove	Season, month and year	Experiment No.	Palms No. in control	Palms No. in Bioveria	Palms No. in Velifer	Palms No. in Bioveria + L77	Palms No. in Bioveria X2
Females	Summer Jun. 2021	1	52	53	-	-	
	Autumn Sep. 2021	2	52	49	50*	-	
	Winter Dec. 2021	3	47	50	44	-	
	Spring Mar. 2022	4	57	66	63	-	
	Summer Jun. 2022	5	57	60	-	59**	
	Autumn Oct. 2022	6	51	59	-	51	
	Spring Mar. 2023	7	52	104	-	-	19
	Summer Jun. 2023	8	56	94	-	-	16
	Autumn Sep. 2023	9	51	82	-	-	14
Males	Autumn Sep. 2021	10	9	12	14*	-	
	Winter Dec. 2021	11	11	10	12	-	
	Spring Mar. 2023	12	13	16	15	-	
	Summer Jun. 2022	13	13	15	-	16**	
	Autumn Oct. 2022	14	16	15	-	15	
	Autumn Sep. 2023	15	20	20	-	-	14

* Velifer was applied at concentration of 0.02%.

** L77 was applied at concentration of 0.025%.

Table 3. Entomopathogenic fungi commercial product information

Commercial name	Fungus	Isolate	Manufacture	Formulation	Application method	Dose
Bioveria *	*Beauveria bassiana*	GHA	LAM international	Emulsion	Spraying	0.2%v/v
Velifer	*Beauveria bassiana*	PPRI 5539	BASF	Emulsion	Spraying	0.2%v/v

* Botanigard is the original name of the emulsion given by the producer LAM international while Bioveria is the product name termed by RIMI company, which distributes the product in Israel.

2.4. Climate data

The climate databases of the study area were collected from the archives of the Northern Arava Research and Development Center. Monthly Mean, maximum and minimum temperature at 2 meters (C°), RH (%), solar radiation intensity (W/m²), and the monthly mean of the reference evapotranspiration (ET0) by FAO 56 Penman Monteith equation (Allan et al., 1998) were recorded for each month during the experiments. The average for each month for these factors was determined using hourly measurements and a standard time base of 24 hours. The weather station is located approximately 7 Kilometers South-west of our field trials location.

2.5. Sensors for RPW activity

"IoTree" sensors by "Agrint Sensing Solutions INC" were used to detect RPW activity on the palms. Each palm had equipped with a sensor installed on his trunk in a height of 1 meter from the ground (Figure 2). After a calibration period of two weeks, the sensor becomes active. The sensor's mechanism of action is based on his ability to recognize the specific vibration of the RPW larvae in the palm's trunk. Each sensor vibration information is transferred to a hub, which sends it to a server on real time. Then, the vibration duration and frequency are being processed and compared to the vibrations coming out of other palms. After processing, the palm is categorized as clean, suspect, or infested with RPW. The information is showed in Agrint website or mobile application. Palms are numbered and displayed on a satellite map with indication of their infestation level. Palm marked in green is considered clean, yellow marked palm is suspect and red marked palm is considered infested by the RPW.

2.6. Fungal survival evaluation

2.6.1 Soil samples collection for fungal propagules survival quantification

Soil samples were collected from each of the groves. Samples were collected in EPF application day, before and after the application. Before each application, five samples were collected from each group in the males grove, while 10 samples were taken from the females grove. Following the application, two samples were taken from each group treated with EPF in the males grove, and three samples were taken from each EPF-treated group in the females grove. Five different spots around the palm trunk were sampled by a trowel. Samples taken prior to the application, are served to examine EPF survival rates from the last application. Post application samples determines the initial number of EPF CFU and validating the emulsion vitality. The difference in CFU quantity counted immediately after application and on the last day of application (before the next application) is served to measure CFU loss during each experiment.

2.6.2. Moisture content determination

Each sampled palm soil was well mixed, while approximately 10 grams of the wet sample were extracted, poured, and sewed on 90 mm petri dish. Number of sample soil and the sample weight was written on the plate's cover. Then, the plate was left to dehydrate for one week in a dark room. After dehydration, the sample was weighted again to determine its final weight, for moisture content determination. The moisture content percentage was calculated by this formula:

$$\frac{Initial\ soil\ weight - final\ soil\ weight}{Initial\ soil\ weight} * 100 = \%\ Moisture\ content$$

2.6.3. CFU/G dry soil quantification

While determining the moisture content for each sample, we also conducted a quantitative analysis of CFU/G dry soil. First, 1 gram of wet soil from each sample was mixed with 10 ml sterile water containing 0.01% (v/v) triton-x surfactant for batter extraction. The sample was transferred for two hours into a rotating incubator with a temperature of 25 degrees Celsius and a speed of 200 rpm. Finally, 3 replicates of 50 µl of the suspension were uniformly sewed on 90 mm Petri dishes that contained a fungus-selective Sabouraud Dextrose Agar (SDA) supplemented with 1µl/1ml Chloramphenicol and 1µl/2ml Dodine. Sewed samples were transferred to incubation in a dark room with a temperature of 28 degrees Celsius for 5-7 days according to the colonies appearance and densities. After incubation period, CFU was counted and doubled by 200 to revert the dilution factor. To calculate the weight of dry soil, each sample retrieved moisture content was reduced by this formula:

$$1g - \frac{moisture\ content}{100} = \ dry\ soil\ weight\ (g)$$

Finally, the normalized CFU count was divided by the normalized dry soil weight to attain the quantity of CFU/G dry soil.

2.7. Statistical analysis

All statistical analyses were conducted using JMP® software, version pro-16. (SAS institute Inc., USA) The number of infected and healthy palms along with CFU survival data for each group, were recorded using spreadsheet software developed by Microsoft Excel. (Microsoft, USA). CFU count from Soil samples was repeated three times to obtain a representative data. A fixed significance level (α) of 0.05 was used for all tests.

2.7.1. EPF palm protection efficacy test

To test whether EPF treatments had a significant effect as a protective measure against the RPW in each season, we used Chi-square or Fisher exact tests. At the end of each season, each group infested, and healthy palms were counted. Healthy, and infested palms in each group were categorized separately and their frequency registered with respect to the total palms in the group. For example, if 3 out of total 50 palms in a group were infested during an experiment, the frequency of infested palms category registered as 3 out of 50 palms while in the healthy category, frequency registered as 47 out of 50 palms. In the next stage, "fit y by x" function was used to test the relationship between the treatment and the frequency of infected and healthy palms. Treatment or season were set as the x factor, in accordance with analysis type, and palms health was set as the y factor. Frequency of each group's healthy or infested palms was set as Frequency value. In a case where 20% or more of the cells in the Chi-square test table had expected count of less than 5, Fisher's exact test was used. To compare the efficacy rates of EPF preparations on each season, healthy palms frequencies of the different EPF treatments were compared to each other without including the control group. To test the seasonal variations in efficacy of each EPF preparation, the healthy palm rate of each treatment was compared by itself to the control group.

2.7.2. Effect of climate variables on EPF treatment efficacy

Healthy to total palms ratio was recorded in the control or EPF groups on each month as described in the previous section. To avoid sample size distortions, the number of infested palms on each month was reduced from the group's total number of palms in the next month, until the next EPF application. For example, if 2 out of 52 palms were infested in RPW in the control group in January, in February, those two palms were removed from the total palms of the group, and healthy to total palms ratio was determined from corrected number of total 50 palms, for this group. Since in March, EPF were applied again, the total number of palms was reset according to the number of fitted palms according to experiment protocol. After the summation of the healthy to total palms ratio on each month, efficacy of EPF treatments was determined in relation to the control group, using Henderson-Tilton's formula. Originally, this formula was used to calculate the efficacy of insecticides when sample sizes are not equal among the treated and control groups (Henderson and Tillton, 1955). We modified it to calculate the protection efficacy of EPF against control treatment. The adjusted formula is:

$$Effiacy\,\% = (1 - \frac{N(T,\ Total) * N\,(C,\ Healthy)}{N\,(C,\ Total) * N\,(T,\ Healthy)}) * 100$$

Where-

N (T, Total) = Total number of treated palms.

N (C, Total) = Total number of Control palms.

N (T, Healthy) = Number of palms remained healthy in treatment group.

N (C, Healthy) = Number of palms remained healthy in control group.

The formula's results presented as quantitative percentages, where's higher ratio of healthy palms among EPF treatments over the control, will yield higher positive value. Lower ratio of healthy palms among EPF treatments, will produce lower negative value. Identical efficacy rates among EPF and control treatments will result in 0%. Statistical analysis was performed using fit y by x function, where's monthly ET_0 values were set as the affecting variables (X factor). Monthly calculated efficacy percentages were set as the dependent variables (Y factor). The regression between these variables was examined on each grove separately. Since there is multicollinearity between temperature, RH and UV, ET_0 was chosen to serve as an index, which ascends with conditions of higher UV and temperatures, and low RH.

2.7.3. Seasonal variations of EPF survival rate on female and male groves

EPF survival rates were evaluated for each grove on different seasons. CFU loss per day was calculated for each sampled palm by this formula -

$$\frac{\log(CFU_{after\ spraying} + 1) - \log(CFU_{end\ of\ exp.} + 1)}{Experiment\ num.\ of\ days}$$

CFU/g dry soil numbers were registered after spraying and in the end of the experiment for each sampled palm. A Logarithm was applied for each sample to normalize data distribution and stabilize the variance across the different samples. Before the Logarithm application, 1 CFU was added for each sample to avoid zero values in the data. To normalize the time factor, CFU loss per day was calculated. The Log-transformed values within each group were calculated in this way: the final value at the end of the experiment was subtracted from the initial value recorded at the beginning of the experiment, and this difference was then divided by the number of days the experiment lasted. The calculated data for each experiment was registered to an excel sheet and each sample was categorized by farm and season. CFU loss per day defined as the dependent factor. Before each ANOVA test, all dependent factors were first subjected to Levene's test to assess the equality of variances. (α <0.05),

Season and grove impact on EPF survival

Whole model analysis was carried out using fit model option on JMP software. Season, grove, and the combination of these two factors were defined as the contributing factors, using the cross option. Rank transformation was applied to CFU loss per day values to achieve equal variances. A Post-hoc Tukey-HSD was conducted to examine differences in survival between tested seasons.

Season impact on EPF survival in each grove

For each grove by itself, each season was considered as the independent variable (x factor). When equal variances were observed, a one-way ANOVA test was performed. In cases where variances found to be unequal, Welch's t-test was utilized to assess the seasonal variances. In case of significance variations have found between seasons, multiple comparisons were performed between survival rates for each pair of seasons. Each comparison underwent another Levene's test to check its homogeneity. If variances were equal, a pooled-t test applied; Otherwise, t-test was used. Bonferroni correction was applied to adjust the level of significance by dividing the significance level by the number of comparisons. Hence, the adjusted Alpha level was set as α=0.05/3= 0.0167.

EPF survival rates difference between the groves

Evaluation of the differences between EPF survival in the male and female groves on each season was performed. Grove was defined as the independent factor in each season. Seasons comparisons consisted equal variances in survival rates were subjected to pooled-t test, while unequal variances analyzed by Welch's t-test.

2.7.4. EPF survival and palms health association

To examine the relationship between the quantity of EPF found in the soil to the relative number of healthy palms in each group, Chi-square test was carried out. The relationship has been tested using "fit y by x" function. On each farm, Average CFU count was calculated for each EPF and control group, from the samples collected in each group at the end of each season, prior to the next application of EPF. Similarly to the previous section, 1 CFU was added to each sample and a logarithm was applied. The calculated log CFU average at the end of the season was defined as the x factor, when the palms health in the group were defined as the y factor. Palms health and their frequency calculated in the same way as in section 2.7.1. To create the graph that shows the relationship between the two variables, a probability formula based on the Chi-square results was created and saved in date sheet. Then, graph builder function was used when CFU mean survival defined as x factor and the calculated probability for healthy palm was defined as the y factor.

3. Results

3.1. Climatic variables

Table 4 presents the summary of mean, maximum, and minimum recordings of the temperature, RH, and radiation across the experiment's different seasons. **At the Arava Valley** during the **summer** experiments period, the average temperature was 32.9°C, with a mean solar radiation intensity of 312 W/m² and a RH of 39%. In the **autumn**, the average temperature was 23.6°C, with a mean radiation flux of 171 W/m² and a RH of 51%. In the **winter** experiment period, average temperature was 15.8°C, with a mean solar radiation intensity of 126 W/m² and a RH of 53%. In the **spring** experiments, the average temperature was 23.7°C, with a mean radiation flux of 247 W/m² and a RH of 39%. Detailed data on climate conditions for each month is provided in Supplementary Table S1.

Table 4. Summarized climatic data measured during the study by seasons. Data includes mean, maximum and minimum values of temperature, relative humidity, radiation, and evapotranspiration (ET_0) for each experiment season.

Season	Temperature (°C)			Relative humidity (%)			Radiation (W/m²)			ET_0 (mm/day)
	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean

Summer	32.9	46.2	21.5	39	74	6	312	1026	0	7.35
Autumn	23.6	40.9	6.8	51	96	13	171	921	0	3.12
Winter	15.8	26.9	3.1	53	92	15	126	825	0	1.92
Spring	23.7	42.4	6.5	39	92	6	247	1037	0	4.68

3.2. Protection efficacy of EPF treatments to palms under varying climate conditions and agricultural practices

3.2.1. Seasonal effect on protection efficacy of EPF treatments

Female grove

Summer

Experiments were conducted in the female date palm grove during the summer seasons of 2021, 2022 and 2023, June-September (Table 5). Either one of the experiments has not shown statistically significance results for EPF treatments in protection against RPW infestation. The first summer experiment (No. 1) was conducted in June 2021, 96% of the palms in the Bioveria group were healthy, while 100% in the control group were healthy (χ test, p=0.0959). Overall, only two out of 105 palms in the experiment were infested by RPW this season. In the second summer experiment (No. 5), while 89% of the palms in the control group were healthy, in Bioveria and Bioveria+L77 treatment groups 90% and 84% of the palms were healthy respectively (χ test, p=0.5611)**.** In the third summer experiment (No. 8), 93% of the palms in the control group were healthy, while in Bioveria and Bioveria X2 treatment groups 88% and 81% of the palms were healthy respectively (χ test, p=0.3876).

Autumn

Experiments were conducted in the female date palm grove during the autumns of 2021, 2022, and 2023, from September/October to December (Table 5). In the first autumn experiment (No. 2), 100% of the palms in the Bioveria group remained healthy versus 92% in the control group (Fisher's exact test, p=0.1179). The second autumn experiment (No. 6) performed in October 2022. The results show 98% healthy palms in the Bioveria and bioveria+L77 group, compared to 96% healthy palms in the control group (Fisher's exact test, p=0.8698). The third autumn experiment (No. 9) performed in September 2023. In the end of the experiment, 93% of the palms in both the Bioveria and Bioveria X2 groups were healthy, while in the control group 88% remained healthy (Fisher's exact test, p=0.8206).

Winter

A single winter experiment (No. 11) was performed in the female grove from December 2021 to March 2022 (Table 5). None of the 141 palms in the treatment and control groups were infested with the RPW. As a result, it was not possible to measure the effectiveness of EPF protection.

Spring

The spring experiments were conducted in the female grove in 2022 and 2023 from March to June (Table 5). At the end of the first experiment (No. 4), the Bioveria and Velifer groups had 91% and 92% healthy palms, respectively, while in the control group, 95% of the palms remained healthy (Fisher's exact test, p=0.7348). In the second spring experiment (No. 7), the Bioveria and Bioveria X2 groups had 92% and 84% healthy palms, respectively, while the control group had 98% healthy palms (Fisher's exact test, P=0.1049).

Male grove

Summer

The summer experiment (No. 13) conducted in the male grove from June to September 2022 resulted in 87% and 80% healthy palms in the Bioveria and Bioveria+L77 treatments, respectively, compared to 100% healthy palms in the control group (Fisher's exact test, p=0.2942) (Table 5). In the summer experiment of 2023, preventive EPF treatments were applied in the grove, but an error in the sensors disrupted their proper indication. Therefore, the experiment was not further analyzed.

Autumn

Autumn experiments were conducted in the male date palm grove during September/October-December of 2021 and 2022 and 2023 (Table 5). The first experiment (No. 10) resulted in 92% healthy palms in the Bioveria group versus 67% in the control group (χ test, p=0.1464). In the second experiment (No. 14), 87% of the palms in both Bioveria and Bioveria+L77 groups remained healthy, while 87.5% of the palms in control group remained healthy (Fisher's exact test, p=1.00). In the third experiment (No. 15), 100% of the palms in the Bioveria group remained healthy, compared to 93% in the Bioveria X2 group and 87% in the control group (Fisher's exact test, p=0.1808).

Winter

Winter experiment (No. 11) was conducted in the male grove during December to March 2021 (Table 5). Results indicated 70% and 92% healthy palms in the Bioveria and Velifer groups, respectively, compared to 82% healthy palms in the control group (Fisher's exact test, p=0.3875). Analyzing altogether EPF-treated palms resulted in 86% healthy palms (n=22) compared to 82% healthy palms (n=11) in the control group (Table 7) (Fisher's exact test, p=1.00).

Spring

Spring experiment (No. 12) was conducted in the males grove during March to June 2022 (Table 5). Results indicated a significant protection rate, as 100% of the palms in both Bioveria and Velifer groups remained healthy, while only 75% of the palms in the control group remained healthy (Fisher's exact test, p=0.0216). The spring experiment of 2023 was not analyzed due to errors in the sensors.

Table 5: Experiments summary by grove, period of application, experiment number, number of total palms and the ratio of palms that remained healthy

Grove	Season and month of application	Exp. No.	Control No. of palms (Healthy %)	Bioveria No. of palms (Healthy %)	Velifer No. of palms (Healthy %)	Bioveria+L77 No. of palms (Healthy %)	Bioveria X2 No. of palms (Healthy %)	P-value
Females	Summer Jun. 2021	1	52 (100%)	53 (96%)	-	-	-	0.4952 @
	Autumn Sep. 2021	2	52 (92%)	50 (100%)	-	-	-	0.1179 @
	Winter Dec. 2021	3	47 (100%)	50 (100%)	44 (100%)	-	-	-
	Spring Mar. 2022	4	55 (95%)	64 (91%)	61 (93%)	-	-	0.7674 @

	Summer Jun. 2022	5	54 (91%)	59 (90%)	-	55* (84%)	-	0.4697 #
	Autumn Oct. 2022	6	51 (92%)	59 (97%)	-	49 (98%)	-	0.3879 @
	Spring Mar. 2023	7	52 (98%)	104 (92%)	-	-	19 (84%)	0.1049 @
	Summer Jun. 2023	8	56 (95%)	91 (88%)	-	-	16 (82%)	0.2141 @
	Autumn Sep. 2023	9	51 (88%)	82 (93%)	-	-	14 (93%)	0.8206 @
Males	Autumn Sep. 2021	10	9 (67%)	12 (92%)	-	-	-	0.2722 @
	Winter Dec. 2021	11	11 (82%)	10 (80%)	12 (92%)	-	-	0.7094 @
	Spring Mar. 2022	12	12 (75%)	16 (100%)	13 (100%)	-	-	**0.0223** **@**
	Summer Jun. 2022	13	10 (100%)	16 (87%)	-	15* (93%)	-	0.7749 @
	Autumn Oct. 2022	14	16 (87.5%)	15 (80%)	-	13 (85%)	-	0.8803 @
	Autumn Sep. 2023	15	20 (85%)	20 (100%)	-	-	14 (93%)	0.1808 #

* L77 was applied at concentration of 0.025%. # Calculated by Chi-square test. @ Calculated by Fisher's exact test. Note: Due to human error, in trials 2 and 10, Velifer was applied in a concentration of 0.02% instead of 0.2%. As this conidia concentration is insufficient to protect the palms from the RPW, the results for these specific experiments results were subtracted.

3.2.2. Treatment and seasonal effect on protection efficacy of EPF

Females grove

Comparison of protection efficacy among EPF treatments and the control palms, did not result in significant results (Table 6), while seasonal changes significantly effected infestation rate among treatment groups compared to the control (Table 7). In summer, Bioveria treatment showed 91% healthy palms, followed by Bioveria+L77 (84%) and Bioveria X2 (81%) (χ test, p=0.2443). In autumn, Bioveria+L77 had 98% healthy palms, while Bioveria and Bioveria X2 had 96% and 93%, respectively (Fisher's exact test, p=0.4574). Healthy palms rate in the spring were the highest among Velifer (94%), followed by Bioveria (92%) and Bioveria X2 (84%) (χ test, p=0.5006). Both Bioveria and Velifer treated palms remained healthy during the winter season. Although not statistically significant, the efficacy rates of Bioveria treatments varied across different seasons. The effectiveness of Bioveria in protecting palms compared to the control showed seasonal variation, with the highest efficacy observed in autumn. During this season, 96% of the Bioveria-treated palms remained healthy, compared to 91% of the control palms (χ test, p=0.0643). In contrast, the treatment showed lower efficacy in summer, with 91% of the treated palms remaining healthy versus 94% in the control group (χ test, p=0.1226). The least effective season for Bioveria treatment was spring, with 92% of treated palms healthy compared to 96% in the control (χ test, p=0.1192). Notably, in winter, none of the palms in either group were infested by the RPW. In the winter, similar to the Bioveria treatments, none of the palms in the Velifer group were infested by the RPW. During spring, both the Velifer and control groups maintained 96% healthy palms (Fisher exact test, p=0.4632). Bioveria + L77 treatments demonstrated significant differences in efficacy across seasons. In autumn, 98% of the treated palms remained healthy compared to 91% in the control group (Fisher exact test, p=0.1246). Conversely, in summer, only 84% of the treated palms were healthy versus 94% in the control group (Fisher exact test, p=0.0207). Although not statistically significant, the efficacy of Bioveria X2 treatments also varied. In autumn, 93% of the treated palms were healthy compared to 91% in the control group (Fisher exact test, p=1.0000). Summer treatments were less effective, with 81% healthy palms in the Bioveria X2 group versus 94% in the control group (Fisher exact test, p=0.0795). Spring treatments showed the least effectiveness, with 84% of the treated palms remaining healthy compared to 96% in the control group (Fisher exact test, p=0.0692)

Males grove

None of the EPF treatments resulted in superior protection over the others (Table 6). Additionally, seasonal variations did not significantly affect any specific treatment (Table 7). In the summer, Bioveria+L77 treatment showed higher rates of efficacy (93%) over Bioveria treatment (88%) (Fisher exact test, p=1.0000). In autumn experiment, similar rates of 93% and 91% were observed among Bioveria X2 and Bioveria treatments, respectively, while Bioveria + L77 treatment was less efficient with 85%. (Fisher exact test, p=0.7276). In winter experiments, Velifer treatment showed 92% healthy palms, compared to 80% in Bioveria treatment (Fisher exact test, p=0.5714). Both Velifer and Bioveria treatments resulted in 100% healthy palms in the spring season. Bioveria treatment efficacy varied over the seasons, with 100% in spring compared to 75% in the control (Fisher exact test, p=0.0752). 91% healthy palms against 82% in the control group in the autumn (Fisher exact test, p=0.1839). 88% compared to 100% healthy palms in the control, in the summer Fisher exact test, p=0.5077). In the winter, 80% and 82% healthy palms were observed in the Bioveria and control group, respectively (Fisher exact test, p=1.0000). Velifer exhibited 100% efficacy against 75% in the control, in spring season (Fisher exact test, p=0.0957). while in winter it exhibited 92% versus 82% in the control (χ test, p=0.5901). Bioveria + L77 treatment resulted in 93% healthy palms compared to 100% healthy control palms, in summer (Fisher exact test, p=1.0000). In autumn,85% against 82% healthy palms observed in Bioveria + L77 and control groups, respectively (Fisher exact test, p=1.0000) Bioveria X2 treatment was applied only in autumn and showed 93% healthy palms compared to 82% in the control group (Fisher exact test, p=0.6714).

Table 6: Seasonal comparison of EPF treatments in the female (left) and male (right) groves.

Treatment	**Females**				**Males**			
	Summer	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**
Bioveria No. of palms (Healthy %)	203 (91%)	191 (96%)	50 (100%)	168 (92%)	16 (88%)	47 (91%)	10 (80%)	15 (100%)
Velifer No. of palms (Healthy %)	-	-	43 (100%)	61 (94%)	-	-	12 (92%)	13 (100%)
Bioveria + L77 No. of palms (Healthy %)	55 (84%)	49 (98%)	-	-	15 (93%)	13 (85%)	-	-
Bioveria X2 No. of palms (Healthy %)	16 (81%)	14 (93%)	-	19 (84%)	-	14 (93%)	-	-
P-value	0.2443 #	0.4574 @	-	0.5006 #	1.0000 @	0.7276 @	0.5714 @	-

Calculated by Chi-square test. @ Calculated by Fisher's exact test.

Table 7: Seasonal impact on EPF treatments efficacy in the female (top) and male (bottom) groves.

Females Grove					
Season	**Control (No. of palms, Healthy %)**	**Bioveria (No. of palms, Healthy %, p-value)**	**Velifer (No. of palms, Healthy %, p-value)**	**Bioveria + L77 (No. of palms, Healthy %, p-value)**	**Bioveria X2 (No. of palms, Healthy %, p-value)**
Summer	162 94%	203 91% 0.1226 #		55 84% **0.0207 @**	16 81% 0.0795 @
Autumn	154 91%	191 96% 0.0643 #	-	49 98% 0.1246 @	14 93% 1.0000 @
Winter	48 100%	50 100% -	43 100% -	-	-
Spring	107 96%	168 92% 0.1192 #	61 96% 0.4632 @	-	19 84% 0.0692 @
Males Grove					
Season	**Control (No. of palms, Healthy %)**	**Bioveria (No. of palms, Healthy %, p-value)**	**Velifer (No. of palms, Healthy %, p-value)**	**Bioveria + L77 (No. of palms, Healthy %, p-value)**	**Bioveria X2 (No. of palms, Healthy %, p-value)**
Summer	10 100%	16 88% 0.5077	-	15 93% 1.0000 @	-
Autumn	45 82%	47 91% 0.1839	-	13 85% 1.0000 @	14 93% 0.6714 @
Winter	11 82%	10 80% 1.0000	12 92% 0.5901	-	-
Spring	12 75%	15 100% 0.0752 @	13 100% 0.0957 @	-	-

Calculated by Chi-square test. @ Calculated by Fisher's exact test.

3.2.3. Combined seasonal effect on palm protection efficacy of EPF treatment

Prevention of RPW infestation by EPF treatments, regardless of the type of EPF preparation, revealed different seasonal efficacy rates among the female and male groves (Table 8).

Summer

Examination of all three summer experiments in the female grove, revealed significant higher infestation rate among the EPF treated palms, compared to the control group (χ test, P-=0.0383). Similar trend was observed in the male grove summer experiment, but without statistical significance (Fisher's exact test, P=0.5638).

Autumn

When examining all three autumn experiments in the female grove, EPF treatments were significantly protecting palms from RPW infestation, showing 96% healthy palms (n=254), while in the control group 91% remained healthy (n=154) (χ test, P=0.0353; Table 7). Similarly, the three autumn experiments altogether in the male grove, resulted in superiority to EPF treatments in palm protection, as 91% (n=74) of EPF-treated palms remained healthy versus 82% (n=45) in the control group. However, this difference was not statistically significant (Table 8; χ test, P=0.1915).

Winter

In both farming practices, EPF treatments showed ineffectiveness during the winter, when compared to the control palms. It is worth mentioning that the overall infestation rate in the winter is relatively low.

Spring

During the spring, EPF treatments demonstrated effectiveness in the male grove (Fisher's exact test, P=0.0223), while the opposite trend was observed in the female grove, though with no statistical significance (χ test, P=0.0911).

Table 8. Seasonal comparison of experiments of each grove: number of palms and healthy percent in control and combined EPF treatments and comparison tests P-value.

Grove	**Season**	**No. of repeats**	**Total No. of palms in the control group (Healthy %)**	**Total No. of palms in the EPF treatments (Healthy %)**	**P-value**
Females	Summer	3	162 (95%)	275 (89%)	**0.0383 #**
	Autumn	3	154 (91%)	254 (96%)	**0.0353 #**
	Winter	1	48 (100%)	93 (100%)	-

	Spring	2	107 (96%)	248 (92%)	0.0911 #
Males	Summer	1	10 (100%)	31 (90%)	0.5638 @
	Autumn	3	45 (82%)	74 (91%)	0.1915 #
	Winter	1	11 (82%)	22 (86%)	1.00 @
	Spring	1	12 (75%)	28 (100%)	**0.0223 @**

Calculated by Chi-square test. @ Calculated by Fisher's exact test.

3.2.4. General protection efficacy of EPF treatments in different seasons

As demonstrated in table 9, when examining the results in the Arava without considering the effect of the agricultural practice or type of EPF preparation, only in the autumn season EPF application had proven to be effective in protecting palms from the RPW. EPF treatment in the summer seasons had shown inferiority in palms protection, when compared to the control group. In **autumn** experiments, 89% of 199 palms in the control groups remained healthy compared to 95% out of 328 in the EPF treated palms (χ test, P=0.0142). In **summer**, the opposite trend was found as 95% of the 172 control group palms remained healthy while 89% out of 306 in the EPF treatments (χ test, P=0.0250). In **winter** results had shown relatively low and similar infestation rates; both control (total 59 palms) and EPF (total 115 palms) exhibited 97% healthy palms (Fisher's exact test, P=1.00). In **spring**, similar healthy palm percentages were observed in both groups**;** 94% of 119 palms were healthy in the control group versus 92% of 276 in the EPF treated palms (χ test, P=0.5329).

Table 9. General comparison of healthy palms rate and percentage in control versus combined EPF groups in each season with its statistical test P-value

Season	**No. of palms in control group (Healthy %)**	**No. of palms in EPF treatments (Healthy %)**	**P-value**
Autumn	199 (89%)	328 (95%)	**0.0142 #**
Summer	172 (95%)	306 (89%)	**0.0250 #**
Winter	59 (97%)	115 (97%)	1.00 @
Spring	119 (94%)	276 (92%)	0.5329 #

Calculated by Chi-square test. @ Calculated by Fisher's exact test.

3.3. Environmental factors effect on EPF treatment efficacy

The effect of environmental factors, as expressed by the reference evapotranspiration (ET0), on the efficacy of EPF treatments was evaluated for male and female groves as described in section 2.7.2. EPF treatments effectiveness was significantly reduced by environmental factors in the female grove, whereas the effect in the male grove was opposite, but insignificant (Figure 7).

Female grove

A negative association was observed between ET0 values and efficacy rates in the female grove, with EPF treatment efficacy strongly decreasing as ET0 values increased ($R^2 = 0.20$, p-value = 0.0004). This relationship is illustrated by a descending trendline (Figure 7). Lower ET0 values up to 4 were associated with positive efficacy rates. However, at an ET0 of around 4, a turning point occurred where EPF treatments became ineffective and were even less efficient than the control as ET_0 values increased further.

Male grove

In the male grove, a positive trend was observed between climate variables and EPF efficacy rates, although no significant correlation was found ($R^2 = 0.018$, p = 0.0004) (Figure 3). Lower ET0 values below 3 resulted in relatively low efficacy rates. With further increases in ET0 values, there was a steady increase in the mean efficacy, as demonstrated by the trendline, yet with widely skewed predictions, indicated by the wider area around the trendline.

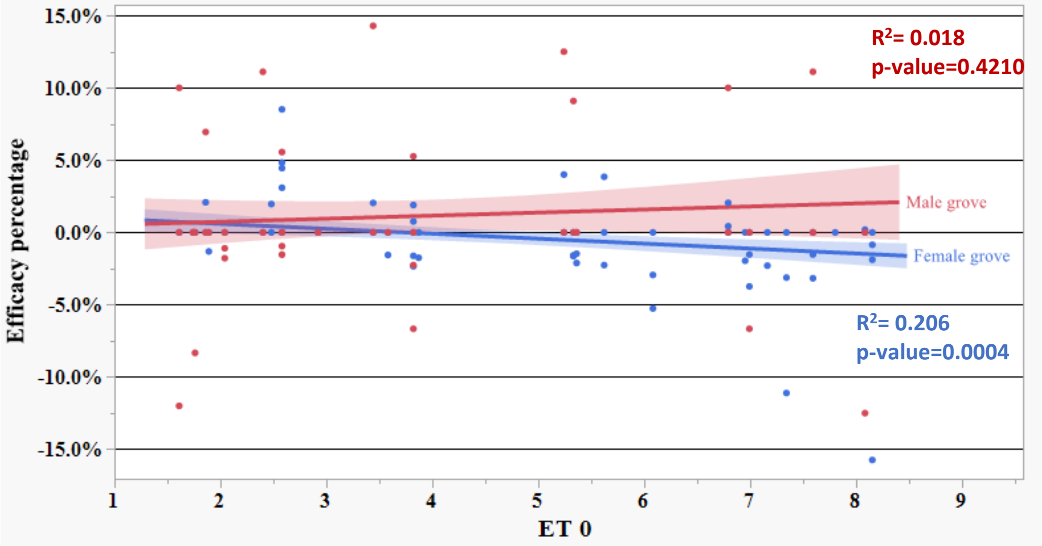

Figure 3. The relationship between climate variables (expressed as ET0 values) and the efficacy of EPF treatments compared to the control in the male (red) and female (blue) groves. Each dot represents an observation, and the linear regression trendline signifies the predicted efficacy according to ET0 values. A wider area around the trendline represents higher

scattering of predictions. Positive efficacy percentages indicate a lower infestation rate for EPF treatments than the control, while negative efficacy percentages indicate a higher infestation rate.

3.4. EPF survival under different climate conditions

3.4.1. Season, grove type and their combined effect on EPF survival

Whole model effect was performed to test the effects of season, agricultural practice, and season*grove combined effect on EPF survival in the soil. Levene's test for homogeneity of variances reveled unequal variances among CFU loss per day results (Levene's test, F= 3.9952, p=0.0039). Consequently, rank transformation was applied on this variable.

Results has not showed significant effect for combined season and grove on EPF survival (Whole model effect: season*grove – F= 0.2115, DF=2, P=0.8101). Similar survival rates were found in both groves across all the seasons (Whole model effect: agricultural practice – F= 0.9069, DF=1, P=0.3454). Generally, without considering the effect of grove variables effect, the season had a significant effect on EPF survival (whole model effect: season – F= 6.0848, DF=2, P=0.0043). Post hoc assay Tukey-HSD revealed a significantly high CFU loss in summer compared to autumn and spring. Spring and autumn EPF survival rates, were approximately identical (Figure 4).

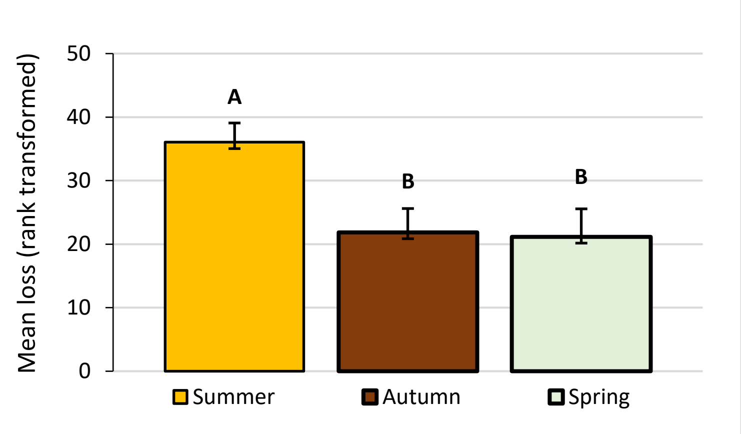

Figure 4. Mean CFU loss per day (ranked) as recorded in each season in both the female and male groves in the Arava. Different upper-case letters designate significant difference by Tukey-HSD comparison. (α<0.05)

3.4.2. Season effect on EPF survival in each grove

Female

The impact of seasonal variations on CFU loss in the female grove was investigated, with findings revealing significant differences across different seasons. Initial analysis using Levene's test indicated unequal variances among seasons (F=7.5666, p = 0.0021). Consequently, Welch's ANOVA was employed, revealing a significant effect of season on CFU survival (Welch's test, F=6.3266, p=0.0172). Accordingly, multiple comparisons t-tests were conducted between CFU loss per day for each pair of seasons, after verifying Levene's equivalence of variance. Sum up results are presented in Table 10. A significant difference in survival rates was observed between summer and autumn (Levene's test, F =8.7233, p = 0.0066; T test, t = 3.2017, p = 0.0082), However, comparisons between summer and spring did not reveal a significant difference in EPF survival (Levene's test, F = 17.3730, p = 0.0004; T test, t = 2.0426, p = 0.0910). Similarly, autumn and spring did not differ significantly in survival rates within the female grove (Levene's test, F = 0.5315, p = 0.4780; Pooled T test, t = 0.1044, p = 0.9183). Bonferroni correction was applied, setting an alpha level of 0.0167 to account for multiple comparisons.

Male

Results in the male grove showed insignificant difference in EPF survival among the tested seasons. (One-way Anova, F=1.8820, p=0.1783) Nevertheless, like the female grove trend, in the summer, the largest CFU loss per day observed.

Table 10. Multiple comparisons including seasons mean EPF difference, in the female grove.

(I) Season 1	**(J) Season 2**	**Levene's test p-value**	**Mean CFU loss difference (I-J)**	**Std. error**	**Anova test p-value**
Summer	Autumn	0.0066	0.0201	0.0063	**0.0082** **#**
Summer	Spring	0.0004	0.0190	0.0093	0.0910 #
Spring	Autumn	0.4780	0.0018	0.01038	0.9183 @

Calculated by T-test.

@ Calculated by polled T-test.

3.4.3. EPF survival variance among groves

The difference in EPF survival was examined in the male and female groves in the Summer, spring, winter, and autumn seasons. EPF survival rates did not differ among the groves in all the tested seasons. Levene's test was used to assess variance equality in means. In the summer, survival rates were slightly higher in the male group, without statistically significant results (Levene's test, F =25.5357, p < 0.0001; Welch's t-test, t = 1.2603, p = 0.2331; Table 11). The autumn season showed very similar survival rates in both groves (Levene's test, F =0.0207, p = 0.8875; Pooled t-test, t = 0.0449, p = 0.9647; Table 11). In the spring season, survival differences were the highest among all the seasons, when more EPF survived in the female grove, but as previously mentioned, results did not differ significantly (Levene's test, F =3.1399, p = 0.1068; pooled t-test, t = 0.5396, p = 0.6013; table 11). Figure 5 illustrates the average daily CFU loss for each grove across different seasons and years. The data provides a descriptive comparison of CFU loss trends over time.

Table 11. Statistical tests results for seasonal comparison in survival rates, between the male and the female groves. Each season degrees of freedom, variance equality test result, and t-ratio and p-value are specified.

Season	**DF**	**Levene's test P-value**	**t-ratio**	**P-value**
Summer	11.24	**>0.0001**	1.2603	0.2331 @
Autumn	15	0.8875	0.0449	0.9647 #
Spring	10	0.1068	0.5396	0.6013 #

Calculated by pooled T-test.

@ Calculated by Welch's T-test.

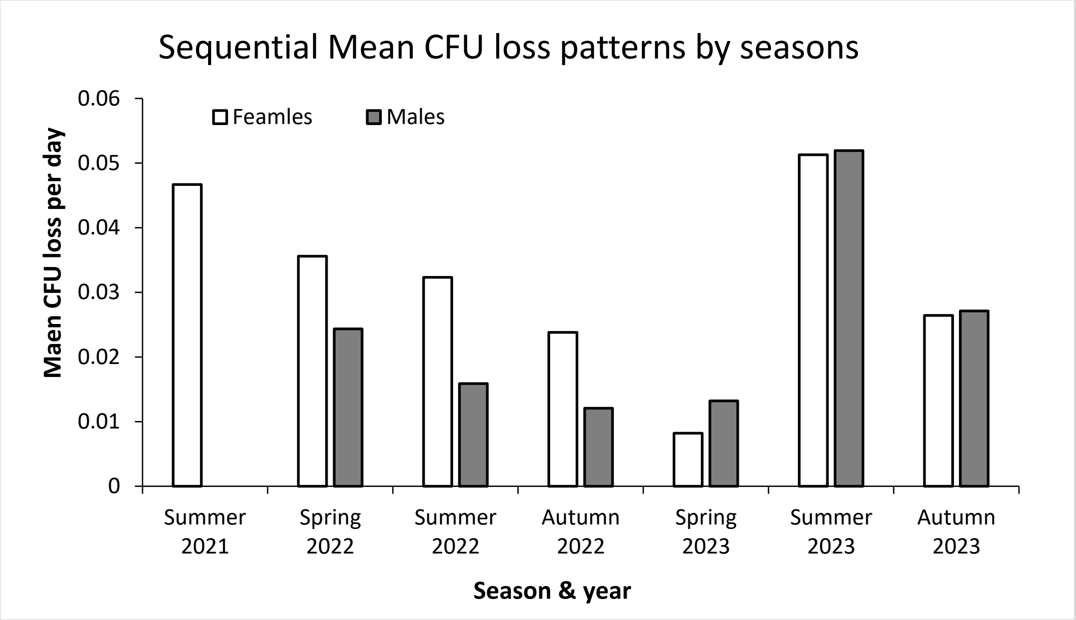

Figure 5. A descriptive comparison bar graph presenting mean CFU loss for female and male groves, ordered chronologically by seasons and years.

3.5. EPF survival relationship to healthy palms ratio

The regression between the quantity of EPF survived in soil and healthy palms ratio in EPF and control groups for each experiment, has been tested, as previously described in section 2.7.4.

3.5.1. Females grove

Logistic fit test result demonstrates a significant positive association between CFU levels and palm tree health in the female grove (χ test, p=0.0382). As showed in figure 6, upward trendline in healthy palm probability is evident as the mean log CFU values increase. When the mean logarithmic CFU count at the end of the experiment was 0, indicating of absence of EPF in soil, health probabilities ranged from 88% to 95% in EPF and control groups, respectively. The summed calculated probability for any palm with 0 CFU to be healthy was around 91%, as demonstrated by the trendline. As the mean logarithm of CFU reached 1 in EPF treatment group, health probability rose to 92%. 1.5-2.5 CFU/g soil found in the control groups, showed approximately 94% healthy palms. Further increase to 4 CFU/g soil in EPF groups, raised the prediction values to almost 96% healthy palms in the females grove.

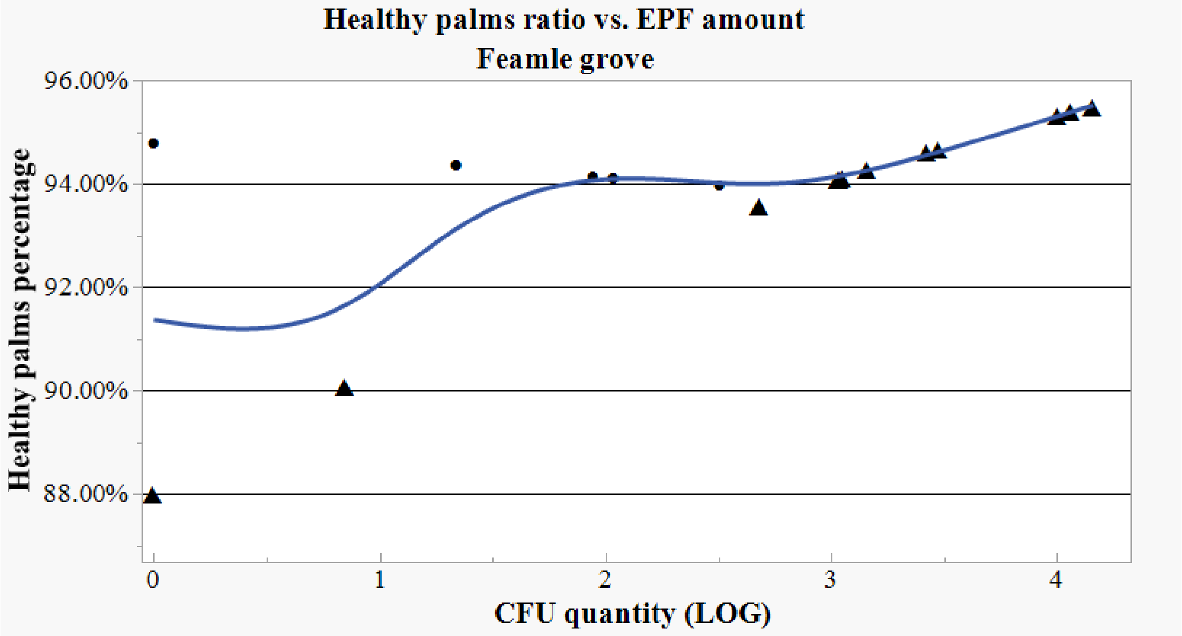

Figure 6. Relationship between soil EPF levels and palms health in the female grove.

● - Control group predictions.

▲ - EPF groups predictions.

3.5.2. Males grove

Positive association between the quantity of EPF and the palm's health observed in the male grove, but without statistical significance (χ test, p=0.4567). Compared to the females grove, different regression trend has been observed in the male grove. As showed in figure 7, when EPF was not found in the soil and the count recorded 0 CFU, approximately 84% healthy palms were predicted. An increase in CFU quantities from 0 to 1 CFU, increased healthy palms rate to 90%. As survival values rose to mean log of 2 CFUs, steady increase in the palm health prediction was observed, reaching its peak, with approximately 96% healthy palms predicted. However, further increase to 3 and 4 CFUs corresponded to reductions in the predicted percentages of healthy palms percentages to 90% and 86%, respectively.

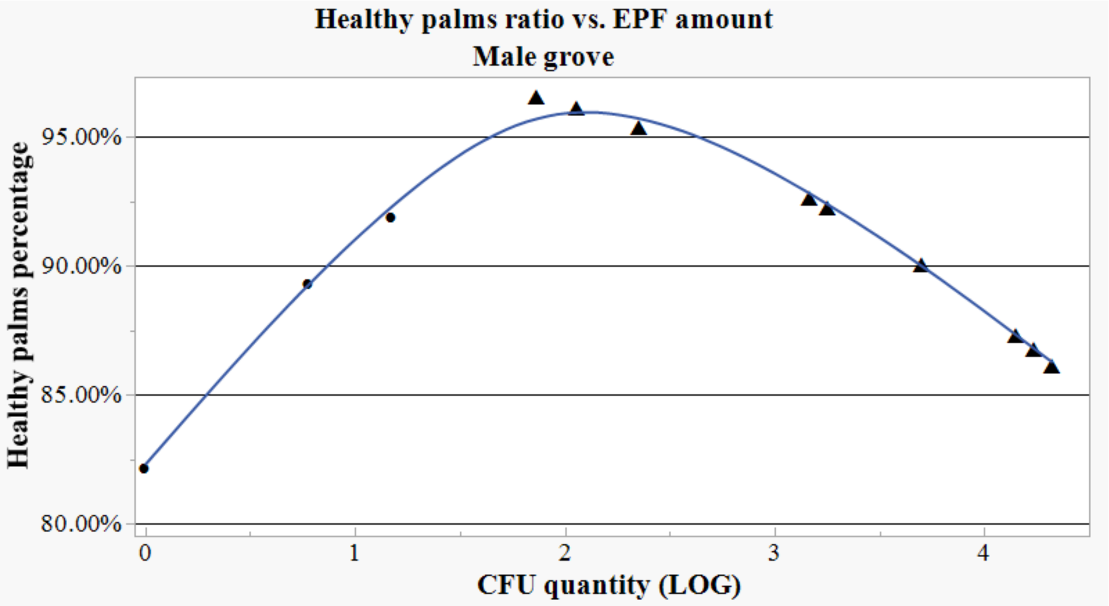

Figure 7. Relationship between soil EPF levels and palms health in the male grove.

● - Control group predictions.

▲ - EPF groups predictions.

3.5.3. Both groves

Logistic fit test result demonstrates a significant positive association between CFU levels and palm tree health when analyzing the results in both groves altogether (χ test, p=0.0437). As demonstrated in figure 8, upward trendline in healthy palm probability is evident as the mean log CFU values increase. When the mean logarithmic CFU count at the end of the experiment was 0, indicating of absence of EPF in soil, health probabilities ranged from 82% to 93% in EPF and control groups, respectively. The summed calculated probability for any palm with 0 CFU to be healthy was around 91%, as demonstrated by the trendline. As the mean logarithm of CFU reached 1 in EPF and control groups, health probabilities rose to 90% and 93%, respectively. In the control groups, soil with 1.5-2.5 CFU/g slightly increased the ratio of healthy palms, reaching its peak at approximately 94%. An increase in CFU/g soil among EPF-treated groups steadily raised the ratio of healthy palms, with 2, 3, and 4 CFU/g soil resulting in palm health ratios of 92%, 93.5%, and 94.5%, respectively.

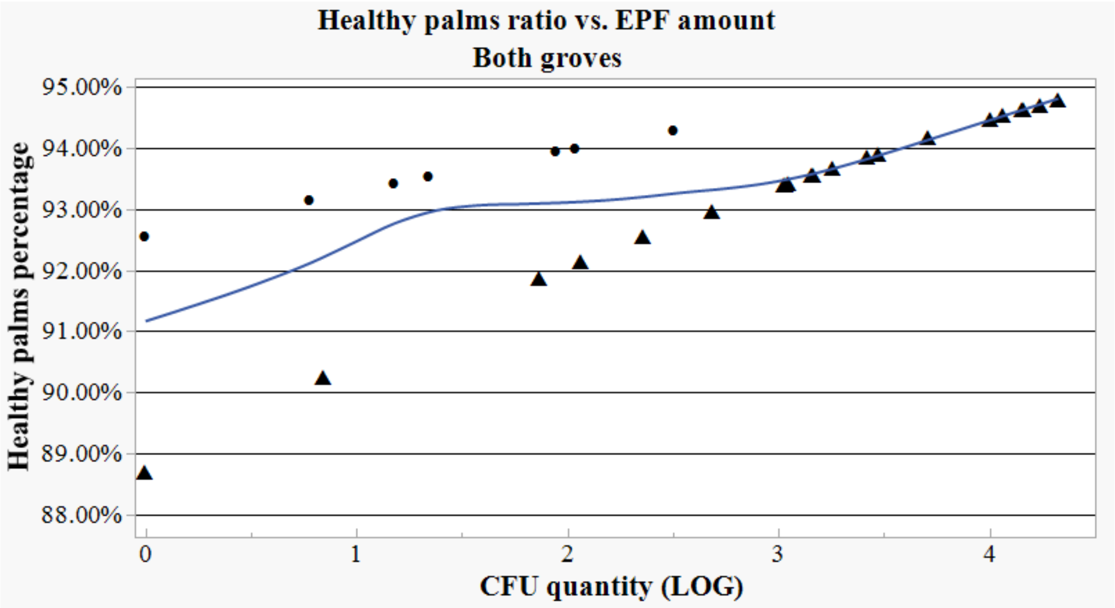

Figure 8. Relationship between soil EPF levels and palms health in both male and female groves.

● - Control group predictions.

▲ - EPF groups predictions.

4. Discussion

EPF serve as effective tools against various pests in different crops, including RPW, but environmental factors affect the efficacy of EPF (Mascarin & Jarosnski, 2016; Ment et al. 2017; Qayyum et al., 2021; Sabbahi & Hock, 2024). Our main objective was to study the efficacy of EPF prophylactic application against the RPW and its survival and the relation between the two while considering the biotic and abiotic factors in the palm groves. Seasonal data on the survival and efficacy of EPF, along with their relationship in female and male date palms, is important for EPF assimilation in agriculture practices as a biologically friendly pesticide against the RPW. Moreover, this information can be further enhance the exiting knowledge of EPF, which can improve EPF as treatments in other crops and climatic areas. EPF prophylactic treatments against the RPW had the highest efficacy following autumn application in the female grove and spring application in the male grove. The various preparations of EPF treatments did not affect the protection efficacy rate. Regarding the climatic data, ET_0 values majorly affected the efficacy of EPF treatments only in the female's grove. For example, mean ET_0 values less than 4 correlated with effective EPF applications, whereas mean values exceeding 5 resulted in palm infestation rates inferior to the control. Regarding EPF survival rates, EPF propagule survival was the lowest in the summer in both groves. Yet a major difference in EPF survival between the summer and the autumn observed in the female grove. Generally, a significant positive correlation between the quantity of EPF propagules that survived in the soil and the rate of healthy palms was demonstrated. However, when each grove was analyzed individually, this correlation was only observed in the female grove.

4.1. Seasonal variation of EPF survival

Seasonal changes significantly affected EPF survival, which is indicated by the number of fungal propagules at the end of the experiment period and, consequently, the calculated CFU loss per day. The summer season in the Arava Valley is characterized by extremely hot and dry environmental conditions (Ginat et al. 2011) which are not favorable for B. bassiana persistence (Ment et al., 2017). Indeed, in most summer soil samples, no CFUs were detected. Based on our calculations of CFU loss per day, it can be inferred that the extinction of EPF propagules likely occurred earlier than anticipated, resulting in a greater daily loss during the summer period. Autumn and spring had climatic conditions that were optimal or near-optimal in terms of EPF survival. These results correspond with a previous study in Egypt by Hussein et al. (2010), which found that *B. bassiana* was more abundant during spring and autumn than summer and winter. Also, it reported that the factors with the most significant effect. B. *bassiana* prevalence in the soil, were maximum and minimum temperatures and minimum humidity levels. The comparison of fungal propagules at the end of the experiment among the groves revealed mostly higher persistence in the male grove. However, the effect of the grove itself, or in combination with the effect of the season, did not show a significant effect on EPF survival. This observation may suggest a limited protective effect of the palm canopy on the fungal propagules, or it could be attributed to the smaller amount of experimental data from the male grove compared to the female grove.

4.2. Efficacy of prophylactic EPFs application in different seasons

Climatic conditions have a strong effect on EPF survival, pathogenicity, and, hence, efficacy (Reviewed by Ment et al., 2017). Previously, it was found that during the summer season in the Arava Valley, RPW activity was absent, with no palm infestation due to the extremely high temperatures in this area (Mendel et al., 2024b). In the present study, RPW infestation rate was compared among EPF-treated date palms and untreated control palms throughout the study's years and seasons. Autumn EPF application resulted in the highest protection efficacy among all tested seasons. During autumn, ET_0 mean rates were approximately 3, and characterized by seasonal low radiation flux and mean temperatures of 23.6ºC. These conditions are expected to enable *B. bassiana* high rates of survival, infectivity and sporulation. (Ment et al. 2017; Zaman et al., 2020). Generally, favorable climatic parameters for EPF development were accompanied by significant EPF survival and palm protection.

Spring trials showed ambiguous results. Generally, the EPF application has not shown an advantage over the control group in protecting palms from RPW. Comparison between the spring and the autumn climate factors revealed higher ET_0 values, characterized by very similar temperatures in both seasons but lower relative humidity and higher UV radiation in the spring. Our assumption is that these differences in values decreased EPF survival and pathogenicity, which resulted in lower EPF efficacy in this season. Analysis of the results on each grove revealed efficient protection in the male grove but not in the female grove. These variations can be explained by the UV radiation protective shield effect, provided by the shorter palms and lower canopies in the males grove. Another potential explanation is that the application of ammonium nitrate and urea in the male grove stimulated EPF growth and sporulation (Lingg and Donaldson, 1981; Cojocaru and Lumînare, 2021). This may have compensated for the loss of fungal propagules during the trials.

Summer trials were characterized by high temperatures, low RH, and high solar radiation, Hence, decreased EPF efficacy in protecting palms from RPW was observed. The summer season abiotic factors are not favorable for *B. bassiana*. The mean ET0 value recorded was 7.35, with a mean temperature exceeding 30 degrees Celsius, while RH was less than 50% in all months of summer experiments. Field trials conducted by Tehri et al. (2015) similarly demonstrated the ineffectiveness of *B. bassiana* in controlling *Tetranychus urticae* populations when applied during hot and dry crop seasons. However, the astounding and unpredictable results were the significantly higher rates of RPW-infested palms among EPF-treated palms over the control palms. When regarding each grove individually, this phenomenon was repeated only in the females grove. We have failed to find similar effects in previous experiments. Though the inability to repeat this experimental setup to gain further data for analysis, another speculation may be the effect of thermal stress on EPF and its volatile organic compounds, which manipulate arthropods' behavior (Liu et al. 2008; Ramírez-Ordorica et al., 2022; Ma et al., 2024).

4.3 Effect of EPF preparations on seasonal protection efficacy

Applications of different EPF treatments, which include different *B. bassiana* strains, surfactants, or double dose had no effect on the protection rate against RPW infestation in both groves. Although statistically insignificant, the Veilfer application during the winter and spring showed similar or higher protection rates compared to the Bioveria. This solid *B. bassiana* formulation was proven before to control populations of RPW when dusted around the crown, stems, and petioles of Canary Palms (Güerri-Agulló, 2012) and Washingtonia palms (Ment et al., 2023). Despite its promising potential, Veilfer was unavailable for all further trials. Silwet L-77 surfactant addition to Bioveria, did not impact its effectivity significantly. The most surprising results were the lack of impact in Bioveria double dose applications, which were previously proven to positively correlate with efficacy rates (Kaur et al., 2011). However, exercising caution when drawing conclusions about differences among treatments is essential. Apart from the Bioveria treatments, EPF treatments were applied with a low number of repetitions and relatively small sample sizes, which could impact the credibility of these results. Analyzing the seasonal variations of each EPF preparation against the control group revealed significant differences in treatment efficacy only in the female grove, for Bioveria + Silwet L-77 treatments, with autumn applications demonstrating superior protection compared to summer applications. Again, it is possible that the relatively low sample size in this group were not sufficient to achieve statistical significance.

4.4 Association between EPF CFU level and palm protection rates

Summer trials were characterized by a significant loss of EPF propagules comparing to autumn trials. These two seasons were also opposite in their efficacy in RPW prevention; autumn trials resulted in significant protection while, summer trials resulted in the opposite effect. Similar correlation between *B. bassiana* soil persistence and its conidia infectivity of the lettuce aphid *Nasonovia ribisnigri* (Mosley) was found in semi-field experiments (Govinda et al., 2015).

Relying on a previous study in which the survival of EPF propagules was found similar and equivalent between trunk and soil of treated palms (Livne, 2024), in the current study EPF persistence was evaluated for soil samples only. Therefore, we decided to set EPF CFU in the soil as a measure for fungal persistence. Also, as the protection mechanism following EPF application relies on fungal propagule administration to the egg-laying hole created by the RPW female (Matveev et al., 2023), fungal persistence is expected to correlate with palms' health. Indeed, the results indicated that EPF CFU/gr of soil surrounding treated palms was positively correlated with palms' health. Although not statistically significant, the male grove regression pattern demonstrated a positive correlation between CFU rates and prophylactic effect in the control palms. Conversely, EPF treated palms showed a decrease in efficacy, when CFU rates increased. First, regarding the control palms, we mentioned that EPF treated, and control groups were arranged in clusters, with at least 15 meters separating the palms of different groups. Although the recorded rates of Log 1 CFU, representing 10 CFU per gram of soil, are relatively low in terms of EPF rates, they were retrieved approximately 3 months after application from untreated soil. The increase in CFU levels, accompanied by lower infestation rates, suggests that EPF conidia can disperse and establish throughout the grove, affecting untreated palms as well. The negative regression in treated palms, however, remains unclear. However, we can hypothesize that in the male grove, the fungal persistence is relatively high due to UV protection provided by lower canopies. Nevertheless, pathogenicity and virulence are influenced by temperature and humidity, and thus, they may not be correlated with spore survival.

4.5 Research limitations and Future prospects

Our research focused on the effects of each season's environmental conditions on the survival of EPF *Beauveria bassiana* and its efficacy in preventing the RPW males and females in commercial date palm groves. Our study included 250 female palms and 53 male palms which a portion of them excluded from the experiments due to our experiment structure. Practically, in most of the cases these sample sizes were not large enough to provide significant results. The RPW typically targets a relatively small percentage of palms, which further highlights this limitation. Additionally, it is important to note that the climate conditions recorded during our experiments were obtained from a weather station situated in an open field and may not fully depict the actual conditions found under date palms. Livne, (2024) observed significantly reduced solar radiation levels and slight temperature variations within a grove of date palms compared to a nearby open-field weather station. Moreover, morphological differences between the palms, which derive from the palm's variety and growth characteristics, can alter the fronds and leaves' number and length (Zaid, 2002), which, in turn, can decrease the solar radiation and temperature in the palm trunk and surroundings. Another limitation in our field-test was the multicollinearity of the climatic factors, temperature, UV, and RH. Since it is not possible to isolate and assess the influence of one factor by itself, we used ET_0 values, which are very prominent and can represent a general index of these abiotic factors. Regarding soil samples, having adequate manpower would be ideal for quantifying EPF from soil samples on a monthly basis and assessing EPF survival patterns more accurately in the different seasons and groves. Furthermore, our comparison of CFU loss per day rates assumes that EPF has a linear declining rate for this examination, which does not describe EPF loss comprehensively. In addition, 0 CFU counted at the end of a season could suggest an even higher rate of EPF propagule loss. Due to human error, in the autumn of 2021, the Velifer groups in the males and females groves, were sprayed with 0.02% of emulsion instead of 0.2%. Applying EPF with this reduced number of spores is insufficient to protect palms from RPW according to the prior test we conducted. Furthermore, soil samples were taken from the male grove in December 2021, and several samples from both groves on different seasons were lost and, therefore, subtracted from the data analysis. Our suggestion for future investigations is that they will include the examination of a larger number of palms, which can yield more significant results in varying environmental conditions. Another study can test EPF survival in the trunk and/or in the soil of the palms on a monthly basis to characterize the survival of EPF in each season. Moreover, assessment of pathogenicity and virulence under seasonal variations could expose another important element. Studies that will include different EPF species, strains, and formulations can also enrich the existing knowledge and promote the use of EPF as a biologically friendly insecticide.

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Supplementary Table 1. Monthly recordings of mean, maximum, and minimum values of temperature, relative humidity, solar radiation, and mean evapotranspiration rates at the study area in the Arava

Month	**Temperature (°C)**			**Relative Humidity (%)**			**Radiation (W/m²)**			**ET0 (mm/day)**
	Mean	**max**	**min**	**Mean**	**max**	**min**	**Mean**	**max**	**min**	**Mean**
Jun. 21	31	43.6	21.9	38	68	12	345	1035	0	7.8
Jul. 21	34.1	44.7	25.9	36	68	9	331	1024	0	8.15
Aug. 21	34.5	46.2	26.1	36	68	6	305	988	0	6.95
Sep. 21	30.8	40.9	19.9	46	83	16	244	921	0	5.24
Oct. 21	27.5	36.6	19	47	76	19	175	840	0	3.44
Nov. 21	22.2	33.7	12.9	48	86	14	135	698	0	1.86
Dec. 21	17.2	26.9	8.3	49	83	19	107	617	0	1.61
Jan. 22	13.8	24.5	3.1	57	92	22	120	725	0	1.76
Feb. 22	16.4	26.2	7.6	54	90	15	150	825	0	2.4
Mar. 22	17.3	32.7	6.5	44	80	9	198	962	0	3.58
Apr. 22	25.5	42.4	14.2	34	75	5	261	1035	0	5.33
May. 22	28.3	41.9	16.3	33	72	3	310	1031	0	6.79
Jun. 22	31.8	43.9	23	36	71	9	338	1024	0	7.59
Jul. 22	33.3	43.5	23.6	37	67	10	334	1009	0	8.081
Aug. 22	33.8	43.5	25.3	43	68	13	289	952	0	6.99
Sep. 22	31.5	40.2	22.3	45	74	15	236	888	0	5.36
Oct. 22	28	41	20.2	50	90	17	180	782	0	3.82
Nov. 22	22.3	30.6	12.6	53	90	21	151	712	0	2.58
Dec. 22	17.5	27.1	8.8	63	94	27	133	612	0	1.74
Jan. 23	16.3	29.8	6.8	58	96	21	146	667	0	2.04
Feb. 23	16.3	33.1	7.6	50	86	12	185	839	0	2.921
Mar. 23	20.4	32.2	11	48	92	10	219	963	0	3.87
Apr. 23	23.4	37.2	13.1	39	69	10	N.A	1037	0	2.48
May. 23	27.4	42.1	18.6	35	82	6	N.A	1026	0	6.08
Jun. 23	31	44	21.5	38	69	10	319	1026	0	7.34
Jul. 23	34.8	45.9	25.1	34	67	10	332	1014	0	8.15
Aug. 23	33.9	43.6	25.5	45	69	9	288	994	0	7.16
Sep. 23	31.9	44	22.9	43	70	13	241	878	0	5.62
Oct. 23	28.6	36.9	20.8	48	78	20	177	822	0	3.82
Nov. 23	23.7	35.6	12.4	52	87	20	153	676	0	2.58
Dec. 23	18.8	29.2	11.1	59	93	28	130	640	0	1.89