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Efficacy of the Entomopathogenic Fungus *Metarhizium anisopliae* var. *acridum* in Controlling *Heteracris littoralis* (Rambur, 1838). Using plant oil Carrier Substance Combined with Essential oils

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Abstract

Using sunflower oil and essential oils (garlic, parsley, and basil), the study assessed the effectiveness of *Metarhizium anisopliae var. acridum* (isolate EV CH 077) against the grasshopper *Heteracris littoralis,* a major threat to agriculture in Egypt. Ten treatments with different concentrations of essential oils (10%, 30%, and 50%) mixed with sunflower oil as a carrier were used in field experiments in the Baharia Oases throughout two summer seasons (2022 and 2023). Analysis was done on synergistic effects, lethal time (LT_{50} and LT_{90}), and mortality rates.

The results showed that, in comparison to other treatments, *Metarhizium anisopliae* plus 50% parsley oil (T6) produced 100% mortality by day 15 of the first season and day 12 of the second. Garlic oil was less effective, but basil oil (50%) showed excellent efficacy as well (96.53% mortality). The increased pathogenicity of fungal formulations with essential oils was demonstrated by the 86.11% death rate of sunflower oil alone (T0). In treatments with essential oils, especially parsley (4.69 days in season 1; 5.14 days in season 2), LT_{50} values were significantly lower, suggesting faster fungal action. Significant changes between treatments were confirmed by statistical analysis (ANOVA, Tukey test, $p < 0.05$), with parsley oil demonstrating the strongest synergy.

The study highlights the potential of using essential oils produced from plants in conjunction with *Metarhizium anisopliae* as a sustainable substitute for chemical pesticides. Particularly, parsley oil proved to be a very successful adjuvant, increasing fungal virulence and decreasing time-to-kill. These results support integrated pest control plans by providing a sustainable way to reduce environmental and non-target hazards while reducing *Heteracris littoralis* infestations.

Keywords: *Metarhizium anisopliae*, essential oils, biocontrol, *Heteracris littoralis*, synergistic effect, sustainable pest management.

1. Introduction

Grasshoppers represent a considerable menace to global agricultural output, resulting in enormous economic losses due to their excessive crop consumption. *Heteracris littoralis* is identified as a significant pest detrimental to numerous farmed crops in areas such as Egypt **(Sharaby et al., 2011).** The major way of managing grasshopper outbreaks consists of applying chemical pesticides. Although it is generally inexpensive, rapid, and efficient, broad-spectrum insecticides can constitute a substantial hazard to human health and nontarget organism populations, which damages the environment **(Dakhel et al., 2020).** This involves the investigation of alternative, ecologically friendly pest management solutions.

According to **Mesquita et al. (2023),** entomopathogenic fungi, including Metarhizium anisopliae, have shown promise as biocontrol agents against a variety of insect pests, particularly Some of the entomopathogenic fungi are significant biological agents employed in integrated pest management systems, and they are the primary regulators of insect populations. In contrast to pesticides, entomopathogenic fungi do not readily cause insect pests to develop resistance to infection **(Jiang et al., 2020).**

Additionally, the use of carrier substances can improve the efficiency of fungal spores by strengthening their adherence to the insect cuticle and protecting them from environmental stressors **(Wang C., & St. Leger 2007).** According to **Rondelli et al. (2020),** oil derived from plants has demonstrated the ability to transport entomopathogenic fungi effectively.

The application of *M. anisopliae* for grasshopper control has been investigated in earlier research. For example, several studies have shown that, in both laboratory and field settings, distinct isolates of M. anisopliae are pathogenic to a variety of grasshopper species **(Lomer et al., 2001; Bateman et al., 1993).** To improve fungal virulence and persistence in the field, research has also concentrated on refining formulations and application techniques **(Inglis et al., 2001).** Furthermore, to expand the range of control and produce synergistic effects, entomopathogenic fungi have been combined with other pest management strategies, such as botanical insecticides **(Koppenhöfer and fuzzy 2008).**

Plant-based essential oils have been shown to have insecticidal and repellent qualities, as well as the ability to increase entomopathogen activity **(Isman, 2020).** Plants that produce essential oils and have been shown to have bioactivity against insect pests include garlic (*Allium sativum*), parsley (*Petroselinum sativum*), and basil (*Ocimum basilicum L.)* **(Yang et al., 2009; Pavela, 2016; Regnault-Roger et al., 2012a).**

The synergistic potential of mixing *M. anisopliae* with particular essential oils as a way to increase its efficacy against *H. littoralis* needs more research, even though the effects of *M. anisopliae* and plant-based carriers have been examined alone and in combination.

In particular, it is unclear how adding essential oils of garlic, parsley, and basil to a plant oil carrier affects *M. anisopliae's* pathogenicity against this significant grasshopper pest in Egypt.

Thus, the main goal of this study was to determine how well *Metarhizium anisopliae var. acridum* (isolate EV CH 077), prepared with sunflower oil as a plant oil carrier and in combination with varying concentrations of essential oils of garlic, parsley, and basil, controlled *Heteracris littoralis.* The goal of this study is to determine the best combinations that might improve *M. anisopliae's* capacity for biocontrol to manage grasshoppers sustainably.

2. Materials and Methods

2.1 Test Insect used

The insect species used in the study is the grasshopper, *Heteracris littoralis* Rambur (Orthoptera: Acrididae) **(Rambur, 1838).**

2.2 Study site of the insect

The Baharia Oases serve as a breeding ground for various grasshopper species, including *H. littoralis.*

2.3 Compounds:

Entomopathogenic fungus

Common Name: **(***M. anisopliae* var**.** *acridum***)**

Isolate: EV CH 077

Trade Names: NOVACRID 50 g/ha / 1L carrier /ha

Source: Green Elephant Morocco Agropolis G15-G16, Municipality of Mejjate, Meknes – Morocco, with plant oil as carrier for the fungus.

2.4 Essential Oils:

Garlic, *Allium sativum* L. (*Amaryllidaceae*)

Parsley (*Petroselinum crispum*): (Mill.) *Apiaceae*

Basil (*Ocimum basilicum* L.): *Lamiaceae*

Source: Egypt Factory, industrial zone: Al-Obour city, Cairo, Egypt; El-Captain for extracting natural oils, plants, and cosmetics. The Entomopathogenic fungus (*M. anisopliae* var. acridum) and its carrier (sunflower oil) were combined with each essential oil separately at different concentrations of 10%, 30%, and 50%.

2.5 The experimental design:

More than 80 insects/m2 of mature adults of *H. littoralis* were found in the experimental field, which was extensively infested. During two summer seasons (August 2022 and 2023), a field trial was carried out in which the area was covered with maize (*Z. mays*) after it had been sown for 35 to 40 days.

The purpose of this study was to assess the efficacy of bioagents in combination with essential oils in different concentrations and using sunflower oil as a carrier. The eleven experimental plots that we created are as follows:

C	Plot without treatment acts as the control
T0	*Metarhizium* carried by sunflower oil alone.
T1	*Metarhizium* carried by sunflower oil 90% mixed with basil 10%.

T2	*Metarhizium* carried by sunflower oil 50% mixed with basil 50%.
T3	*Metarhizium* carried by sunflower oil 50% mixed with basil 50%.
T4	*Metarhizium* carried by sunflower oil 90% mixed with parsley 10%.
T5	*Metarhizium* carried by sunflower oil 70% mixed with parsley 30%.
T6	*Metarhizium* carried by sunflower oil 50% mixed with parsley 50%.
T7	*Metarhizium* carried by sunflower oil 90% mixed with Garlic 10%.
T8	*Metarhizium* carried by sunflower oil 70% mixed with Garlic 30%.
T9	*Metarhizium* carried by sunflower oil 50% mixed with Garlic 50%.

Each plot was measuring 50×21 meters, equivalent to 1050 square meters, approximately a quarter of one feddan (one feddan = 1.038 acre). To ensure consistency, we maintained 100 square meter buffer zones (measuring 10×10 meters) between each plot. The number of replicates is five for each treatment.

2.6 Methods of bio-pesticides application:

Using a handheld sprayer (Micro-Ulva®) with a rotating spinning disc (ULV+), the entomopathogenic fungus (M. anisopliae var. acridum) was applied in its assigned plot using the various carrier substances. In the first season, spraying was done from 6:30 to 8:45 am, while in the second season, it was done from 6:45 to 08:10 am. The sprayers and spraying methods were calibrated by Dobson's (2001) recommendations.

Using sunflower oil and sunflower oil mixed with various essential oils in varying concentrations, the orange nozzle was used with a track spacing of 10 meters and a flow rate of 0.078 L/min. a forward speed of 3.9 km/hr, taking wind speed into account, as well as a spraying height of 0.5 meters. Every 50 x 21 m plot, which had a total area of 1050 square meters, was treated with 100 ml of entomopathogenic fungus (M. anisopliae var. acridum).

2.7 Calculation of mortality

In each treatment plot, five 0.5 × 0.5 m cages were arranged as experiment replications. After administering, we collected thirty insects at random from each treatment plot for each cage using a sweep net. These insects were collected and then placed in the proper cages. The cages were placed inside the treated area under field circumstances. The insects fed every day on the treated plants in their treatment plots. Daily mortality counts were kept up to fifteen days after therapy.

2.8 Statistical analysis

SPSS software was used to do a one-way analysis of variance (ANOVA) on the data (Tukey test). $P < 0.05$ was deemed to be statistically significant.

Henderson and Tilton's formula (1955) was used to correct the percentage of nymph mortality. LdP Line software was used to do probit analysis to estimate the LT_{50} and LT_{90} values.

3. Results

3.1 Comparison of Mortality Rates Within and Between Seasons.

During two summer seasons (2022 and 2023), the effectiveness *of Metarhizium anisopliae var. acridum* (isolate EV CH 077) prepared with sunflower oil and essential oils (garlic, parsley, and basil) against *Heteracris littoralis* was assessed.

First Season: Treatment-specific mortality rates differed considerably. By day 15, T6 (50% parsley oil) had the highest mortality rate (100%), followed by T3 (50% basil oil, 96.53%) and T2 (50% basil oil, 93.06%). Mortality in the control group (C) was very low at 4%.

Similar patterns were noted in the second season, with T6 once more reaching 100% mortality by day 12. The effectiveness of T3 (96.15%) and T4 (96.15%) was likewise good. The mortality rate for the control group stayed low at 13.33%.

Comparing the Two Seasons: Mortality rates were consistently higher in the second season, especially for parsley oil treatments (T4–T6), indicating improved fungal performance in comparable field settings.

3.2 Time Required to Achieve 50% (LT_{50}) and 90% (LT_{90}) Mortality.

First Season: T7 (garlic 10%, 4.54 days) and T9 (garlic 50%, 4.57 days) had the quickest LT_{50}, but T0 (sunflower oil alone) needed 6.29 days. T6 had the lowest LT_{90} values (8.68 days), whereas T9 had the highest (14.66 days).

Second Season: T0 (3.15 days) showed a considerable decrease in LT_{50}, suggesting improved fungal activity. Additionally, T6 (parsley 50%) had a low LT_{50} (5.14 days).

The synergistic impact of parsley oil was demonstrated by the fact that LT_{90} stayed the shortest for T6 (9.48 days).

Key Finding: Fungal lethality was enhanced by essential oils, especially parsley at 50%, with LT_{50} and LT_{90} values consistently lower than those of sunflower oil alone.

3.3 Statistical Significance of Treatments

Within Seasons: Significant differences between treatments were found using ANOVA (Tukey test, *p* < 0.05). T6 (100% mortality) was significantly different from T0 (86.11%) and T7–T9 (84.62–89.58%) throughout the first season. The second season also showed similar significance.
Between Seasons: Probably as a result of fungal adaptation or better environmental circumstances, the second season demonstrated increased efficacy (*p* = 3.74e-13) with lower LT values and greater mortality rates.

3.4 Analysis of Slope, Regression, and Chi-Square Values

Slope: Steeper slopes (e.g., T6: 4.79 in season 1; 4.82 in season 2) indicated rapid pathogenicity, correlating with higher mortality rates.

Regression (R): High R-values (≥0.94) confirmed strong linear relationships between time and mortality for all treatments.

Chi-Square (X^2): All X^2 values were below tabulated thresholds, validating the robustness of the probit models.

Consistency across Seasons: Despite seasonal variability, statistical parameters (slope, R, X^2) remained stable, affirming the reliability of the formulations.

3.5 Enhancement of Fungal Efficacy by Essential Oils

Mortality Increase:

Basil Oil: T1 (10% basil) increased mortality from 86.11% (T0) to 89.58% in the first season. Higher concentrations (T2–T3) further improved efficacy (93.06–96.53%). Parsley Oil: T6 (50% parsley) achieved 100% mortality in both seasons, underscoring its potent synergy with the fungus. Garlic Oil: While effective, garlic formulations (T7–T9) showed lower enhancement compared to parsley, with mortality peaking at 89.58%. Synergistic Effect: Parsley oil at 50% (T6) emerged as the most effective adjuvant, reducing both time-to-kill and final mortality rates.

Table 1: The mortality of *H. littoralis* (Rambur, 1838) using (*Metarhizium* + sunflower oil & sunflower oil mixed with essential oil) first season.

Days	C	T0	T1	T2	T3	T4	T5	T6	T7	T8	T9
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	13.3	10	21	0	13.3	0	20.33	6. 7	13.3
3	0	13. 3	23.3	23.3	36.7	23.3	23.3	20	23.3	13.3	40
4	0	20	23.3	33.3	36.7	23.3	23.3	20	43.3	23. 3	40
5	0.67	32.89	53.02	42.95	46.31	32.89	36.24	59.73	66. 44	46. 31	59.73
6	0.67	46. 31	76.51	53.02	66.44	32.89	36.24	79.51	66. 44	53.02	59.73
7	0.67	59.73	76. 51	73.15	83.22	42.95	46.31	89.93	66. 44	56. 38	69.8
8	0.67	63.09	79.51	76.51	83.22	63.09	66.44	89.93	66. 44	69.8	69.8
9	0.67	79.87	83.22	76.51	86.58	76.51	76.51	89.93	76.51	76. 51	79.51
10	2.67	82.88	85.82	85.82	89.73	82.88	85.82	89.73	79.45	82.88	79.45
11	2.67	82.88	85.82	89.73	89.73	82.88	85.82	89.73	86.3	86. 3	79.45
12	2.67	82.88	85.82	89.73	89.73	89.73	89.73	96.53	86.3	86.3	86.3
13	4	86.11	89.58	93.06	96.53	89.58	93.06	100	89.58	89.58	89.58

Days	C	T0	T1	T2	T3	T4	T5	T6	T7	T8	T9
14	4	86.11	89.58	93.06	96.53	89.58	93.06	100	89.58	89.58	89.58
15	4	86.11 **a**	89.58 **b**	93.06 **c**	96.53 **d**	89.58 **b**	93.06 **c**	**100 f**	89.58 **b**	89.58 **b**	89.58 **b**

Columns with different letters within the same concentration are significantly different (α. < 0.05) (ANOVA) (Tukey test).

p-value equals 2.84037e-10, statistic F equals 34.649744

Table 2: The Mortality of *H. littoralis* (Rambur, 1838) using (*Metarhizium* + sunflower oil& sunflower oil mixed with essential oil), second season

days	c	T0	T1	T2	T3	T4	T5	T6	T7	T8	T9
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	11.3	10	15. 7	0	12.5	6. 7	12	8	0
3	3.33	10.34	24.14	24.14	31.03	27.59	24.14	13.79	20.79	13.79	13.79
4	3.33	17.24	24.14	31.03	41.38	34.48	24.14	24.14	20.79	27.59	13.79
5	6.67	35.71	53.57	46.43	46.43	42.86	35.71	35.71	25	50	25
6	6.67	42.86	75	53.57	78.57	42.86	35.71	71.43	28.57	53.57	32.14
7	6.67	46.43	75	71.43	82.14	57.14	46.43	78.57	42.86	57.14	39.29
8	6.67	53.57	78.57	78.57	82.14	67.86	67.86	78.57	57.14	71.43	42.86
9	10	66.67	81.48	77.78	85.19	81.48	74.07	85.19	66.67	74.07	62.96
10	10	74.07	88.89	88.89	85.19	92.59	85.19	92.59	74.07	81.48	74.07
11	10	81.48	88.89	88.89	85.19	92.59	85.19	96. 3	81.48	85.19	85.19
12	10	88.89	88.89	88.89	92.59	92.59	92.59	100	81.48	85.19	85.19
13	13.33	88.46	88.46	92.31	92.31	96.15	96.15	100	84.62	88.46	88.46
14	13.33	88.46	88.46	92.31	96.15	96.15	96.15	100	84.62	88.46	88.46
15	**13.33**	88.46 **b**	88.46 **b**	92.31 **c**	96.15 **d**	96.15 **d**	96.15 **d**	100 **f**	84.62 **a**	88.46 **b**	88.46 **b**

Columns with different letters within the same concentration are significantly different (α. < 0.05) (ANOVA) (Tukey test).

p-value equals 3.74145e-13, statistic F equals 70.057899,

Table 3: values of LT, Slope, Chi square and Regression to Entomopathogenic Fungus and its Mixtures with Essential oils on Grasshopper at first Season.

NO.	MIXTURE	LT50	LT90	SLOPE		X2		R	
1	T0	6.2868	14.3994	3.5609	+/- 0.2012	12.713	tabulated 19.65	0.9813	tabulated 0.553
2	T1	4.8092	12.5132	3.086	+/- 0.1630	31.2258	tabulated 21	0.9632	tabulated 0.532
3	T2	5.163	12.9613	3.2061	+/- 0.1727	29.3826	tabulated 21	0.9928	tabulated 0.532
4	T3	4.2122	11.5738	2.9196	+/- 0.1622	34.4214	tabulated 21	0.9771	tabulated 0.532
5	T4	6.2829	14.1904	3.6221	+/- 0.2026	34.508	tabulated 19.65	0.9609	tabulated 0.553
6	T5	5.7935	13.6477	3.4441	+/- 0.1744	44.3618	tabulated 21	0.963	tabulated 0.532

NO.	MIXTURE	LT50	LT90	SLOPE		X2		R	
7	T6	4.6911	8.6817	4.794	+/- 0.2465	44.8011	tabulated 19.65	0.9421	tabulated 0.553
8	T7	4.544	14.3744	2.5624	+/- 0.1535	14.7859	tabulated 21	0.9785	tabulated 0.532
9	T8	5.8115	13.4672	3.5113	+/- 0.1763	7.4977	tabulated 21	0.9927	tabulated 0.532
10	T9	4.5682	14.6636	2.5303	+/- 0.1528	9.3358	tabulated 21	0.9859	tabulated 0.532

X2 = Chi square

R= Regression

Table 4: Values of LT, Slope, Chi square, and Regression to Entomopathogenic Fungus and its Mixtures with Essential oils on Grasshopper at the Second Season.

NO.	MIXTURE	LT50	LT90	SLOPE		X2		R	
1	T0	3.1495	16.1605	0.9264	+/- 0.1127	365.3768	tabulated 21	0.4028	tabulated 0.532
2	T1	4.9313	13.3531	2.9624	+/- 0.1615	29.0141	tabulated 21	0.9652	tabulated 0.532
3	T2	5.0953	12.9113	3.1739	+/- 0.1724	37.74	tabulated 21	0.9914	tabulated 0.532
4	T3	4.3215	12.0652	2.8742	+/- 0.1634	30.2304	tabulated 21	0.9773	tabulated 0.532
5	T4	5.3317	12.2744	3.539	+/- 0.2043	50.6399	tabulated 19.65	0.9649	tabulated 0.553
6	T5	5.7505	13.0756	3.5923	+/- 0.1797	54.0686	tabulated 21	0.9555	tabulated 0.532
7	T6	5.1386	9.4774	4.8208	+/- 0.2223	39.9821	tabulated 21	0.9586	tabulated 0.532
8	T7	6.7552	17.7409	3.0563	+/- 0.1680	35.9418	tabulated 21	0.9598	tabulated 0.532
9	T8	5.6957	14.0321	3.2729	+/- 0.1696	7.8268	tabulated 21	0.9918	tabulated 0.532
10	T9	7.2618	15.0588	4.0461	+/- 0.2146	29.4028	tabulated 19.65	0.9703	tabulated 0.553

4. Discussion

The results of this investigation offer strong proof that *Metarhizium anisopliae var. acridum* is an effective biocontrol agent against *Heteracris littoralis,* especially when combined with certain essential oils and sunflower oil. The observed differences in lethal time (LT) values and rates of mortality between treatments demonstrate how important essential oil type and concentration are in determining the total bioinsecticidal action.

Because of its large host range and capacity to infect more than 200 insect species, *M. anisopliae* has been extensively researched for application in biological control **(Swoboda, 2022).** The elevated mortality rates seen in the treated plots, particularly those that employed sunflower oil as a carrier, align with previous research demonstrating the efficacy of *Metarhizium spp*. against a range of orthopteran pests **(Shah & Pell, 2003).** It can enhance fungal spore adherence and cuticle penetration, which is a crucial stage in the infection process **(Prior et al., 1988).** that support the findings of **Bateman et al. (1993) and Moore et al. (1993),** who used cotton seed oil formulations including *Metarhizium flavoviride* to combat *Schistocerca gregaria,* demonstrated higher insect infectivity even at high temperatures and low humidity. Additionally, oil protects fungal conidia from sunlight's ultraviolet rays **(Moore et al., 1993).** Conidial formulations of entomopathogenic fungi have been prepared using a variety of vegetable oils in varying quantities. This is consistent with our research, which showed that the fungus was more effective when it was carried around by plant oils.

The formulation of *M. anisopliae* that contained 50% parsley oil (T6) performed exceptionally well, regularly achieving 100% mortality in both seasons. This was the most notable outcome. This discovery highlights the robust synergistic relationship between *M. anisopliae* and parsley essential oil, indicating that some chemicals in the oil greatly increase the virulence of the fungus. Although essential oils of garlic and basil also increased *M. anisopliae*'s effectiveness, their effects were not as strong as those of parsley oil. Variations in the essential oils' chemical makeup and their distinct ways of acting on the target insect could be the cause of this discrepancy in effectiveness. For example, **Yang et al. (2009)** found that garlic essential oil has an insecticidal effect. Our research demonstrates that garlic oil contributes to grasshopper mortality, albeit less so than parsley oil.

Our findings provide compelling evidence that essential oils can increase the activity of entomopathogens, as suggested by other studies. According to studies, essential oils can enhance the effectiveness of entomopathogenic fungi by acting as adjuvants **(Bitencourt et al., 2022).** Certain essential oils can change an insect's physiology and cuticle integrity, which could facilitate fungal infections **(Regnault-Roger et al., 2012b).** The higher mortality rates seen with oils of garlic, parsley, and basil imply that these essential oils might have certain mortality qualities in addition to helping to transfer the fungal spores **(Regnault-Roger et al., 2012b; Isman, 2000).** That might work together to raise the grasshopper populations' total mortality rate. This result is consistent with earlier studies showing that some plant extracts can increase the virulence of entomopathogenic fungi by either acting as attractants or by interfering with the physiological functions of the insect. Since essential oils (Eos) have shown promising benefits against insect pests, efforts to assess whether their active ingredient compositions are suitable for use in integrated pest control programs have advanced quickly. Due to their unique, volatile, and lipophilic properties, EOs can penetrate insect cuticles and interfere with physiological functions.

According to the characteristics of each Eos, the current study demonstrated that effectiveness increases with the type utilized. For example, parsley had the greatest influence on enhancing the effectiveness of *Metarhizium,* followed by basil, while garlic oil appeared to have the least effect on mortality. The unique lipophilicity and volatility of EOs allow them to penetrate insect cuticles and interfere with physiological functions, leading to biochemical malfunction and death **(Lee et al., 2002).** Additionally, this quick action shows that some EOs are neurotoxic to some pests **(Kostyukovsky et al. 2002).** Because of their lipophilic chemical makeup, EOs can enter insects and result in metabolic malfunction and death **(Lee et al., 2004)**. The chemical components that function as toxins are not the only elements that affect the toxicity of essential oils (EOs); numerous other factors also have a significant influence. EOs can cause toxicity based on the toxin's location of entry, molecular weights, and methods of action. According to reports, insects can inhale, consume, or absorb common essential oils with insecticidal properties through their skin **(Ozols and Bicevskis, 1979).** Additionally, it has been demonstrated that extracted essential oils contain biologically active components that have antibacterial **(Ntezurubanza et al. 1984),** nematocidal **(Chatterjee et al. 1982),** insecticidal **(Chogo and Crank 1981; Chavan and Nikam 1982**), or fungistatic **(Reuveni et al. 1984)** qualities. Because parsley contains phenolic compounds and essential oil, it has anti-inflammatory, antibacterial, diuretic, and hypoglycemic qualities (**Taiz and Zeiger, 1998**). In general, the majority of the oil in parsley seeds is made up of oxygenated phenylpropenes and monoterpenes, including α- and β-pinene. According to **Abd El-Hamid (1993),** essential oils of onion, parsley, and cumin showed tolerable levels of toxicity against Schistocerca gregaria, a desert locust.

Essential oils derived from plants have been extensively studied as possible substitutes for synthetic pesticides because of their significant insecticidal action and environmentally friendly nature **(Isman and Grieneisen, 2014; Ebadollahi et al., 2020).**

Furthermore, studies conducted in both lab and field settings have shown that the entomopathogenic fungus *M. anisopliae* is highly effective at controlling insect pests **(Reddy et al., 2014).** To maximize the effectiveness of pest control, it has been strongly advised that EPF be used in conjunction with other control methods, such as plant-derived essential oils (EOs) **(Mohamed, 2009; Batta and Kavallieratos, 2018).** The faster action of the fungal treatment is further shown by the observed decrease in LT50 and LT90 values when *M. anisopliae* was coupled with essential oils. In pest management, where quick pest mortality can help avoid significant crop damage, this is especially crucial. It's notable that sunflower oil alone (T0) also displayed a lower LT50 in the second season; this could point to either increased fungal activity or gradual field situation adaptation.

There are a number of reasons for the improved fungal performance shown in the second season, including greater mortality rates and shorter LT values. Some of these include the possibility that the fungal isolate has adapted to the local environment, minor changes in the microclimate that encourage fungal infection, or even a residual effect from the first season's application that may have changed the soil microbiome in a way that is advantageous to the fungus.

5. Conclusion

Our findings are important from the standpoint of pest management. A viable substitute for traditional chemical pesticides is the application of *Metarhizium anisopliae* in conjunction with essential oils produced from plants. Given the detrimental effects of broad-spectrum insecticides on non-target creatures and human health, this strategy is in line with the growing demand for sustainable and ecologically friendly pest management methods. The fact that insect pests do not readily become resistant to entomopathogenic fungi, in contrast to their capacity to become resistant to conventional insecticides, lends more credence to the effectiveness of *M. anisopliae*. Metarhizium anisopliae is regarded as a useful biological control agent that provides a secure and efficient method for agricultural and environmental applications.

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