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Seasonal Activity of the Pomegranate Fruit Moth *Ectomyelois ceratoniae* Zeller and its Possible Integrated Control in Ain al-Tamr, Karbala Governorate, Iraq

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Abstract

The pomegranate fruit moth, *E. ceratoniae* Zeller, is a significant pest of pomegranate worldwide. Seasonal monitoring and integrated control trials were carried out in pomegranate orchards of Ain al-Tamr, Karbala Governorate (Iraq) during the 2024 growing season (15 May–15 September 2024). Regular adult male flight began in early May and continued until mid-September, peaking in mid-August (35.15 male moth per trap) at a mean ambient temperature of 35.85°C and a relative humidity of 20.47%. Field trials evaluated botanical oils (thyme and juniper, 7% v/v), the entomopathogenic fungus *Beauveria bassiana* (commercial formulation), their combinations, and the insecticide imidacloprid (Imidor 200 SL) under a randomized complete block design. Treatments and monitoring (pheromone traps) were repeated following two sprays (7 June and 7 July 2025). Combined *B. bassiana* + 7% thyme oil provided control comparable to Imidor, reducing fruit infection to 3.3% and trap captures to 1 male per trap, whereas *B. bassiana* alone reduced infection to ~10% and trap captures to five males per trap. Overall, treated plots averaged 13.31% infection, compared to 55% in controls, after the second spray. Statistical analyses (ANOVA, post hoc tests) confirmed significant treatment effects. These findings indicate that integrating *B. bassiana* with botanical oils can effectively reduce *E. ceratoniae* infestation and may serve as a sustainable alternative to routine chemical control in Ain al-Tamr pomegranate orchards.

Keywords: Ain al-Tamr, *Beauveria bassiana*, botanical oils, *Ectomyelois ceratoniae*, integrated pest management, pomegranate

Introduction

Pomegranate (*Punica granatum* L.) is an economically important fruit crop in Iraq and globally, valued for its nutritional and commercial properties (Morland et al., 2019; Noreen et al., 2025). The carob/pomegranate fruit moth, *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae), causes severe losses by larval boring into fruit arils and pulp, facilitating secondary infections and preharvest drop (Abedi et al., 2019; Rat and Mamay, 2024). The pest can produce multiple generations per year, depending on the climate, and its larvae are protected inside fruit, making control difficult (Teimouri et al., 2014; Nay and Perring, 2005).

Current management relies heavily on insecticides; however, the development of resistance and concerns about residues motivate the evaluation of integrated pest management (IPM) approaches that combine biological and botanicals. *Beauveria bassiana* is a well-documented entomopathogenic fungus effective against many lepidopteran pests (Aziz et al., 2014). Botanical essential oils — notably thyme (*Thymus* spp.) and juniper oils — have shown repellent/insecticidal effects against multiple insect pests and can enhance biological control when used in combinations (Verdi et al., 2019; Semerdjieva et al., 2021). *Beauveria bassiana*, *Thymus* spp. and *Juniperus* spp. Oils are considered potential green alternatives to chemical insecticides for controlling lepidopteran pests. Pathogenicityed studies on their use include pathogenicity, biochemical effects of *B. bassiana* with natural oils and bioformulations, as well as compatibility aspects, all detail a comprehensive approach towards sustainable agriculture (Al-Ghafar et al., 2024). Crude extracts of *B. bassiana* showed strong larvicidal activity against *Tuta absoluta* (Lepidoptera: Gelechiidae), LC_{50}, LC_{90} values being 25.94 and 33.56 μg.mL^{-1} after 24 h exposure accompanied by significant inhibition in

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acetylcholinesterase and detoxification enzymes inside the host (Mahot et al., 2025). Certain vegetable oils, particularly soybean oil, enhance conidial germination and mycelial growth for *B. bassiana*. Recently, Karami et al. (2022) studied botanical insecticides tested the essential oil of *Thymus vulgaris* and its two main components, thymol and carvacrol, against the mulberry pyralid *Glyphodes pyloalis*. Higher toxicity could be expressed in terms of LD_{50}= 2.82 µg per larva for the oil, which also manifested drastic changes in detoxifying enzyme activities, thus confirming its physiological effect on lepidopteran larvae. More recently, a study by Mousavi et al. (2024) developed a wettable powder formulation from thyme extracts that reported high reductions in survival and development of *Helicoverpa armigera* (Noctuidae) under laboratory and semi-field conditions, there has been growing interest in the bioactivity of Juniper essential oils. Kowalska et al. (2023) evaluated *Juniperus horizontalis* essential oil and its major compound sabinene, reporting considerable larvicidal and antifeedant effects against *Tenebrio molitor* larvae. More recently, Trandafir et al. (2022) confirmed that juniper oils display both toxic and repellent activities towards various insect pests. However, data on Lepidoptera is still very much limited. An apparent gap in research persists regarding the integration of *B. bassiana* with thyme and juniper oils against *Ectomyelois ceratoniae* under field conditions, as most available studies have been conducted under laboratory conditions, primarily on other host lepidopteran species. Little is known about (1) possible synergistic or antagonistic interactions among the above agents, (2) the influence of formulation and environmental factors on persistence and efficacy, and (3) non-target and ecological effects in orchard ecosystems. Knowledge generated from such a study can help develop an integrated pest management (IPM) strategy for pomegranate fruit moth in Iraq. Advances in encapsulation and formulation (Abada et al., 2019) further enhance the efficacy of botanicals. The present study aims to (1) describe the seasonal activity of *E. ceratoniae* in Ain al-Tamr and (2) evaluate the efficacy of *B. bassiana*, thyme, and juniper oils, and their combinations compared to imidacloprid, under field conditions.

Materials and Methods

Study Area

The study was conducted in pomegranate orchards in Ain al-Tamr District, Karbala Governorate, Iraq. Coordinates of Ain al-Tamr approximate centroid: 32.3906°N, 43.5607°E. (Figure 1) map of Karbala Governorate highlighting Ain al-Tamr and orchards.

Figure 1. Map showing Karbala Governorate and the location of Ain al-Tamr study orchards (Ayn al-Tamr), with indicated orchard cluster locations and experimental blocks.

Timeline

Seasonal monitoring (pheromone traps) was performed from 15 May 2024 to 15 September 2024 to capture the active adult flight period. Field treatments for control trials were applied in 2025, with the first spray on 7 June 2025 and the second spray 30 days later (7 July 2025). Weekly monitoring (trap counts and fruit infection assessments) continued for 21 days after each spray.

The monitoring year (2024) established baseline flight phenology; treatments were implemented in the subsequent season (2025), when infestations were expected based on the 2024 phenology.

Experimental Design and Treatments

A randomized complete block design with three replicates was used. Each replicate plot consisted of 5 contiguous trees (this is the experimental unit). Treatments were assigned at the plot level. Total trees per treatment= 3 orchards × 3 replicates/orchard × 5 trees per replicate= 45 trees per treatment.

The treatments are: 1. Control: distilled water spray (DW); 2. *Beauveria bassiana* (commercial formulation) - 30 g.16 L^{-1} distilled water; spray volume 6 L per tree (details below). Provide manufacturer and strain - currently recorded as: *Beauveria bassiana* commercial product, [PLACEHOLDER: product name and manufacturer]; 3. Thyme oil 7% (v/v): prepared by mixing 7 mL thyme oil.100 mL^{-1} of carrier [study mixed 3 mL with 93 mL DW to make 7% working solution per tree, this replicates the field method; scaled to sprayer volume as below]. 4. Juniper oil 7% (v/v). 5. *B. bassiana* + 7% thyme oil (synergistic application: fungus applied first, then oil spray 3 days after). 6. *B. bassiana* + 7% juniper oil (exact timing as

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above). 7. Chemical control: Imidacloprid (Imidor 200 SL) at 125 ml.100 L^{-1} (recommended dose).

Application method and calibration

A 16-L knapsack sprayer was used. For calibration, sprayer output was measured to apply 6 L per tree. The sprayer was calibrated by spraying a known area and measuring the volume of liquid used. For each tree, the spray solution volume and concentration were prepared so that each tree received 6 L of the working solution.

For *B. bassiana* treatment, 30 g commercial powder [or ready formulation] mixed in 16 L distilled water as per manufacturer's instructions. Trees were sprayed from the bottom up to ensure coverage of fruit cups and the lower canopy.

Data Analysis

Data was tested for normality and homoscedasticity (Shapiro-Wilk and Levene tests). Where necessary, the number of males per trap was determined using a square root or log(x+1) transformation; infestation rates were determined using an arcsine square root transformation before analysis of variance. A two-way ANOVA (treatment × time) was performed on the number of traps and infestation rates. Differences between treatment means were compared using Tukey's HSD (α=0.05). All statistical tests were performed using SAS 2010 (SAS Institute, 2012), and graphs were generated using Excel (or R). ANOVA statistics reported include F, degrees of freedom (df), and p-value.

Results and Discussion

Seasonal Activity of E. ceratoniae Adult Males

The monitoring of *Ectomyelois ceratoniae* in pomegranate orchards of Ain al-Tamr, Karbala Governorate, from May 15, 2024, to September 15, 2024, revealed clear seasonal fluctuations in adult male populations.The first signs of regular flight activity were observed in early May, with 7.13 males per trap at an average temperature of 24.76°C and relative humidity of 32.3% (Table 1). Populations gradually increased and reached a peak in mid-August, with 35.15 males per trap, coinciding with an average temperature of 35.85°C and relative humidity of 20.47% (Table 1), before declining toward the end of the monitoring period. These results align with previous studies reporting that *E. ceratoniae* populations peak during the warmest months when fruits mature and sugar content increases, providing optimal conditions for larval development (Yaghobi et al., 2020; Liu and Zhang, 2025).

Treatment Efficiency in Controlling E. ceratoniae

First application

The efficacy of different treatments was evaluated through weekly monitoring of both infestation rates and male attraction to pheromone traps. The combined application of *Beauveria bassiana* and 7% thyme oil resulted in the lowest infestation rate of 3.3% (Table 3), comparable to that of the chemical insecticide Imidor, which also achieved a 3.3% infestation rate. Treatments with *B. bassiana* alone or juniper oil recorded slightly higher infestation rates, 10% and 5.6–7.1%, respectively, yet still significantly lower than the control (55%) (Table 3). Male attraction to pheromone traps (Figure 3a) decreased similarly

Table 1. Effects of temperature and relative humidity on the 2024 seasonal population of *E. ceratoniae* males attracted to pheromone traps

Date	Temperature average (°C)	Relative humidity (%)	No of *E. ceratoniae* males
2024/5/15	24.76	32.3	7.13
2024/5/30	29.91	20.38	10.31
2024/6/15	34.84	13.82	14.34
2024/6/30	37.16	17.81	17.41
2024/7/15	37.68	15.16	23.12
2024/7/30	36.67	16.85	28.24
2024/8/15	35.85	20.47	35.15
2024/8/30	36.95	21.27	25.64
2024/9/15	34.103	20.1	8.25
Average	34.213	19.79	18.84

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under the combined treatment, with only one male per trap, compared to 25 males per trap in the untreated control plots.

The observed effects can be explained through the mechanisms of action of the biological and botanical agents used. *B. bassiana* infects insect hosts by penetrating the cuticle and proliferating within the hemocoel, producing lethal enzymes and secondary metabolites that disrupt insect physiology (Figure 4c) (Aziz et al., 2014; Demirel, 2025).

Beauveria bassiana is a contact fungus. Its conidia attach to the host cuticle, germinate, and develop appressoria, which penetrate through the exoskeleton. The fungus secretes hydrolytic enzymes, chitinases, and proteases, which break down the insect's exoskeleton, allowing the fungus to invade the hemocoel. Inside the hemocoel, *B. bassiana* multiplies, consuming the host tissues while releasing toxic metabolites, which ultimately result in the death of the insect (Amri et al., 2014). This process involves the production of ROS together with other secondary metabolites that interfere with cellular functions, leading to apoptosis of host cells. For instance, in the case of the tomato, *B. bassiana* perturbs key molecular pathways related to primary and secondary metabolism, as well as growth and defence-related processes, thus indicating a complex interaction between the fungus and its host (Proietti et al., 2023).

Thyme essential oils, particularly those rich in thymol and carvacrol, manifest insecticidal activity primarily by breaking down the cuticle and cell membranes of the targeted insect pests. This, therefore, increases permeability, which later results in an outward flow of cellular contents. Thyme oil also results in the overexpression of heat shock proteins (HSPs) and antioxidant enzymes within *E. ceratoniae*; thus, these responses indicate oxidative stress within the insect, with damage (Figure 4b) being minimized by protective mechanisms. Most essentially, through fumigant toxicity research on thyme essential oil against different life stages, including eggs, larvae, and adults of *E. ceratoniae,* substantial reductions in survival were observed at all tested levels, indicating potential as a biopesticide for this pest (Farahani and Bandani, 2023).

Juniper essential oils exhibit antimicrobial and insecticidal properties due to their unique chemical composition. The major compounds are α-pinene and sabinene, among others. These constituents disrupt the nervous system of insects, paralyzing them and eventually leading to their death. Studies have shown that juniper oils possess antioxidant activities, which may contribute to their insecticidal properties by causing oxidative stress in pests. Other researchers have discovered that juniper essential oil can inhibit the growth of plant pathogenic fungi, indicating a broad spectrum of activity that can be utilized in integrated pest management (IPM) strategies (Wu et al., 2022).

The mode of action between *B. bassiana*, thyme, and juniper essential oils on *E. ceratoniae* has been individually studied. More research is required to fully understand their interaction when combined in mixtures or applied simultaneously to the target pest. The effects of possible synergistic as well as potential antagonism between *B. bassiana* and plant-derived essential oils used for integrated pest management should be investigated through environmental effect/ non-target biocontrol agent studies, which will enable sustainable applications in agriculture.

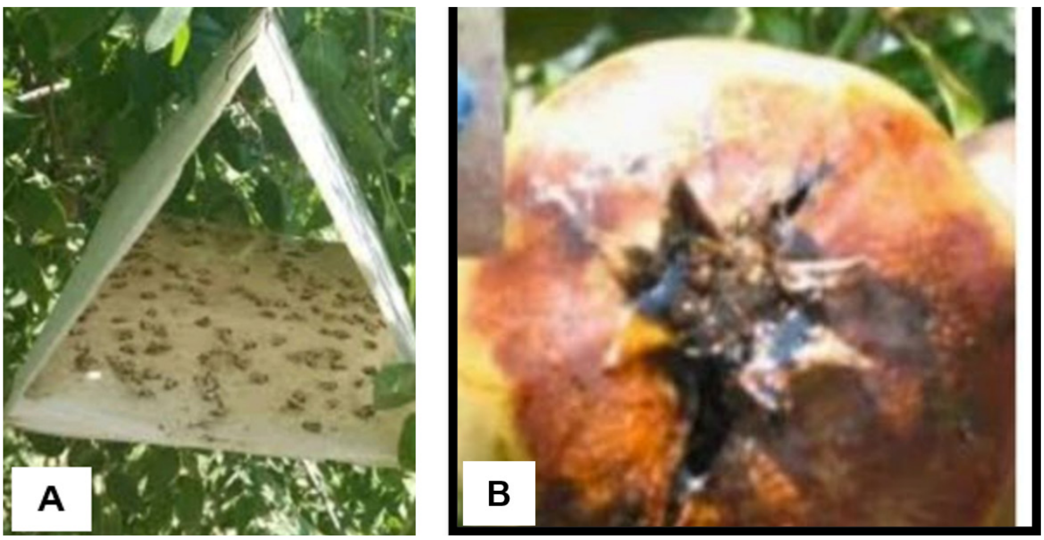

Figure 3. Pheromone trap (A), damage symptoms on pomegranate fruits (B)

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Thyme oil contains thymol and carvacrol, which act as neurotoxins and feeding deterrents, interfering with larval development and oviposition (Abada et al., 2019; Verdi et al., 2019). Juniper oil exhibited a moderate effect, likely due to its lower concentrations of bioactive terpenoids. Yet, it still contributed to reducing pest populations when used as part of an integrated approach (Semerdjieva et al., 2021).

The integration of *B. bassiana* with thyme oil demonstrates the synergistic potential of combining biological and botanical control measures, yielding results comparable to those of chemical insecticides while providing environmental and economic benefits. This integrated approach reduces chemical residues on fruits, minimizes environmental contamination, and potentially lowers production costs (Abbas and Al-Khazraji, 2025; Hamad et al., 2024). However, limitations include the study being conducted at a single location and for only one growing season, limiting broader generalization. Additionally, statistical post-hoc analysis is required to confirm that *B. bassiana* + thyme oil is truly equivalent to Imidor, despite identical numerical results (Table 3). Future research should involve multi-site, multi-season studies and economic assessments to

Table 2. *E. ceratoniae* infection rate and male population affected by the first run application with the individual and interacting experimental factors

Treatments	7 DPA		14 DPA		21 DPA	
	Infection rate %	No. of trapped males	Infection rate %	No. of trapped males	Infection rate %	No. of trapped males
B. bassiana	5	2	10	5	13.3	6
7% Thyme EO	5	3	11.6	4	13.3	5
7% Juniper EO	6.6	4	11.6	6	13.3	8
B. bassiana + 7% Thyme EO	10	4	15	7	11.6	7
B. bassiana + 7% Juniper EO	10	3	16.6	5	11.6	7
Imidacloprid	20	2	16.6	4	13.3	6
Control	30	11	40	16	45	20
Average	12.37	4.14	17.34	6.71	17.34	8.42
$L.S.D_{0.05}$	1.84	0.891	2.73	1.731	3.13	2.382

Notes: *Values are means of three replications, DPA is days post-application, and EO is essential oil.

Table 3. *E. ceratoniae* infection rate and male population affected by the 2nd run application with the individual and interacting experimental factors

Treatments	7 DPA		14 DPA		21 DPA	
	Infection rate %	No. of trapped males	Infection rate %	No. of trapped males	Infection rate %	No. of trapped males
B. bassiana	11.6	6	10	6	10	5
7% Thyme EO	13.3	5	10	3	8.3	4
7% Juniper EO	15	7	11.6	7	8.3	3
B. bassiana + 7% Thyme EO	6.6	5	6.6	3	3.3	1
B. bassiana + 7% Juniper EO	6.6	6	6.6	4	5	2
Imidacloprid	6.6	5	5	3	3.3	1
Control	50	22	50	23	55	25
Average	15.57	8	14.25	7	13.31	5.85
$L.S.D_{0.05}$	3.23	2.171	2.82	1.621	2.16	1.214

Notes: *Values are means of three replications, DPA is days post-application; EO is essential oil. Second application.

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validate the sustainability and cost-effectiveness of this IPM strategy. Overall, these results confirm that *E. ceratoniae* populations in Ain al-Tamr exhibit strong seasonal trends, peaking during the warmest months of fruit maturation. The integrated use of *B. bassiana* with thyme oil provides a highly effective, environmentally friendly, and sustainable alternative to chemical insecticides, aligning with modern IPM principles and improving fruit quality and yield.

Conclusions

The pomegranate fruit moth, *Ectomyelois ceratoniae*, exhibits activity in the Ain Tamr district from early May to late September, reaching a population peak in mid-August. Infestation intensity increases as the harvest stage approaches. Effective suppression of *E. ceratoniae* can be achieved through integrated pest management (IPM) strategies, which emphasize the use of biological control agents, such as *Beauveria bassiana*. When combined with vegetable oils, *B. bassiana* demonstrates enhanced efficacy while reducing reliance on synthetic insecticides. The mixture of *B. bassiana* and thyme oil provides control performance comparable to Imidacloprid. Continuous monitoring of moth populations from May to September, particularly during mid-August and harvest periods, is recommended. Adoption of these IPM practices supports sustainable pomegranate cultivation and minimizes environmental risks.

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