



## ORIGINAL ARTICLE

## Influence of Environmental Factors on the Overwintering Survival of *Eurytoma amygdali* Enderlein Larvae in Almond Orchards

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## KEYWORDS

Almond seed wasp;  
Irrigation;  
Overwintering;  
Soil depth;  
Soil texture;  
Survival;  
Temperature

## ABSTRACT

Almond is one of the oldest nut crops and currently holds the largest share in nut production. The almond seed wasp (*Eurytoma amygdali* Enderlein) is a significant pest of almond trees in Iran. Given the importance of this pest and the insufficient information on factors affecting its population dynamics, this study aimed to investigate the environmental factors influencing the survival of overwintering larvae of the almond seed wasp in a region of Bardsir (Kerman province). Several experiments were conducted in this area. In the first experiment, the effect of temperature on the survival rate of the almond seed wasp was examined, revealing that higher air temperatures correlated with increased survival rates ( $96.55 \pm 3.45\%$ ). The second experiment assessed the impact of soil depth (up to 30 cm) on larval survival, finding no significant differences across various depths within this range. The third experiment studied the influence of irrigation on the larvae's survival, indicating that reduced or absent irrigation resulted in lower larval survival rates ( $17.24 \pm 7.14\%$ ), while excessive irrigation increased survival rates ( $55.17 \pm 9.4\%$ ). Finally, the fourth experiment examined soil texture, demonstrating that decomposed animal manure ( $82.76 \pm 7.14\%$ ) plays a critical role in retaining moisture, thereby enhancing the survival of the pest's larvae. These findings provide valuable insights into the environmental conditions that favor *E. amygdali* survival, suggesting potential strategies for pest management in almond orchards.

## Introduction

Almond is one of the oldest cultivated nut crops, currently holding the largest share in nut production (Tehraniifar *et al.*, 2002; Imani *et al.*, 2021). Almond has various uses in the food, industrial, and cosmetic sectors, and its oil is widely utilized in pharmaceutical industry. Annually, thousands of tons of this product are used in the food industry in the form of white almond kernels, slices, powder, and crushed almonds (Tehraniifar *et al.*, 2002; Ansari and Gharaghani, 2019).

The almond seed wasp, *Eurytoma amygdali* Enderlein, is one of the most important pests of almonds. This wasp belongs to the family

Eurytomidae. Some species in this family are parasitic, but most are herbivorous (Izadi and Samih, 2010). Both the almond seed wasp and the pistachio seed wasp (*E. plotnikova*) are significant pests of this family in Iran (Izadi and Samih, 2010). The almond seed wasp is a serious pest of almonds in several countries, including Middle East and central Asian countries (Abdul-Rassoul and Mohammed, 2017; Ibrahim *et al.*, 2008; Khanmohamadi *et al.*, 2019; Zerova and Fursov, 1991), Southeastern European (Skendžić *et al.*, 2021; Haltrich and Viktor, 1998) and Mediterranean countries (Arambourg *et al.*, 1983; Lozano, 2018), as well as the Azerbaijan, Armenia

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Georgia, (Zerova and Fursov, 1991) and Romania (Cioaca *et al.*, 2022). There is a high risk of introduction to countries with almond crops where *E. amygdali* has not yet been reported, such as in the USA (California), Australia (PHA, 2013) and in northern Africa. In Iran, this species can destroy up to 90% of the crop (Mohamadi Khoramabadi and Arzani, 2009). This pest has one generation per year, although a portion of the population may complete its life cycle over two, three, or even four years. (Tzanakais and Veerman, 1994). Female wasps lay their eggs inside almond fruits, and the larvae feed on the almond embryo, completing their growth by mid-summer before entering diapause (Margaritopoulos and Tzanakakis, 2006). Pupation occurs in late winter to early spring, with adults emerging through an exit hole in the almond shell. Within a few days, females begin laying eggs in green, unripe almonds using their long ovipositors (Kouloussis and Katsoyannos, 1991). The newly hatched larvae feed on the kernel and embryo sac (Duval and Millan, 2010). By mid-summer, the larvae reach their full size and enter diapause, remaining inside the damaged almond shell, which often mummifies on the trees (Kouloussis, 2008).

Among climatic factors, temperature has a significant impact on the growth, development, and survival of insects (Mansingh, 1974). In Iran, Damanabi and colleagues (Damanabi *et al.*, 1980) studied the biology of the almond seed wasp in East Azerbaijan. According to their research, the almond seed wasp is a univoltine species (one generation per year), although part of its population, due to diapause, extends its life cycle over two, three, or even four years. The pest overwinters as a fully grown larva inside the almond fruit. Although more than two eggs may be laid within one fruit, no more than two larvae have been reported to reach full development inside a single almond fruit, and usually, only one larva exists within each fruit (Margaritopoulos and Tzanakakis, 2006). The use of animal manure increases the survival rate of the almond seed wasp. Roshandel and

Noorbakhsh (2005) investigated the effect of cold temperatures on the population and infestation rate of the almond seed wasp. Jafari Nodooshan and Shamszadeh (2006) reported that plowing the soil around almond trees can significantly reduce the population of this wasp. Skendžić *et al.* (2021) investigates the biology and ecology of the almond seed wasp, *E. amygdali*, specifically focusing on its behaviors. It analyzes how climate change impacts the overwintering strategies of this pest, emphasizing the importance of understanding these dynamics for effective pest management. In this experiment, the following factors were studied: the survival rate of almond seed wasp larvae at freezer temperatures and in orchard conditions, the survival rate of larvae at different soil depths, the survival rate of larvae under various irrigation conditions, and the survival rate of larvae in soils with different textures.

## Materials and Methods

The experiments were conducted in an almond orchard located 2 kilometers from Bardsir (Kerman province), with geographical coordinates of 56°34' E longitude and 29°55' N latitude, and at an altitude of 2000 meters above sea level. The climate of this region is temperate, characterized by cool and mild summers and cold, snowy winters. The almond variety in this orchard was "Kaghazi" or "Yalda" (Nonpareil) and the soil texture was sandy. The irrigation method used in the orchard was flood irrigation. This research was carried out through several experiments, described as follows:

### **Experiment 1: Investigating the effect of temperature on the survival of almond seed wasp**

To examine the effect of temperature on the survival of the almond seed wasp, infested almond fruits containing larvae were collected from the aforementioned orchard. Groups of ten fruits were placed inside mesh bags, which were well-ventilated and considered as experimental units with ten replications. Based on the region's climatic conditions

and simulating different winter scenarios (warm winter, cold winter, semi-cold winter, and moderate winter), the experiment included the following treatments:

-Orchard Treatment ( $T_1$ ): From November 20, 2022, to December 20, 2022, for 30 days, the bags containing infested almond fruits were randomly hung on the branches of almond trees.

-Freezer Treatment ( $T_2$ ): In this treatment, the bags containing infested fruits were stored in a freezer at  $-18^\circ\text{C}$  for 30 days.

-Freezer-Orchard Treatment 1 ( $T_3$ ): The bags containing infested fruits were stored in a freezer at  $-18^\circ\text{C}$  for 15 days. Then, from December 3, 2022, for 15 days, they were randomly hung on the branches of almond trees.

-Freezer-Orchard Treatment 2 ( $T_4$ ): The bags containing infested fruits were stored in a freezer at  $-18^\circ\text{C}$  for 7 days. Then, from November 25, 2022, for 23 days, they were randomly hung on the branches of almond trees.

After completing the experimental periods, the infested fruits were transferred to the laboratory, and the larvae inside were carefully extracted without damage. The larvae from each treatment were individually placed in separate Petri dishes at room temperature. After they moved and became active, the survival rate of each treatment was calculated.

#### ***Experiment 2: Investigating the effect of soil depth on the survival of almond seed wasp***

This experiment began on December 10, 2022 (the date of the first snowfall of winter). As in the previous experiment, mesh bags containing ten infested almond fruits with almond seed wasp larvae were used. To examine the effect of the soil depth where larvae were placed, three treatments were designed based on whether soil tillage had been performed in the fall:

-Surface Soil Depth ( $T_1$ ): In this treatment, ten bags containing infested almond fruits were randomly placed on the surface of the orchard soil. The bags remained until the first almond blossoms opened, and

then they were transferred to the laboratory. On March 16, 2023, the survival rate of each treatment was calculated using the method from the previous experiment.

-15 cm Soil Depth ( $T_2$ ): In this treatment, the bags were placed in a 15 cm deep pit in random parts of the orchard, covered with soil. The survival rate calculation was done as in the previous treatment.

-30 cm Soil Depth ( $T_3$ ): This treatment followed the same procedure as  $T_2$ , but the bags were buried at a depth of 30 cm.

#### ***Experiment 3: Investigating the effect of irrigation on the survival of almond seed wasp***

To investigate the effect of rainfall and irrigation on the pest's survival, infested almond fruits with larvae were collected from the orchard on December 10, 2022, and placed ten per plastic pot at a depth of 5 cm. These pots were well-ventilated and considered experimental units. The treatments were as follows:

-No Irrigation and No Rainfall ( $T_1$ ): Ten pots containing infested almond fruits were covered with orchard soil, and no irrigation was applied. The pots were placed in a section of the orchard where they would not be exposed to rainfall. The pots remained until the first almond blossoms opened, and on March 16, 2023, the survival rate was calculated.

-No Irrigation but Rainwater ( $T_2$ ): In this treatment, no irrigation was applied, but the pots were placed where they could receive rainwater. The survival rate was calculated similarly to  $T_1$ .

-Irrigation and Rainwater ( $T_3$ ): This treatment followed the same procedure as  $T_2$ , but irrigation was applied according to the orchard's irrigation schedule, and the pots also received rainwater.

#### ***Experiment 4: Investigating the effect of soil texture on the survival of almond seed wasp***

For this experiment, starting on December 10, 2022, plastic pots containing infested almond fruits with larvae were used. To examine the effect of soil texture where the infested fruits were placed, three

treatments were designed as follows:

-Orchard Soil ( $T_1$ ): Ten pots were filled with soil from the orchard. The pots remained until the first almond blossoms opened, and then they were transferred to the laboratory. On March 16, 2023, the survival rate was calculated using the method from the previous experiments.

-Sandy Soil ( $T_2$ ): The pots were filled with fine sand. The survival rate was calculated similarly to the previous treatments.

-Decomposed Animal Manure ( $T_3$ ): This treatment followed the same procedure as  $T_2$ , but the pots were filled with a mix of decomposed animal manure (an equal mixture of various livestock manures).

### Data analysis

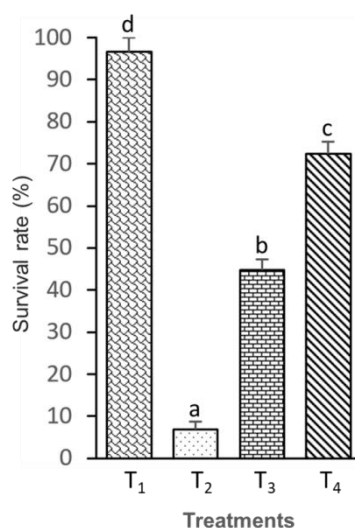
The statistical analyses of the experiments in this study were conducted using the Stat Plus (2009) statistical software. The data analysis process was divided into two stages: Calculation of Means and Identification of Differences: In the first stage, the means of the obtained data from each experiment

were calculated in order to identify any differences between the means. Determining Significant Differences: In the second stage, the significant differences between the means were determined at a 5% confidence level ( $p < 0.05$ ). To compare the obtained means from the experiments, a One-Way ANOVA was used. To examine the significant differences between experimental groups, the Fisher's LSD test was employed at a 5% probability level.

### Results

#### *Experiment 1 (Effect of temperature on pest larvae survival)*

The results obtained from the experiment showed (Fig. 1) that the highest survival rate of the almond seed wasp pest was observed in the garden treatment ( $T_1$ ) ( $96.55 \pm 3.45\%$ ), while the lowest survival rate was found in the freezer treatment ( $T_2$ ) ( $6.9 \pm 1.79\%$ ). A comparison of the means indicated that there were significant differences between all treatments ( $P \leq 0.006$ ).



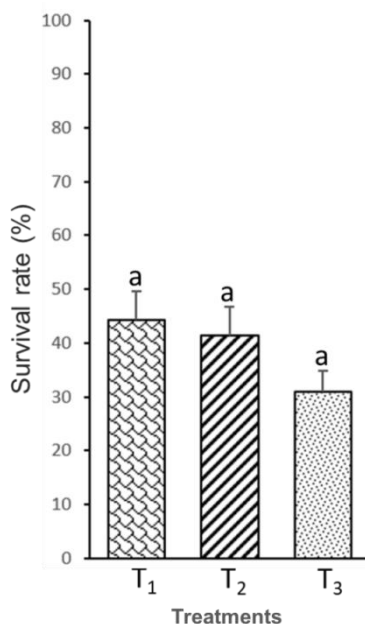
**Fig. 1.** The effect of ambient temperature on the survival of the almond seed wasp pest.  $T_1$ : Orchard treatment,  $T_2$ : Freezer treatment,  $T_3$ : Freezer-Orchard treatment 1,  $T_4$ : Freezer-Orchard treatment 2.

#### *Experiment 2 (Effect of soil depth on pest larvae survival)*

*survival)*

The results obtained from the experiment showed (Fig. 2) that although the survival rate decreased with increasing depth of almond fruits containing larvae

placed in the soil, soil depth did not have a significant effect on the survival rate of the almond seed wasp pest larvae.



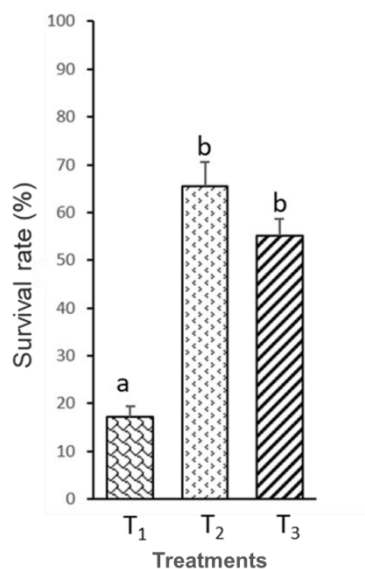
**Fig. 2.** The effect of soil depth on the survival of almond seed wasp larvae. T<sub>1</sub>: Zero depth-soil surface treatment, T<sub>2</sub>: 15 cm soil depth treatment, T<sub>3</sub>: 30 cm soil depth treatment.

**Experiment 3 (Effect of irrigation on pest larvae**

**survival)**

The results obtained from the experiment showed (Fig. 3) that the highest survival rate of the almond seed wasp larvae was observed in treatment T<sub>2</sub> (65.52±4.98%), while the lowest survival rate was noted in treatment T<sub>1</sub> (17.24±2.14%). The results

indicated that treatment T<sub>1</sub> had a significant difference compared to treatments T<sub>2</sub> and T<sub>3</sub> ( $P \leq 0.0024$ ). However, there was no significant difference between treatments T<sub>2</sub> and T<sub>3</sub>.

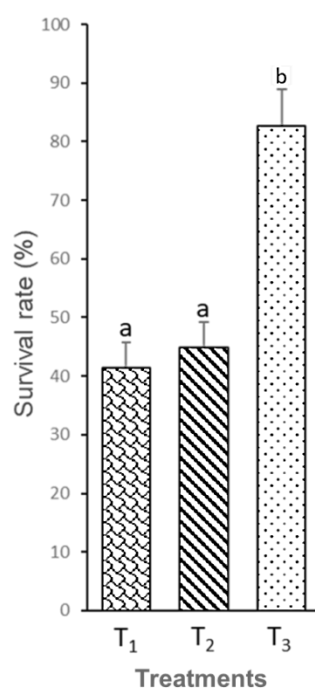


**Fig. 3.** The effect of irrigation on the survival of almond seed wasp larvae. T<sub>1</sub>: No irrigation and no rainfall treatment, T<sub>2</sub>: No irrigation but rainwater treatment, T<sub>3</sub>: Irrigation and rainwater treatment.

**Experiment 4 (Effect of soil texture on pest larvae****survival)**

The results obtained from the experiment showed that the highest survival rate of the almond seed wasp larvae was observed in treatment T<sub>3</sub> (82.76±6.14%), while the lowest survival rate was noted in treatment

T<sub>1</sub> (41.38±4.31) (Fig. 4). Comparison of the means indicated that treatment T<sub>3</sub> had a significant difference compared to treatments T<sub>1</sub> and T<sub>2</sub> ( $P \leq 0.0027$ ).



**Fig. 4.** The effect of soil texture on the survival of almond seed wasp larvae.

T<sub>1</sub>: Orchard soil treatment, T<sub>2</sub>: Sandy soil treatment, T<sub>3</sub>: Decomposed animal manure treatment.

**Discussion**

Based on the results obtained from this experiment, temperature can significantly affect the survival of almond seed wasp larvae. Specifically, as the temperature decreases (colder weather-cold winter), the mortality rate of the mentioned pest larvae increases. Consequently, a reduction in temperature leads to a decrease in the population of adult almond seed wasps for the following year. The results of this experiment align with those of a study conducted by Roshandel and Noorbakhsh in 2005, which concluded that cold affects the population and infestation levels of almond seed wasps, consistent with the findings of this research. Khanmohamadi colleagues (2016) demonstrated that the almond seed wasp, *Eurytoma amygdali*, as a univoltine pest, spends nearly nine

months of the year in diapause at the last larval stage inside damaged fruits. In this study, changes in simple sugars, lipids, proteins, glycogen, trehalose, glucose, supercooling points (SCP), and cold hardiness were measured in the diapausing larvae. The results indicated that as glycogen levels decrease and simple sugars and sugar alcohols increase, the larvae's cold tolerance improves. It was also shown that this insect utilizes a "freeze-avoidant" mechanism to survive, as no larvae survived once their body fluids crystallized. However, with the ongoing phenomenon of global warming, characterized by rising temperatures and milder winters, the implications for pest populations become concerning. Warmer winters may not only reduce the mortality rates of almond seed wasp larvae

but also enable these pests to thrive and reproduce more successfully. This could lead to higher populations of almond seed wasps and increased infestation levels in the following growing seasons. The alignment of these findings with the research by Roshandel and Noorbakhsh (2005) underscores the need for a deeper understanding of how climate change affects pest dynamics. The topic of climate change's impact on pest populations, particularly the almond seed wasp, is closely related to the findings of Skendžić and colleagues (2021). In their research, they investigate the biology and ecology of the almond seed wasp in Georgia and analyze how climate change influences its overwintering strategies. As the climate continues to warm, it is essential for farmers and agricultural scientists to adapt their pest management strategies to address the changing behaviors and life cycles of pests like the almond seed wasp.

According to the results of this study, soil depth ranging from the surface to a depth of 30 centimeters had no effect on the survival of almond seed wasp larvae. Therefore, it can be stated that tilling the soil to this depth, provided it does not damage the fruit containing the larvae, does not effectively control the mentioned pest. Jafari Nodooshan and Shamszadeh reported in 2006 that tilling under trees could significantly reduce the population of this pest, which contradicts the results of this research. The differing results between this study and the findings of Jafari Nodooshan and Shamszadeh (2006) regarding the impact of soil depth on the survival of almond seed wasp larvae can be attributed to several factors:

- 1- Experimental Conditions: Variations in experimental settings, such as geographical location, climate, and soil composition, can lead to different outcomes. If Jafari Nodooshan and Shamszadeh conducted their research in a region with distinct environmental conditions or different agricultural practices, this could explain the disparity in results.
- 2- Methodology: The specific methodologies employed in each study may vary, including the techniques used for tilling,

the timing of the experiments, and how the larvae were assessed for survival. Differences in how experiments were designed and conducted can significantly influence the results.

- 3- Temporal Factors: Agricultural practices and pest dynamics may change over time due to evolving farming techniques, pest resistance, or climate change impacts. Thus, findings from earlier studies may not be applicable to current conditions. In conclusion, the differences in results highlight the complexity of pest management and the need for further research that considers various environmental and methodological factors. Understanding these nuances is crucial for developing effective strategies for controlling pests like the almond seed wasp in diverse agricultural contexts.

The findings of this research indicated that soil moisture, resulting from irrigation, rainfall, and soil texture, plays a significant role in the successful overwintering of pest larvae. This is due to the fact that environmental dryness reduces the moisture content of the larvae's bodies, leading to their death. Therefore, environments with decomposed animal manure, due to adequate moisture, increase the survival and persistence of the mentioned pest. These results are consistent with findings from a study by Damanabi *et al.* in 1980, which examined the biology of the almond seed wasp in East Azerbaijan. According to their research, the almond seed wasp is a univoltine species, with a portion of its population extending its life cycle through diapause, completing its life cycle in two, three, or even four years. Its overwintering occurs in the larval stage within the almond fruit. Although more than two eggs may be laid within a single fruit, no more than two larvae have been reported to reach the final developmental stage within one fruit, with usually only one larva present in each fruit. The use of animal manure enhances the survival of almond seed wasps.

Several studies have shown that environmental factors significantly impact the survival of pests, including *E. amygdali*. In our experiments, it was observed that the survival rate of this pest's larvae in

typical orchard conditions—considering temperature, humidity, and soil texture—ranged between 40% and 50%. This finding aligns with previous research indicating that temperature and moisture levels are critical determinants of larval survival in various agricultural systems. Moreover, the ability of environmental conditions to impose substantial mortality on pest populations has been documented in other pest species, where adverse weather conditions have been linked to increased larval mortality. Our results suggest that managing these environmental factors could be crucial in controlling the population of *E. amygdali* and potentially minimizing damage to almond orchards. Given the results of this research, in addition to understanding the factors affecting the successful overwintering of larvae and predicting pest populations for the following year, it is possible to employ practical agricultural methods within an integrated pest management framework. The approach of collecting and destroying infested fruit is highlighted as a key method for controlling the survival of overwintering larvae of the almond seed wasp (Nourbakhsh, 1998). This practice is effective because it directly removes the source of the pest's survival and reproduction, thus minimizing its impact on future crops. Research indicates that proper sanitation and management practices, such as removing and destroying affected almonds, significantly reduce pest populations and can be part of an integrated pest management (IPM) strategy.

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### Conflict of interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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