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Exploring the Efficacy of Essential Oils in Laboratory Conditions for Controlling Mediterranean Fruit Fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae)

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ABSTRACT

The Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), poses a significant threat to agriculture worldwide. This study examines the potential insecticidal effects of essential oils from *Mentha arvensis* and *Cinnamomum zeylanicum* on controlling *C. capitata* under laboratory conditions. Even at low concentrations, toxicity assays indicated that both essential oils significantly increased the mortality of adult Medflies. The concentration-dependent effect of these oils on *C. capitata* mortality is demonstrated, with *Mentha arvensis* achieving 100% mortality within 48 hours at 1% concentration and *Cinnamomum zeylanicum* exhibiting rapid efficacy, reaching a low LC_{50} value after only 1 hour of application. The concentration and application time of essential oils were found to have a significant impact on their effectivness. This study highlights the potential of essential oils for controlling *C. capitata* populations. Essential oils offer a sustainable and eco-friendly alternative for managing *C. capitata* but further studies are necessary for their successful incorporation into integrated pest management programs.

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Introduction

The Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is a serious agricultural pest of global importance due to its invasive behavior and devastation of a broad variety of fruit crops. The medfly, which is native to sub-Saharan Africa, has extended its geographic range quickly, becoming a cosmopolitan species found on five continents (Gomulski et al., 2008; CABI/EPPO, 1998). Its adaptability to diverse climatic conditions and polyphagous nature, with a voracious hunger for more than 350 botanical species spanning 65 plant families, make it a tough agricultural challenge (Papadopoulos et al., 1996; Weems, 1981; Liquido et al., 1991).

In conventional farming, the management of *C. capitata* has mainly relied on the substantial use of synthetic insecticides and proteinic baits laced with toxic substances (Roessler, 1989). However, the continuous use of these compounds caused significant environmental and to health issues. Concerns include acute and chronic poisoning of pesticide applicators and consumers, damage to non-target organisms, disruption of pollination systems, pollution of groundwater, and causing insecticide resistance (Nerio et al., 2010; Picollo et al., 2005).

As a result of these concerns, there is an increasing interest in creating ecologically benign alternatives for the control of *C. capitata*. Due to their bioactive properties, which include insecticidal, repellent, and antifeedant effects, essential oils derived from numerous plant sources have emerged as promising candidates for this purpose. (Isman, 2006; Kilic et al., 2019).

The possible insecticidal effects of *Mentha arvensis* and *Cinnamomum zeylanicum* essential oils against *C. capitata* have been gaining considerable interest. These oils are renowned for their diverse chemical compositions, which include compounds with insecticidal effects, such as menthol and cinnamaldehyde. Previous studies have provided persuasive information that these essential oils have the ability to elicit toxicity in adult *C. capitata* (Passion et al., 1999; Miguel et al., 2010; Benelli et al., 2012; Benelli et al., 2013; Papanastasiou et al., 2020).

This study aims to determine the insecticidal effects of *M. arvensis* and *C. zeylanicum* essential oils on adult *C. capitata* under laboratory conditions. This research contributes to our understanding of the potential of these essential oils as viable alternatives for treating medfly infestations by determining mortality rates and concentration-dependent responses. In addition to this, this study explores the concentration and timing factors that impact the efficiency of essential oils, therefore shedding light on their practical implementation in integrated pest management strategies.

Materıals and Methods

Ceratitis capitata Culture

Ceratitis capitata adults and larvae were collected from infested orchards.

The population has been consistently maintained under semi-mass rearing conditions, utilizing the rearing procedures outlined by Caceres (2002). The maintenance of the colony took place in controlled climatized chambers, with a consistent temperature of 25 ± 2°C, relative humidity of 75 ± 5%, and a photoperiod of 12:12 hours (L:D) throughout the entire life cycle. Additionally, total darkness was maintained during the pupal development stage. Adult flies were housed in “sandwich” cages (measuring 100 cm in length, 6 cm in width, and 75 cm in height). These cages were equipped with voile cloth on their sides. The diet provided to the adult flies consisted of a combination of refined sugar and lyophilized beer yeast extract in a ratio of 3:1, as described by Walder et al. (2014). Flies were provided unrestricted access to water. Oviposition cages were arranged in a randomized design, and egg deposition occurred on the screens of the cages. Eggs were collected from the cages after a 24-hours and subsequently, to obtain laboratory-reared larvae, the eggs collected from the maternal colony were subjected to 48 hours of bubbling in a water bath at a constant temperature of 24˚C before being introduced into the artificial larval diets.

Essential Oils

The essential oils tested in this study were *Cinnamomum zeylanicum* and *Mentha arvensis*. The essential oils were purchased from the Nature In Bottle company. The source of plants, plant parts and the obtaining methods are shown in Table 1.

Contact Activity Assays

Adult individuals were exposed to direct contact with essential oil residues from *Mentha arvensis* and *Cinnamomum zeylanicum.* This test was chosen to determine the insecticidal effect of essential oils on mortality of adults. This methodology closely emulates the practical scenario of flies coming into contact with treated vegetation, as outlined by Pavela in 2011.

Experimental units comprised of plastic cages 12 cm in diameter by 5 cm height, with a lid which had a cover which featured a hole 7.5 cm in diameter. Preliminary experiments showed similar mortality using ventilated Petri dishes (9 cm) diameter and ventilated lower cages (9 cm diameter), simply to make sure there is no fumigation impact of essential oils. The control was treated with pure acetone (EMSURE® ACS). Filter paper disks of 11 cm diameter were treated with 1 ml of acetonic solutions (0, 0.2; 0.4; 0.5; 0.6; 0.7; 0.8; 1%) of the oils and dried for at least 10 minutes, under a fume extractor. Later, each treated filter paper was fixed on the top of the cage with plasticine at two opposite points. A small plastic container (1 cm diameter) containing adult food as well as a drinking trough was supplied. Afterward, five couples of *C. capitata* adults previously sexed (<72 h old) were introduced in each cage and placed in a climatic chamber at 25°C, RH 75%, L:D 16:8. Mortality was scored after 1, 6, 24, 48, and 72 h. 5 replicates per concentration were carried out.

Statistical Analysis

The assessment of the toxicity was conducted by calculating the percentage of mortality observed on the 1st, 2nd, 24th, 48th and 72th hours following the application of essential oils. Data collected from the insecticide trials were subjected to statistical analysis, considering both the specific study conditions and the date of sampling. To enhance the suitability of percentage data for statistical analysis, an arc-sine square-root transformation was applied. The data were analyzed by the analysis of variance (ANOVA). Mean values were separated using Tukey’s honestly significant difference (HSD) multiple comparisons test at a significance level of $P<0.05$. Dose-response results were subjected to probit analysis using Polo software (Polo Plus, version 1.0) to calculate the 50% lethal concentration (LC_{50}), 90% lethal concentration (LC_{90}).

Results and Discussion

Direct contact with essential oil residues from both essential oils resulted in increased mortality among adult Medflies.

The essential oils of *Mentha arvensis* and *Cinnamomum zeylanicum* caused a substantial increase in mortality over time, even at low concentrations (Table 2).

In the dose response tests of *M. arvensis*, *C. capitata* mortality reached 100% within 48 h at 1% concentration. For *C. zeylanicum* application within 72 hours, the mortality reached 100% at 0.8% concentration. The results show that the concentration of essential oils affected the mortality of *C. capitata* differently ($P<0.05$). These results for *M. arvensis* and *C. zeylanicum* revealed concentration-dependent mortality effects. This finding is consistent with the well-established principle that the efficacy of essential oils as insecticides is influenced by their concentration (Regnault-Roger, 1997). Higher concentrations often result in more rapid and complete insect mortality.

Mentha arvensis and *C. zeylanicum* were capable of controlling *C. capitata* based on the LC_{50} and LC_{90} values at 1, 6, 24,48, and 72 hours (Table 3). When examining the LC_{50} and LC_{90} values for *C. zeylanicum*, the LC_{50} value was determined to be 0.8455 even 1 hour after application. The LC_{90} values found 48 and 72 hours after application were 0.978 and 0.466, respectively. For *M. arvensis,* while the LC_{50} value was 0.724 at 1st hour of application, the LC_{90} value was 0.999 at 6th of application.

The determination of the LC_{50} and LC_{90} values is critical for assessing the insecticidal activity of essential oils. According to the results, *M. arvensis* was able to successfully kill the adults of *C. capitata*, as indicated by their LC_{50} and LC_{90} values. Similar findings have been reported in other studies involving the use of essential oils against fruit fly pests (Benelli et al., 2012).

Table 1. The source of plants, plant parts and the obtaining methods

Common name	Scientific name	Plant parts	Extraction method
Field mint	*Mentha arvensis*	leaf	Steam distillation
Cinnamon	*Cinnamomum zeylanicum*	leaf	Steam distillation

Table 2. The effect of varying concentrations of essential oils on the mortality rate of adult Mediterranean fruit flies at distinct time points of application.

EO	T	Concentration (%)						
		0.2	0.3	0.4	0.5	0.6	0.8	1
Mentha arvensis	1	10±7.07Bd*	16±15.17Cd	32±19.24Cc	40±12.47Cbc	46±5.48Db	48±13.04Cab	68±8.37Ba
	6	14±11.40Be	26±11.40BCd	52±13.04BCc	68±5.47Bb	68±8.37Cb	80±7.07BCab	90±7.07Aa
	24	26±5.47ABf	44±8.94ABe	60±14.14ABd	80±7.07ABc	88±8.37BCbc	92±4.47ABa	96±5.48Aa
	48	32±8.36Ad	46±11.40ABcd	68±10.95ABc	84±5.47Ab	88±8.37ABab	94±5.48ABa	100±0.00Aa
	72	34±8.94Ad	52±8.37Ac	84±8.94Ab	92±8.36Aab	94±8.94Aa	98±4.47Aa	100±0.00Aa
Cinnamomum zeylanicum	1	2±4.47Bd	2±4.47Bd	6±5.47Cd	14±5.47Ccd	22±4.47Dc	46±5.48Db	60±7.07Ba
	6	2±4.47Be	4±5.47ABe	10±7.07Cde	18±8.36Cd	34±5.48Cc	60±7.07Cb	80±14.14ABa
	24	4±5.47ABe	4±5.47ABe	12±4.47Cde	18±8.94Cd	42±4.47Cc	60±7.07Cb	80±14.14ABa
	48	4±5.47ABd	6±5.47ABd	60±7.07Bc	70±7.07Bb	74±8.37Bab	76±5.48Bab	80±14.14ABa
	72	10±7.07Ac	14±5.47Ac	78±4.47Ab	98±4.47Aa	98±4.47Aa	100±0.00Aa	100±0.00Aa

EO: Essential oil; T: Time (h); *Means followed in the same column by the same capital letter and in the same row by the same small letter are not significantly different (P≤0.05; Tukey's HSD test).

Table 3. Dose effects of essential oils on *Ceratitis capitata* adults

Essential oil	Time (h)	LC_{50}	LC_{90}	Slope± S.H	Chi square (χ^2)
Mentha arvensis	1	0.724	2.741	2.217±0.425	2.033
	6	0.416	0.999	3.373±0.365	2.718
	24	0.321	0.762	3.414±0.381	1.871
	48	0.290	0.629	3.811±0.419	2.995
	72	0.260	0.500	4.516±0.504	3.179
Cinnamomum zeylanicum	1	0.845	1.563	4.803±0.585	0.234
	6	0.712	1.248	5.252±0.555	1.028
	24	0.695	1.236	5.123±0.538	1.727
	48	0.469	0.973	4.049±0.403	36.552
	72	0.359	0.466	11.291±1.263	6.7178

Interestingly, the essential oil of *M. arvensis* displayed rapid efficacy, with a low LC_{50} value even at just 1 hour after application. This indicates that *M. arvensis* may also affect Medfly populations in the field, rapidly. This early effectiveness is useful for integrated pest management programs when rapid control is required to prevent additional crop damage.

This study showed that *Mentha arvensis* and *Cinnamomum zeylanicum* essential oils exhibit insecticidal properties that hold promise for controlling *C. capitata*. This potential efficacy can likely be attributed to the presence of bioactive compounds within these oils, such as menthol and cinnamaldehyde, both recognized for their insecticidal characteristics (Papanastasiou et al., 2020)

The results indicated that both *M. arvensis* and *C. zeylanicum* essential oils led to a substantial increase *in C. capitata* mortality over time, even at low doses and concentrations. This rapid mortality effect aligns with previous studies demonstrating the acute toxicity of essential oils to various insect pests (Isman, 2006; Papanastasiou et al., 2017). Essential oils can act quickly to disrupt vital physiological processes in insects, leading to their rapid demise.

It appears that the concentration and timing of essential oil treatments are crucial factors in determining their efficacy. This data underscores the necessity of accurate application methods and dose management to optimize the impact on *C. capitata* populations. For field application, studies on the appropriate timing of essential oil applications are essential (Sethi et al., 2014).

For our country, several researchers have evaluated the effectiveness of a variety of essential oils produced from both native and exotic plant species against *C. capitata*. These essential oils include those from citrus fruits like orange and lemon, as well as those from indigenous Turkish plants such as oregano and thyme. Kutca et al. (2021) and Tabanca et al. (2020) evaluated the insecticidal characteristics of these oils, as well as their effects on larval development, adult mortality, and oviposition inhibition.

The diverse flora of Türkiye provides a valuable resource for investigating the potential of indigenous plant essential oils for *C. capitata* management. In several studies the bioactive compounds in local plant species and their repellent or toxic effects against *C. capitata* have been examined (Kurtca et al., 2021; Benelli et al., 2012; Blythe et al., 2020). These studies have highlighted the significance of improving formulations and application strategies for essential oils. Researchers have investigated several different factors to improve the field stability and efficacy of essential oils (Yusufoğlu et al., 2021).

The agricultural industry in Türkiye has acknowledged the significance of integrated pest management (IPM) for sustainable agriculture. Essential oils are considered an important component of IPM techniques for *C. capitata* (Kurtca et al., 2021). This approach combines many control strategies to successfully manage insect populations. While essential oils provide promise, research has uncovered obstacles such as heterogeneity in efficacy across different environmental conditions and the requirement for consistent administration methods (Kilic et al., 2019; Raff al., 2019). Future studies in Türkiye may concentrate on refining essential oil-based *C. capitata* management strategies, taking concentration, application time, and other variables into account.

Conclusion

The essential oils of *Mentha arvensis* and *Cinnamomum zeylanicum* show environmentally acceptable potential for the management of *Ceratitis capitata*. Their diverse insecticidal properties, such as larval development suppression, oviposition deterrence, and adult mortality, make them promising candidates for integrated pest management programs (Bempelou et al., 2018). However, more study is required to optimize the formulations, enhance their persistence in the field, and identify synergistic combinations with other pest control methods. In addition, it is crucial to comprehend the long-term effects of essential oil applications on the environment and the resistance development on *C. capitata* populations (Bempelou et al., 2018).

Essential oils have considerable potential as a sustainable and environmentally acceptable alternative for the management of *C. capitata*. They are effective against this destructive pest and pose fewer risks to the environment and human health than chemical pesticides. However, more research is required to overcome those challenges and assure successful implementation in integrated pest management strategies for *C. capitata*.

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