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A field study on a plant-based protein hydrolysate containing insecticidal protein from *Metarhizium anisopliae* Metchnikoff against *Bactrocera oleae* (Rossi) (Diptera: Tephritidae)

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ABSTRACT

The present study evaluated the field efficacy of an olive kernel–derived protein hydrolysate optimized with destruxin purified from *Metarhizium anisopliae* for the management of the olive fruit fly, *Bactrocera oleae* Rossi. The experiment was conducted using a completely randomized block design in an olive orchard with approximately 250 trees per hectare planted at 5-m row spacing. To assess the effects of the formulated protein bait on adult population density and fruit infestation, 100 trees of the Zard cultivar were selected per experimental plot, with four replicates assigned to each treatment. Adult capture data obtained using a commercial protein hydrolysate and the destruxin-amended formulation (OKD) revealed two distinct population peaks in 2024, occurring from early June to mid-July and from early August to late September. In 2025, the first peak occurred at a similar time, whereas the second peak was observed earlier, extending from mid-August to mid-September. Regardless of sampling period, OKD consistently resulted in significantly higher total, male, and female captures of *B. oleae* compared with the commercial protein hydrolysate. Capture trends were comparable between OKD and commercial bait traps across both seasons. Sticky yellow traps recorded the lowest numbers of captured adults, whereas captures in OKD- and pheromone-baited traps increased significantly by four- to five-fold, with no significant difference detected between these two treatments. Moreover, the application of OKD resulted in a significant reduction in olive fruit infestation during both seasons, with infestation levels approximately 20 % lower than those recorded in orchards without trapping.

1. Introduction

Bactrocera oleae (Rossi) (Diptera: Tephritidae) is recognized as the most destructive pest of olive cultivation worldwide, owing to the exclusive larval feeding on olive fruits that results in substantial economic losses (Nardi et al., 2005; Daane and Johnson, 2010; von Gleich and Schröder, 2020). Eggs deposited beneath the fruit epidermis hatch into larvae that feed intensively on the mesocarp surrounding the seed, progressing through three instars. This activity causes both quantitative and qualitative damage, including premature fruit drop, reduced fruit weight, and deterioration of oil quality through increased acidity (Pinheiro et al., 2020). Among the most effective management strategies for B. oleae are pheromone- and bait-based traps containing protein hydrolysates, often supplemented with insecticides. These formulations may be deployed within traps or applied directly to foliage; however, foliar applications can lead to pesticide residues, environmental contamination, and adverse effects on non-target organisms, thereby constraining their long-term sustainability (Keswani et al., 2020; Sharma and Sharma, 2021).

To address these concerns, entomopathogenic fungi and their secondary metabolites represent a promising alternative due to their selective activity and environmentally compatible modes of action. Rather than using whole fungal propagules, fungal secondary metabolites offer improved stability, ease of formulation, and reduced ecological risk, making them attractive candidates for integration into IPM programs (Sani et al., 2020). Different entomopathogenic fungi, mainly *Beauveria bassiana* Vuillemin and *Metarhizium anisopliae* Metchnikoff, attach to the insect cuticle and pass through by enzyme production. Once they reach

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the insect hemocoel, they proliferate as blastospores, utilize available nutrients, and secrete secondary metabolites that cause host death (Sani et al., 2020). Fungal secondary metabolites contain bioactive chemicals, including polyketides, non-ribosomal peptides, polyketide-peptide hybrid metabolites, and terpenes, which exhibit antibacterial and antifungal properties in the natural habitat of fungi. Additionally, these compounds act as insecticides and affect the nutrition, lifespan, fertility, and immunity of host insects (Bandani et al., 2000; Molnar et al., 2010). Destruxins are cyclic peptides produced by *M. anisopliae* that have demonstrated various medicinal and insecticidal properties (Pedras et al., 2002). Destruxins influence insect growth and development in various examples such as *Phaedon cochleariae* Fabricius (Coleoptera: Chrysomelidae), *Epilachna sparsa* Herbsta (Coleoptera: Coccinellidae) (Amiri et al., 1999), *Choristoneura fumiferana* Clemens (Coleoptera: Tortricidae) (Brousseau et al., 1996), and B. oleae (Motamedi Juibari et al., 2023).

The olive fruit fly has been introduced into Iran for more than two decades and has caused significant damages to olive fruit in terms of quantity and quality (Moezzipour et al., 2015). Despite various control methods, it remains a constant threat to fruit production due to direct damage to the fruits, highlighting the need to improve control methods. In two previous studies, plant-based protein hydrolysates were prepared and improved by attractants to introduce a new formulation of eco-friendly substances (Motamedi Juibari et al., 2023; Zadjafar et al., 2024). Additionally, a destruxin was purified from native isolates of *M. anisopliae* and showed significant effects on the survival and physiological traits of *B. oleae* (Motamedi Juibari et al., 2023). Despite the well-documented insecticidal activity of entomopathogenic fungi and their secondary metabolites, their practical application in olive fruit fly management has remained largely limited to laboratory bioassays or formulations based on whole fungal spores. Based on our knowledge, no previous study has evaluated the field performance of a prepared protein hydrolysate from olive kernels enriched with a purified fungal secondary metabolite against *Bactrocera oleae*. On the other hand, the present study introduces a novel attract-and-kill strategy by incorporating a lethal concentration of destruxin, purified from a native isolate of *M. anisopliae*, into a plant-based protein hydrolysate bait. By assessing adult fly capture, fruit infestation levels, and comparative efficacy with conventional pheromone traps under orchard conditions, this work provides the first field-based evidence supporting the integration of fungal secondary metabolites into environmentally friendly bait formulations for olive fruit fly management within IPM programs. At the end, the current study was conducted to determine the field efficiency of a nature-based protein hydrolysate containing a lethal concentration of destruxin against *B. oleae* in olive orchards by evaluating adult catch, comparison with pheromone trap and effect on fruit infestation.

2. Materials and methods

2.1. Preparation of protein hydrolysate

Plant-based protein hydrolysate was prepared using commercially provided olive kernels (Palizkasht company) that were dried at 40 °C for 24 h. The dried kernels were ground into a fine powder and passed through a No. 30 mesh sieve. The resulting powder was mixed with n-hexane (pure 95 %) at a ratio of 1:5 (weight/volume) and stirred on a magnetic stirrer for 1 h. After centrifugation at 10,000 rpm for 15 min, the sample was passed through a mesh 60. Then, 100 g of defatted powder was added to 1 liter of distilled water and the pH was adjusted to 10 using 1 N NaOH (AOAC Method983.23, 1990). Incubation was carried out for 3 h, then the mixture was centrifuged at 12,000 rpm for 15 min at 4°C. The supernatant was isolated and its pH was adjusted to using 1 N HCl. A re-centrifugation was performed under the aforementioned conditions, then the precipitates were washed twice with 20 mL of sterile distilled water and freeze-dried (Matsuoka et al., 2012). The freeze-dried sample was enzymatically hydrolyzed by adding 70 mg of the sample to 350 mL of sterile distilled water and adjusting the pH to 8.5 using NaOH. Incubation was carried out at 80 °C for 5 min to remove unwanted enzymes. Once cooled, 3 mg of pepsin was added to the solution and left at 50 °C for 4 h. The enzyme activity was stopped by placing the solution in a water bath at 80 °C for 10 min. Finally, centrifugation was performed at 15,000 rpm for 20 min and the sample was used to determine protein content and utilized as hydrolyzed protein (Matsuoka et al., 2012; Zadjafar et al., 2024).

2.2. Extraction of destruxin

The method used by Ortiz-Urquiza et al. (2010) was employed to extract secondary metabolites from the MASA isolate of M. anisopliae. A liquid medium containing 40 g of glucose and 20 g of peptone was prepared in 1 L of distilled water, inoculated with a concentration of 10^7 conidia/mL of the isolate, and then incubated at 25°C for 7 days on a rotary shaker at a speed of 110 rpm. After 7 days, the medium was filtered through filter paper number 3 and saturated with a 90 % ammonium sulfate solution. The mixture was then centrifuged at 5000 g for 30 min, and the resulting precipitate was filtered through a 7 kDa dialysis bag (Thermo Fisher Co.) for 24 h. The dialyzed solution was then centrifuged at 10000 g for 20 min and filtered using a 0.45 μM syringe filter. The final sample was subjected to liquid chromatography-Electrospray Ionization-Mass Spectrometry/Mass Spectrometry (LC-ESI-MS/MS) analysis as described by Motamedi-Juibari et al. (2023) (Supplementary data 1).

2.3. Preparation of protein hydrolysate amended by destruxin (OKD)

The protein hydrolysate extracted from olive kernels was used as a stock solution, and 1 mL of destruxin at its LC_{90} concentration (previously determined under laboratory bioassays against adult B. oleae following Motamedi-Juibari et al., 2023; see Supplementary Data 1) was added. The mixture was stirred for 30 min and stored at 4°C prior to field application. Stability of OKD is three months in packed flask at laboratory temperature. In orchard condition, it should be replaced every two-three weeks depending on humidity if there is no rainfall.

2.4. Field studies

2.4.1. Meteorological data and location

Field experiments were conducted in a commercial olive orchard located in Rahmatabad region, Rudbar city (Guilan province, Iran) during the 2024 and 2025 growing seasons. Meteorological data, including mean daily temperature, relative humidity, and precipitation, were obtained from the Manjil meteorological station located approximately 5 km from the experimental site (Fig. 1). The orchard selected for the field study was located in Rudbar city, Rahmatabad region, during 2024 and 2025. The experiment was conducted using a completely randomized block design with approximately 250 trees (7–10 m tall, 25–30 years old) per hectare planted in rows 5 m apart.

2.4.2. Comparison of OKD efficiency with a commercial protein hydrolysate

2.4.2.1. Experimental design and plot layout. The experiment was conducted using a randomized complete block design. In each year, the orchard was divided into experimental plots of 1000 m^2, each containing approximately 100 olive trees (cv. Zard). Each treatment was replicated four times per year, resulting in four plots per treatment per year. Plots were separated by buffer zones to minimize interference among treatments.

2.4.2.2. Treatments. Five clearly defined treatments were included:

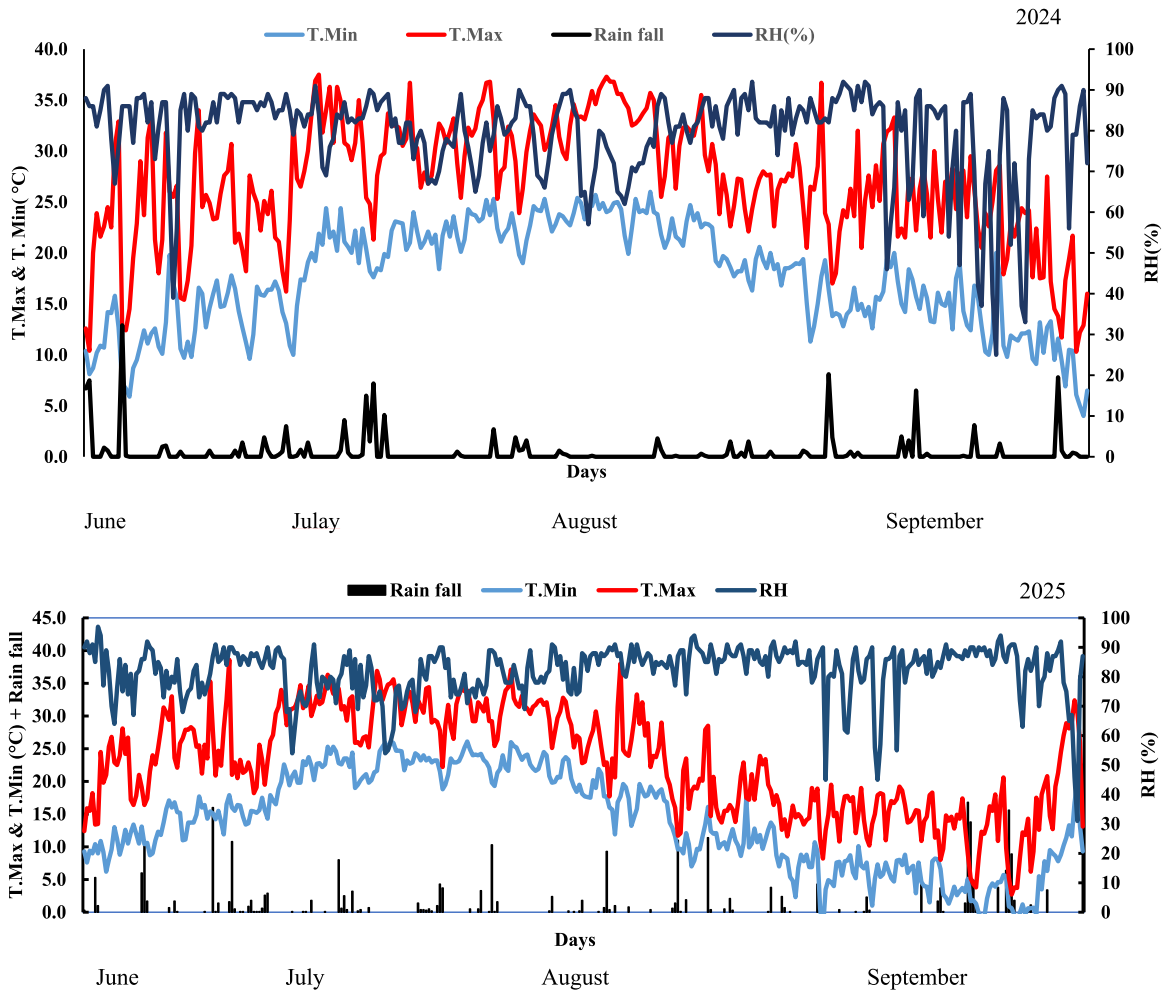

Fig. 1. Mean daily temperature (°C), relative humidity (%), and precipitation (mm) recorded at the Manjil meteorological station (Guilan province, Iran) during the bait spraying period in olive orchards in 2024 and 2025. Data are presented for the months of June to September for both years.

1. OKD in McPhail traps McPhail traps were baited with a plant-based protein hydrolysate amended with a lethal concentration (LC_{90}) of destruxin purified from *M. anisopliae*. One McPhail trap was installed per plot, suspended at a height of 2–3 m within the tree canopy.
2. Commercial protein bait (DACUS BAIT 100® + diazinon) A commercial protein hydrolysate (DACUS BAIT 100®) mixed with diazinon (0.1 %) was applied as a toxic bait spray, following the manufacturer's recommendations. This treatment served as the chemical control.
3. Pheromone traps Species-specific pheromone traps for *Bactrocera oleae* were installed at a density of one trap per plot and used to monitor adult male populations.
4. Sticky yellow cards Five yellow sticky cards (9.5 × 24.5 cm) were placed per plot on five different trees to monitor adult fly activity and assess non-target insect capture.
5. No-trap control plots Plots without traps or bait applications were included solely for fruit infestation comparison and to assess natural population levels.

2.4.3. Timing and application of treatments

All traps and bait treatments were initiated simultaneously at the beginning of the adult flight period in each season. There was no sequential application of treatments. Bait sprays were applied only when adult population density reached the economic threshold of 20 adult males per trap over five days, as determined by McPhail trap monitoring. A maximum interval of 14 days was maintained between successive bait applications.

To evaluate the effect of OKD on the density of olive fruit fly and the level of fruit contamination, 100 trees of the Zard variety were selected in each experimental plot and four replications were considered for each treatment. A commercial protein hydrolysate (DACUS BAIT 100®) mixed with diazinon as a toxic material was used as a control and the protein concentration was considered LC90 (Motamedi Juibari et al., 2023). The size of the experimental plots was 1000 square meters. Both treatments were established on the trees using McPhill trap. At this stage, each of the plots was sprayed as a bait spray. In this experiment, the number of caught males, females and total catch were considered and compared to commercial protein hydrolysate

2.4.4. Determination the population density for baiting time

McPhail and sticky yellow cards measuring 9.5 cm × 24.5 cm were used to detect the adults of *B. oleae*. Briefly, 300 mL of water and protein hydrolysate produced from olive kernels (4 %) was mixed with MASA toxin (8 mg/mL) and poured into McPhill traps. For each plot, one McPhill trap was used in the central part and each trap was installed at a height of two to three meters in the central part of the tree crown. Also, five yellow sticky cards were placed inside each plot on five trees. Sticky yellow cards were included in the experimental design to provide an independent visual monitoring tool reflecting adult fly activity within the tree canopy and to evaluate the ecological selectivity of baiting treatments, as these traps capture flying insects passively and are commonly used for population monitoring rather than mass trapping.

The mixture within McPhill traps was checked weekly and replaced by fresh one. In addition, the olive fruit fly caught along with the non-target insects caught counted weekly (Varikou et al., 2016). Thus, the trap catch rate indicates population status in the orchard or plot.

2.4.5. Determining the time of baiting

The time and number of baiting application was determined based on the statistics of caught adults in McPhail traps in each test plot. When the number of captured adults reached the economic threshold (20 adult males per trap over five days), the trees were sprayed with the prepared protein hydrolysate. The amount of solution used for each tree was 350 mL because the aim of baiting was to attract adult olive fruit fly insects to the baited parts of the trees and consequently reduce the pest population. However, in foliar spraying, the entire tree crown was sprayed, which varied depending on the size of the tree crown. There should be a maximum of 14 days between each foliar spraying stage.

2.4.6. Fruit sampling to determine the level of contamination

In this way, 400 fruits were randomly collected from 10 trees in each plot from four directions: north, south, east and west of the trees and transferred to the laboratory. Then, the fruits were examined under a stereomicroscope to determine the presence of eggs, larvae, pupae and exit holes. The level of fruit contamination indicates the effectiveness of the treatments to reduce *B. oleae* population and the amount of crop damage (Varikou et al., 2017).

2.4.7. The effect of treatments on pest population reduction

A weekly fruit sampling was done to determine *B. oleae* population throughout the season in the control (commercial protein hydrolysate and diazinon 0.1 %) and treated trees by protein hydrolysate and destruxin. Comparisons were done between control and treatment for each sampling session. With this method, the trend of decreasing or increasing pest density in the orchard was evaluated to determine the best foliar spray method for pest control.

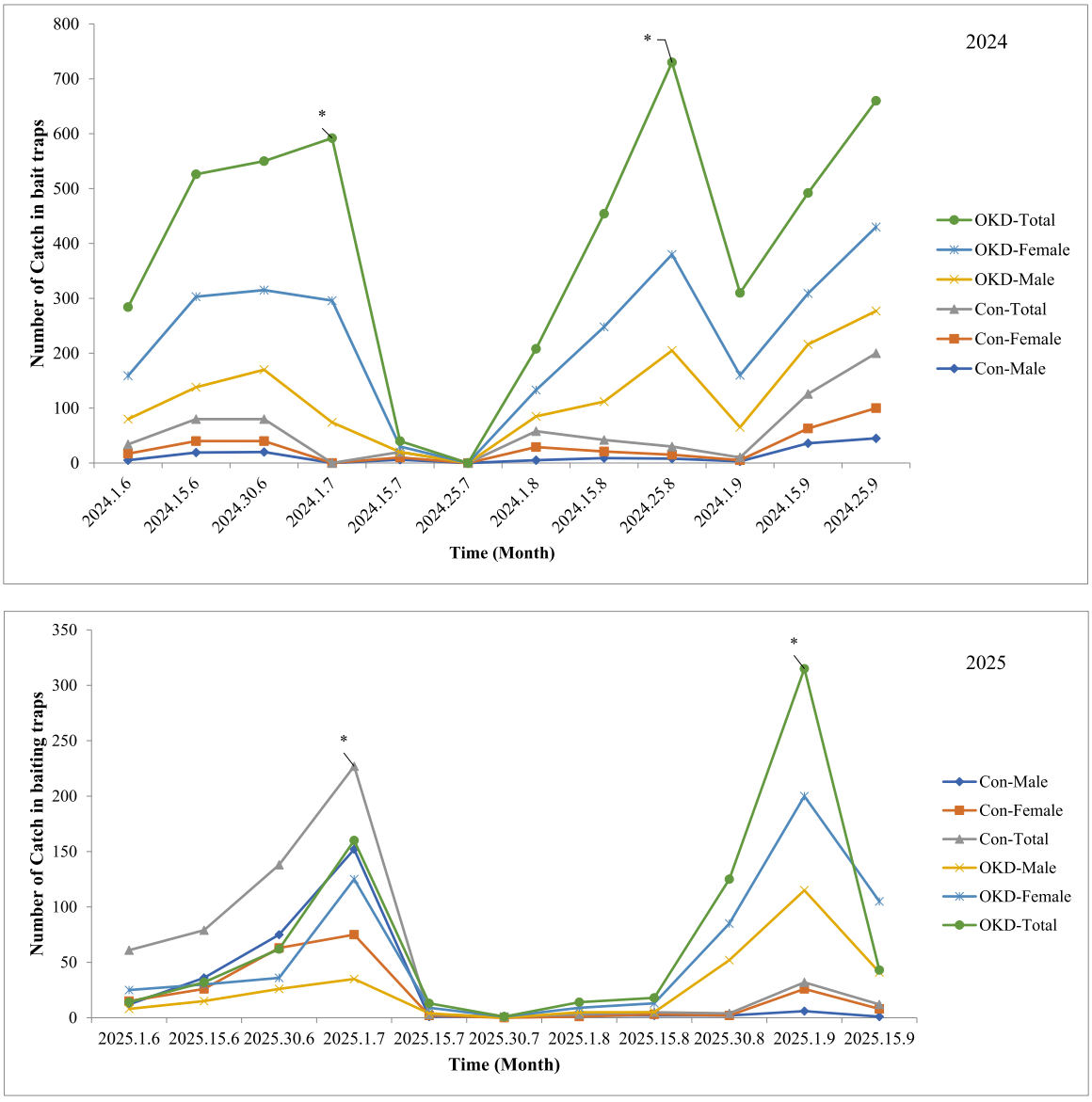

Fig. 2. Mean (± SE) number of *Bactrocera oleae* adults captured per trap per week in olive orchards treated with OKD (protein hydrolysate amended with destruxin), commercial protein bait (DACUS BAIT 100® + diazinon), pheromone traps, and sticky yellow cards during the 2024 and 2025 growing season in Rudbar, Iran. The x-axis represents sampling dates from 2024.1.6 to 2024.25.9 and 2025.1.6–2025.15.9. The statistical highest value has been marked by asterisk at $p \leq 0.05$ (Tukey's HSD test).

2.5. Statistical analysis

Each treatment was replicated four times, and experimental plots were randomly assigned within the orchard to minimize spatial bias. Trap placement and fruit sampling were conducted using identical protocols across all plots to ensure reproducibility. Normality of the data was tested by Kolmogorov–Smirnov method. Data were analyzed by one-way analysis of variance followed by comparison of the means with *t*-test and Tukey post hoc honestly significant difference (HSD) test at $p \leq 0.05$.

3. Results and discussion

Fig. 1 illustrates meteorological data including temperature, relative humidity, and rainfall during the study period. The results of *B. oleae* captures using commercial protein hydrolysate and OKD are shown in Fig. 2. The results showed two peaks of adult catching in 2024 from June 1st to July 15th and August 1st to September 25th. In 2025, the first peak was similar to 2024 but the second peak started from August 15th to September 15th (Fig. 2). Such seasonal shifts in insect population peaks are increasingly attributed to climate-driven alterations in temperature accumulation and humidity regimes, which influence insect physiology, behavior, and microbial interactions (Ali et al., 2023b; He et al., 2025). Apart from the time of sampling, OKD showed the highest catch of total, males and females of B. oleae compared to the values obtained by commercial protein hydrolysate (Fig. 2, $p \leq 0.0001$; Df=10,2). Our data revealed a similar trend of catching in OKD and commercial baiting traps in both years. Additionally, the number of females caught was higher than that of males in both years which may be attributed to the materials used in OKD to enhance attractance for females (Fig. 2; $p \leq 0.0003$; Df=10,2). Female-biased attraction to nutritionally rich baits is consistent with broader evidence showing that protein composition and microbial-derived metabolites strongly influence feeding behavior and physiological readiness for oviposition in insects (Ali et al., 2019; Ali & Xiao, 2025). The lowest catch was recorded during late August to early September which may be attributed to the high temperature prevailing in the area for both years affecting adult activity. The seasonal population dynamics of *Bactrocera oleae* observed in the present study are consistent with patterns reported across Mediterranean olive-growing regions. Two main population peaks, typically occurring in early summer and late summer to early autumn, have been widely documented in Greece, Italy, Spain, and other Mediterranean countries (Haniotakis, 2005; Daane & Johnson, 2010; Kakani et al., 2010). The earlier onset of the second population peak in 2025 compared with 2024 aligns with previous studies demonstrating that temperature accumulation and summer heat stress strongly influence adult activity, survival, and reproductive timing of *B. oleae* (Gutierrez et al., 2009; Varikou et al., 2016). Recent modeling and predictive studies on entomopathogenic fungi further indicate that climate change not only alters insect phenology but also affects the spatial efficacy and persistence of microbial control agents, reinforcing the need for climate-resilient IPM strategies (He et al., 2025; Ali et al., 2023b). High summer temperatures above 32–35 °C are known to suppress adult flight and oviposition, leading to temporary population declines, followed by resurgence when temperatures moderate in late summer or early autumn (Broumas et al., 2002; Pontikakos et al., 2012). Recent syntheses of long-term microbial pest management studies further emphasize that climatic factors strongly modulate the success of biological control agents and bait-based strategies in field conditions, reinforcing the importance of adapting formulations to local environmental dynamics (Mahanta et al., 2025).

The number of caught adults of *B. oleae* showed the lowest number in the sticky yellow trap ($p \leq 0.0001$; Df=10,2). but the caught number statistically increased in both OKD and pheromone traps by 4–5-fold (Fig. 3; $p \leq 0.0001$; Df=10,2). Meanwhile, no statistical difference was recorded in the number of caught adults between OKD and pheromone

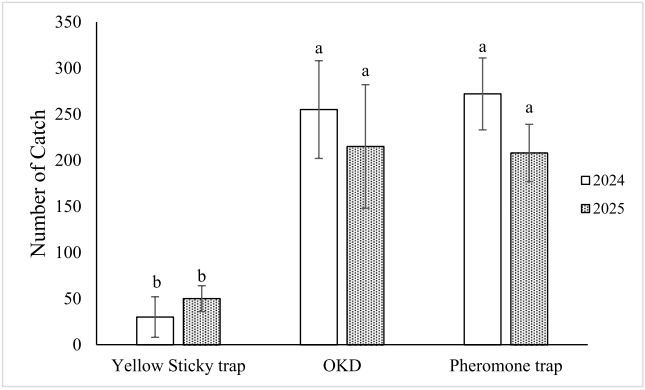

Fig. 3. Comparison of adult *Bactrocera oleae* captures (mean ± SE) using sticky yellow traps, pheromone traps, and the olive-kernel-derived protein hydrolysate amended with destruxin (OKD) during the study period. Columns sharing different letters are significantly different based on Tukey's HSD test ($p \leq 0.05$).

traps (Fig. 3; $p \leq 0.23$; Df=10,2). The lower catch in the pheromone traps may be attributed to the selective attractance of pheromone traps only on *B. oleae*. Comparable selectivity patterns have been reported in other agroecosystems, where targeted attract-and-kill or microbial-based approaches reduced pest pressure while minimizing broader ecological disruption (Ali et al., 2023a). Finally, our records showed a significant decrease in the percentage of infested fruits of olives in 2024 and 2025 when OKD was used in olive orchards as it showed almost 20 % lower fruit infestation compared to the orchard with no trapping (Fig. 4; $p \leq 0.0001$; Df=10,2). These results were in accordance with the findings of Varikou et al. (2015) and Abbasi-Mozhdehi (2019). Similar levels of disease or pest suppression have been documented in plant–microbe interaction studies, where microbial metabolites or biologically derived compounds enhanced host defense or reduced pathogen success (Soylu et al., 2025; Qian et al., 2024; Fu et al., 2024). It was recorded that bait spraying containing Dimethoate, Cypermethrin and Lambda-Cyhalothrin from 100 to 300 µL per mL of protein hydrolysate significantly decreased the *B. olea* population and fruit infestation in Greece (Varikou et al., 2015). Additionally, comparison bait spraying containing pyridalyl demonstrated the intensive attractance of *B. oelae* at the onset of spraying leading to lower fruit infestation (Abbasi-Mozhdehi, 2019).

The superior performance of OKD compared with commercial protein hydrolysate in attracting both male and female adults is in agreement with international findings emphasizing the importance of bait composition and protein quality in olive fly management. Numerous

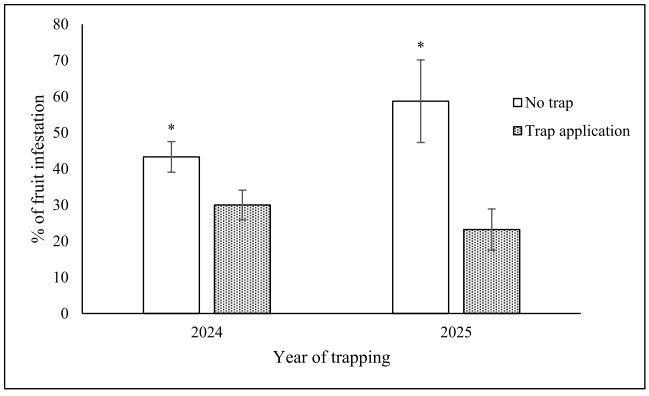

Fig. 4. Percentage of olive fruit infestation (mean ± SE) in orchards treated with the olive-kernel-derived protein hydrolysate amended with destruxin (OKD) compared with untreated orchards during 2024 and 2025. Asterisks indicate significant differences between treatments based on *t*-test ($p \leq 0.05$).

Mediterranean IPM studies have shown that protein hydrolysates derived from locally available plant materials can outperform imported commercial formulations by better matching the nutritional requirements and olfactory preferences of *B. oleae* females, particularly during the pre-oviposition period (Navrozidis et al., 2011; Vassiliou et al., 2017). The higher proportion of females captured in OKD-baited traps is particularly significant, as female-targeted control strategies are widely regarded as more effective in reducing subsequent fruit infestation (Haniotakis et al., 1991; Daane et al., 2015). Emerging evidence suggests that such enhanced bait attractiveness may also interact with the insect gut microbiome, influencing feeding behavior, nutrient assimilation, and susceptibility to microbial and chemical stressors (Yasika and Shivakumar, 2025).

In Mediterranean IPM programs, mass trapping and bait spraying are often combined to suppress adult populations while minimizing insecticide use. Studies from Greece and Spain have shown that protein bait sprays applied in spot treatments can reduce fruit infestation by 15–40 %, depending on bait formulation and application timing (Varikou et al., 2015; Ortega et al., 2018). The approximately 20 % reduction in fruit infestation achieved with OKD in the present study falls well within this effective range and is comparable to reductions reported for spinosad- or pyrethroid-based bait sprays under Mediterranean conditions (Montiel & Jones, 2002; Vontas et al., 2011). Importantly, unlike many conventional bait sprays, OKD integrates a biological toxin, destruxin, rather than synthetic insecticides, addressing growing concerns over resistance development and environmental contamination in olive agroecosystems. This approach is consistent with recent perspectives advocating the enhancement of entomopathogenic fungal resources through metabolic optimization and targeted deployment, rather than reliance on broad-spectrum chemical insecticides (Ahmad et al., 2025). Emerging research further suggests that nutritional cues, secondary metabolites, and microbial associations interact at the gut and behavioral level, influencing insect susceptibility to biological toxins and control agents (Ali et al., 2019; Liu et al., 2025).

The integration of *Metarhizium anisopliae*-derived destruxins into bait formulations represents a novel advancement relative to most Mediterranean IPM approaches, which have primarily focused on entomopathogenic fungi as soil or foliar applications rather than as bait toxicants (Konstantopoulou & Mazomenos, 2005; Quesada-Moraga et al., 2006). Such metabolite-based pest control strategies align with modern pest management paradigms that prioritize biological specificity, resistance mitigation, and reduced reliance on conventional insecticides (Ali et al., 2023a; Ali & Xiao, 2025). Previous laboratory and semi-field studies have demonstrated that destruxins exhibit strong oral and contact toxicity against tephritid flies, including *B. oleae*, by disrupting calcium homeostasis and immune function (Pedras et al., 2002; Skrobek et al., 2008). The successful field performance of OKD in the present study suggests that combining fungal secondary metabolites with protein hydrolysates may overcome some of the limitations previously associated with fungal biocontrol agents, such as slow kill rates and sensitivity to environmental conditions. Such metabolite-based strategies have been identified as a growing and influential research direction in microbial pest control over the past six decades, reflecting their increasing relevance for sustainable agriculture (Mahanta et al., 2025).

4. Conclusions

From an integrated pest management (IPM) perspective, the use of olive kernel-derived protein hydrolysate is particularly relevant for Mediterranean and Middle Eastern olive production systems, where sustainability and circular bioeconomy principles are increasingly emphasized. In Iran, official agricultural statistics reported reports indicated olive production of approximately 150,000 tons in 2024 and 167,000 tons in 2025 (Ministry of Agriculture Jihad, 2025), reflecting the growing importance of effective and sustainable pest management strategies in this sector. (Anonymous, 2025). Similar efforts in Italy and Spain have explored agro-industrial by-products, such as olive mill wastewater and grape pomace, as components of pest management tools, emphasizing waste valorization and reduced dependency on imported inputs (Sánchez et al., 2019; Malheiro et al., 2020). The OKD formulation aligns with these international trends by utilizing locally available olive by-products while reducing spray coverage through a targeted attract-and-kill approach, thereby contributing to the conservation of beneficial arthropods**.** Potential non-target effects of OKD represent an important consideration in olive IPM programs and warrant careful evaluation. Potential effects on non-target and beneficial insects are an important consideration in olive IPM programs. In the present study, **no noticeable increase** in non-target insect captures was observed in OKD-baited traps during **routine monitoring**, suggesting a degree of selectivity toward *B.oleae*. This selectivity is likely **attributable** to the combined use of a protein hydrolysate primarily attractive to tephritid flies and destruxin, which exerts its toxic effect mainly through ingestion. Previous studies have shown that protein bait-based and attract-and-kill strategies, applied as localized treatments, have limited impact on beneficial arthropods compared with broad-spectrum insecticide sprays (Haniotakis et al., 1991; Daane et al., 2015). Moreover, the localized application strategy and the low volume of active ingredients further reduce the likelihood of non-target exposure (Niogret et al., 2011). This study was conducted over two seasons in a limited number of orchards, and inter-annual climatic This study was conducted over two consecutive seasons in a limited number of orchards, and inter-annual climatic variability—particularly temperature extremes—may have influenced adult activity patterns and trap performance. Differences in orchard management practices, including cultivar composition, irrigation regimes, and prior pest control history, were not fully standardized and may have contributed to variability in capture rates and fruit infestation levels. In addition, non-target effects were assessed qualitatively rather than through systematic sampling, and economic feasibility was not evaluated. Further multi-year studies across diverse climatic zones are therefore required to confirm the robustness, ecological safety, and practical applicability of OKD within integrated pest management programs. Nevertheless, future studies should include systematic monitoring of beneficial insect populations to quantitatively confirm the ecological safety of OKD under diverse field conditions. The integration of plant-based protein hydrolysates with fungal metabolites represents a promising direction for next-generation IPM programs against *B. oleae*, particularly in regions such as Iran where large-scale olive production coexists with high biodiversity. Future studies should focus on multi-year validation across different climatic zones, compatibility with parasitoids such as *Psyttalia concolor*, and economic analyses relative to conventional Mediterranean control practices.

CRediT authorship contribution statement

Mohammad Reza Abbasi-Mozhdehi: Methodology, Formal analysis, Data curation. **Arash Zibaee:** Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests. Arash Zibaee reports financial support was provided by University of Guilan. Arash Zibaee reports a relationship with University of Guilan that includes: employment. Arash Zibaee and Other Authors had no involvement in the peer review of this article and had no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to another journal editor." If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to

Table 1
Summary of experimental treatments and their purpose in the field study.

Treatment	Formulation / Tool	Application method	Purpose	Used for comparison with
T1	OKD (olive kernel protein hydrolysate + α-pinene + destruxin)	**Bait spray** on selected trees (spot application)	Population suppression and fruit protection	Commercial protein hydrolysate bait spray; no-trapping control
T2	Commercial protein hydrolysate + diazinon (DACUS BAIT 100®)	**Bait spray** on selected trees (spot application)	Standard chemical bait control	OKD bait spray
T3	OKD solution	**McPhail trap** (liquid bait)	Adult population monitoring and attractancy comparison	Commercial protein hydrolysate trap; pheromone trap
T4	Commercial protein hydrolysate	**McPhail trap** (liquid bait)	Adult population monitoring	OKD trap
T5	Sex pheromone lure	**Pheromone trap**	Selective monitoring of adult males	OKD and protein hydrolysate traps
T6	No bait / no trap	**Untreated orchard area**	Baseline fruit infestation (negative control)	OKD-treated plots

Note: Sticky yellow cards were deployed across all plots solely for *population monitoring and ecological selectivity assessment* and were not considered control or suppression treatments.
This schematic distinction clarifies that (i) **fruit infestation reduction** was assessed by comparing bait-sprayed plots (T1, T2) with untreated plots (T6), whereas (ii) **adult attraction efficiency** was evaluated using trap-based comparisons (T3–T5).

influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.napere.2026.100183.

Data availability

Data will be made available on request.

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