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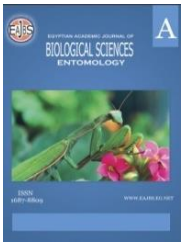

Cold Incubation Effect on The Development of *Bactrocera zonata*. (Saunders), (Diptera:Tephritidae) Stages Under Laboratory Conditions at Fayoum Governorate.

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ABSTRACT

A laboratory experiment was designed to evaluate different incubation periods for the development of *Bactrocera zonata*. Larvae of the same instar were subjected to cold incubation periods for 1-15 days as treatments at 5 ± 1 °C and 50% R.H. The periods to complete their life cycle after cold treatments were counted to detect the coldest period affecting the survival of *B. zonata***.** Results revealed that, compared to the control which completed their life cycle normally, all treatment periods did not affect the life cycle of the cold-treated insects except those of the 9th-day treatment which produced about half of the population, while the treatments from 13th-15th days resulted in the survival of the highest population of insects completing their life cycle. Also, it was noticed that, the preassociativity of *B. zonzta* in the orchards during January month at Fayioum through hanged both of pheromone and nutritive traps.

INTRODUCTION

Most orchards are subjected to the invasion with different pests mainly the polyphagous species *Bactrocera zonata*. (Saunders), (Tephiridae). Which is the most serious pest as it invades more than 50 host families like Mango (*Magifera indica*), peach (*Prunus persica*), guava (*Psidium guajava*) and citrus fruits Fletcher (1987); White and Elson (1992); Allwood *et al.* (1999), Hashim *et al.* (2004). The females deposit their eggs in the mature fruits, which develop into larvae feeding internally on fruit tissues causing decomposition of the fruits thus, yielding great economic losses Hallman et al. (2013). Nowadays, data concerning the presence of that invasive pest under the global climate changes. So, the authors hypothesized that *B. zonata* could be affected by weather changes mainly in the winter season at Fayoum Governorate, Egypt, which is characterized by special topography and a variety of host agricultural and horticultural crops. The idea of this work came from previous notifications by the authors when they notified the survival and development of many fully grown pupae came from samples of fallen guava fruits brought from certain fields and they were kept in the incubator. So, the authors designed an

experiment to detect the impact of the cold incubation of *B. zonata* for different periods on the suitability of that invasive pest under low temperatures to minify its presence in the winter season at Fayoum Governorate, aiming at, the early warning for farmers from that serious pest under the cold weather and its emergence to strong individuals invading the successive hosts, also, to be as a loud sound for the early facing and controlling that pest by means of an IPM strategy to prevent its spreading to the successive hosts. Earlier works by Back and Pembertan (1916) evaluated the cold treatments on fruit flies and proved that no tested eggs or larvae of fruit flies survived in refrigeration for 15 days. Eckenrode *et al.* (1975) reported that the thermal units had been used to predict the seasonal development and emergence of various insects. Previous studies were conducted on the impacts of cold incubation on *Bactrocera zonata* for different purposes as those of Younes and Akel (2010); Follett and Snook (2013); Hallman *et al.* (2011 and 2013) and Abd El-Maaboud *et al.* (2018) with different obtained results. The study of Zingore *et al.* (2020) suggested that the current global climatic changes may affect the invasion of *B.zonata* for horticultural crops, which has a negative effect on agricultural productivity, and as little is known regarding the effect of current climatic changes on *B.zonata*. So, this work was conducted to evaluate the impacts of cold incubation for certain periods on *B.zonata* development aiming at the prediction and the early warning of that serious pest threatening fruit production.

MATERIALS AND METHODS

This work was carried out at the laboratory of Trichogramma Mass-Rearing at Fayoum Governorate, Plant Protection, Research Institute, Agricultural Research Center, Egypt from (December 2022 to January 2023). Treatments were conducted simulating field conditions as the elder larvae and pupae of *B.zonata* may spend different periods subjected to cold weather. So, the experiments were designed aiming at the evaluation of cold incubation on the developmental stages of *B. zonata* mainly the larval stage in infested fruits. Fallen and infested guava fruits from the citrus orchard at Harfoush District-Fayoum Governorate were collected and transferred to the laboratory in paper bags, then separated for the full-grown larvae of *B. zonata* which were the basement of the experiments. Larvae were subjected to cold incubation at 5 ± 1°C and 50% RH for 1-15 days in the incubator as treatments (Where the lowest minimum temperature was recorded during the months of December 2022 and January 2023, 5-8°C, according to the Egyptian Meteorological Authority). After each cold incubation period, the treated larvae were transformed to the standard rearing conditions and maintained until the next development stage. Daily inspection and count for live and dead larvae and to record the time needed to the next stage of *B. zonata* larvae were reared under normal laboratory conditions.

Experimental Techniques:

For each treatment (1-15 cold incubation days) which was repeated three times, about 90 larvae/ for each treatment were settled in small boxes kept at 5 ± 1°C and 50% RH. The treated larvae of *B. zonata* were inspected immediately after each incubation period by direct examination under laboratory conditions, then they were put in large transparent boxes bottomed with a thin layer of fine, sterilized sand for the pupation stage. Then separation for pupae was accomplished and put in separate jars and the developmental rate was calculated successively. The developmental pupae transferred from cold-treated parents' larvae were kept in clean jars bottomed with a thin layer of sterilized sand and covered with a thin layer of cotton cloth tied with rubber and were maintained at the normal rearing conditions of the laboratory till the adult's emergence. The mortality, emerged adults and emergence rate were recorded successively.

The incubation was carried out at the lowest minimum temperature of 5°C, as the appropriate temperature for the growth of larvae was 12 - 15 °C. The larvae were removed from the incubator one treatment after another to monitor the emergence of the adult insect after exposure to the low temperature for a period of one to 15 days.

Traps Preparation:

Pheromone and nutritive traps were hanged according to evolution protocols in a citrus farm-sized one feddan located at Harfoush District, Fayoum Governorate on 1st January 2023. Both traps were hunged to detect the presence activity of *B. zonata* in the orchard fields during the coldest month of the year.

Data Analysis:

Estimating the developmental rates of pupae and adults reflected the cumulative survival of cold-treated larvae to pupae and adults from refrigeration. It was the main criteria used to evaluate the impacts of cold incubation on *B.zonata* development. The periods of development to pupae and adults were counted.

The percentage of developed Pupae was calculated as the number of developed pupae vs. thenumber of cold-treated larvae x 100.of adults' emergence was calculated as the number of emerged adults vs. the number of cold-treated larvae x 100

All data were compared to the control. Calculate the corrected efficacy percentage according to Abbott, (1925).

Statistical Analysis:

Analysis of variance (ANOVA) was used and Duncans multiple range Duncan test (1955) was used formeans separation Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Pupae Development:

Presented data revealed that *B. zonata* larvae subjected to cold incubation for 1, 3, 4, 5, 6, 7, 12,13, 14 and 15 days were similar to controls in development to the next stage indicating 100% pupae development. Followed by the treatment of 2nd - and 8th-day cold incubation indicating 96.66% and 93.3% pupae development. The treatment after 9 days of cold incubation resulted in the least pupae development indicating 53.33%. Figure (1) illustrates that, this may be due to the fact that the first instar larvae and the new hatchlings (eggs) were affected by the decrease in temperature.

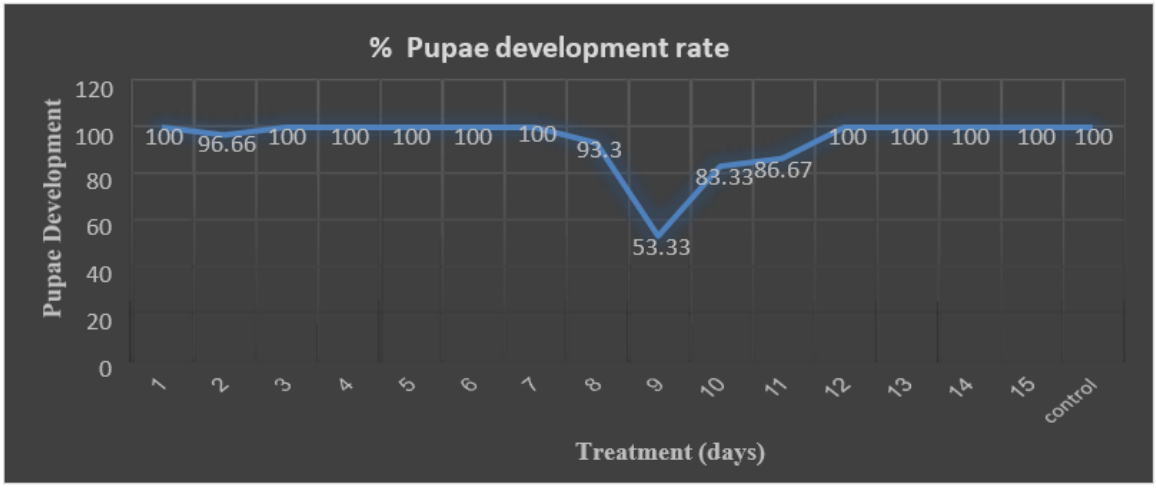

Fig. 1: *B.zonata* Percentages Pupae development after cold incubation treatments and control.

The time needed for development to the pupae stage differed significantly. Compared to controls which turned to full pupae in 5th days, treatments of 1st, 2nd and 10th days cold incubations were similar to control with the average of 5th days. The longest period counted for pupae development was with treatment of 9th days cold incubation as it counted after 10 days. The last period for pupae development averaged only one day and was counted with treatments of 12, 13, 14 and 15 days of cold incubation. **Figure (2)** illustrates that, number of *B. zonata* pupae development days after cold incubation treatments.

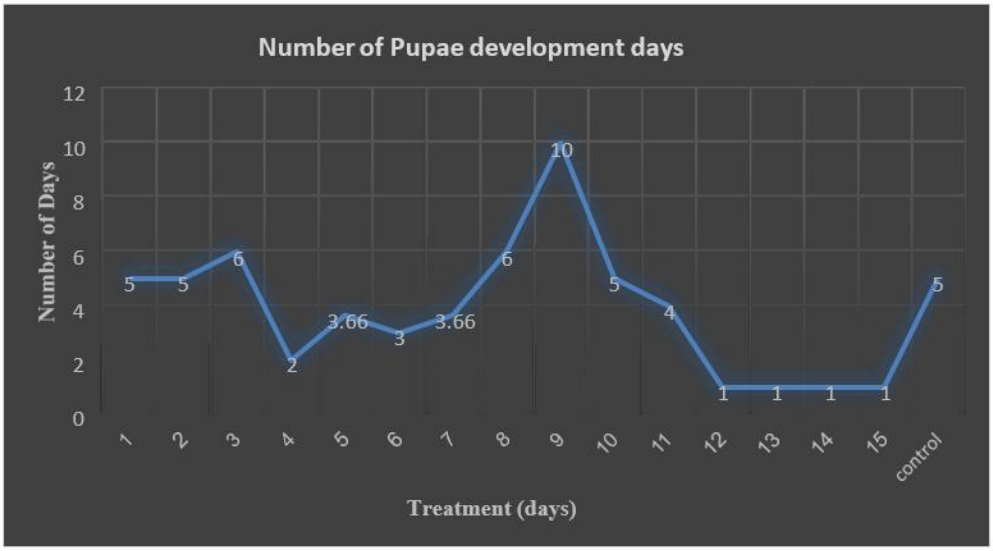

Fig. 2: *B.zonata* Numbers Pupae development days after cold incubation treatments and control.

Adults' Emergence:

Data in Figures (1 and 2) illustrated that It was clear from the results data compared to control emerged *B.zonata* flies from pupae stage indicating 96.66%, the treatments of 2nd and 3rd cold incubation days were similar to controls averaging 96.66% for both treatments. Treatments of 1st, 4th, 5th, 6th, 7th, 12th, 13th, 14th and 15th cold incubation days resulted in adult emergence indicating 100% adults. The obvious effect of cold incubation was recorded with treatment of 9th day cold incubation indicated 53.33% as shown in Figure (3). This may be due to the insect's adaptation to climate change and the emergence of generations resistant to temperature differences, whether low or high.

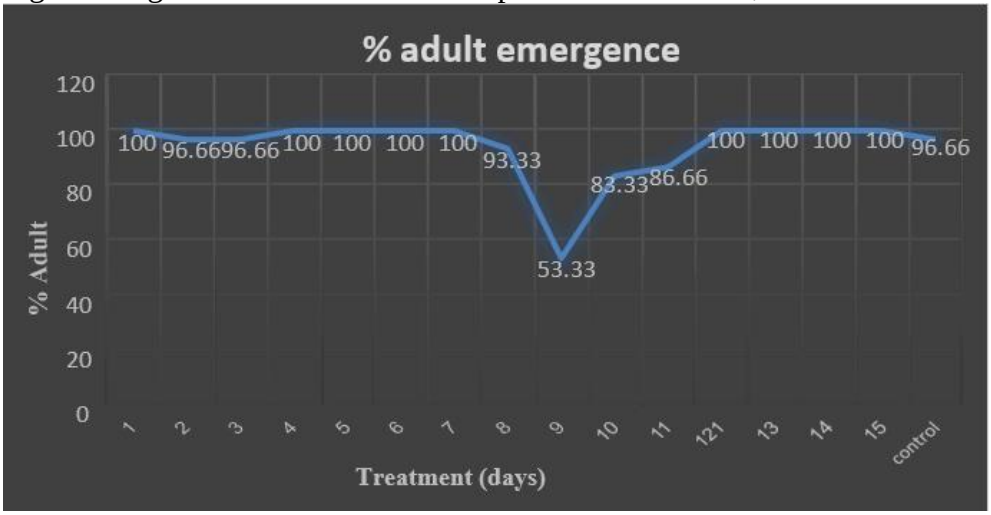

Fig.3: *B.zonata* Percentage adults emergence after cold incubation treatments and control.

The periods of adult's emergence from pupae differed significantly between treatments, compared to adults' control which emerged after 11.5 days of pupae stage, treatments of 1- and 10-days cold incubation resulted in adult emergence after 11 days of pupae stage and it was close to the controls. The longest periods to emerge, adults, were recorded with the treatments of 6- and 7-day cold incubation as they averaged 19 and 17 days respectively. The last period for an adult's emergence after the pupae stage was 2 days and it was counted with the treatment of 15 days of cold incubation as shown in Figure (4).

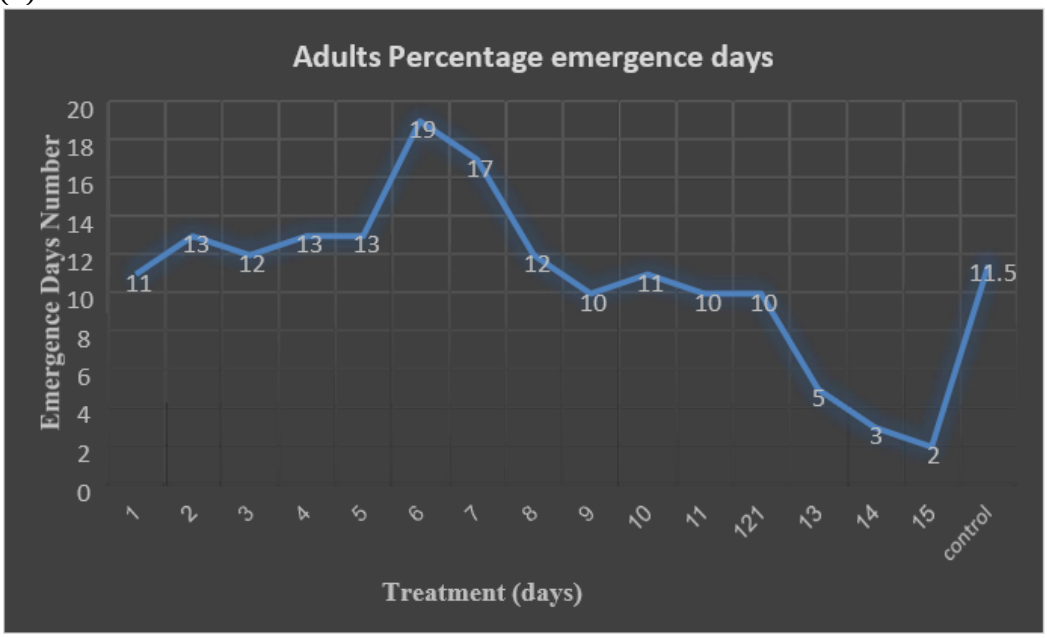

Fig. 4: *B.zonata* numbers adults emergence days after cold incubation treatments and control.

Obtained results in this work revealed that the cold incubation periods of *B.zonata* brought from fields had no to moderate effects on its development except those of 9 days of cold incubation treatment which resulted in the production of about half of the population compared to the control**.** Also, present results revealed that the periods needed to complete the development of the immatures to the next instar differed among the treatments. It must be noted that the laboratory experiments were conducted on field samples to simulate field conditions and to detect what could happen with cold weather aiming at the early prediction and warning of the severe damage caused by that invasive pest in the next season. In addition, our suggestion about *B.zonata* survival after its treatment with cold incubation periods was accomplished by means of the pheromone and nutrition traps were hung on the farm in the coldest months of December 2022 and January 2023 at Fayoum Governorate to be as a monitor for the presence of the peach flies. Both traps proved the presence of complete individuals of *B.zonata* by collecting many individuals from the field. This point is consistence with Zingore *et al.* (2020) when they suggested that the global changing climatic changes could affect the presence of *B.zonata* flies. So, this work was focused on the cold tolerance of that pest after the cold treatments whether in the laboratory or in the fields, the matter demands more attention must be paid by farmers to care of their farms especially the soil beneath the trees by applying the agricultural practices to prevent and suppress that pest by killing the fallen elder larvae going to pupation and pupae found in the soil by applying an IPM strategy, especially with the current climate changes. Present results are consistent with the previous work of Back and Pemberton (1916) who worked on the effect of cold treatments but on another pest, the Mediterranean fruit fly, and they

reported that 3rd instar proved more resistant to cold than 1st and 2nd instar and the 3rdinstar was found alive after refrigeration for 20 days. Many works tested cold treatments on *B. zonata* as those of Hallman *et al.* (2013) who evaluated the impacts of cold treatments for *B. invadens* and *B. zonata* compared with *Ceratitis capitata*, they found that the 3rd instar was the coldest tolerant for the three species, but they concluded that *B. zonata* was not colder tolerant than *C. capitata*. The present results are contrary to those obtained by Follett and Snook (2013) as they reported that, the survival of 3rd instars to the adults' stage generally decreased with increasing the cold incubation duration at 4- 11°C and Abd El-Maaboud *et al.* (2018) who declared that cold treatment of immature stages of *B.zonata* at 1.7°C for 14 days caused mortality for tested larvae. Works of (Ali (2016); Dohino, et al. (2019); Ware *et al.* (2005); Duycket *et al.* (2004); Grout *et al.* (2011); Mohamed (2000); Mohamed and El-Wakkad (2009) and Powell (2003)) evaluated the impacts of cold treatments on Bactrocera immatures with different obtained results which proved the efficacy of cold treatments on immature stages of that pest.

Conclusion and Recommendations:

The experimental data reported in this work revealed that cold treatment had a moderate effect on the development of *B.zonata*. Also, it was undertaken primarily with the hope that it would be an aidin the prediction of the population of *B.zonata* at Fayoum Governorate to face that serious pest invadingmany fruit trees in the following seasons, but whatever its value in this direction it now appears that the results may be of much greater agricultural importance in defining the problem and the agricultural practices accomplished by the farmers themselves to avoid the dispersal of this pest to the alternative hosts. More studies and continuous monitoring and surveys for *B.zonata* mainly with the global climatic changes are required.

The phenomenon of the peach fly's (*B.zonata*) adaptation to low temperatures requires studyingthe DNA to know the physiological changes of the pest. As well as climate changes on pest behavior in theopen field during two years scurvily.

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ARABIC SUMMARY

تأثير التحضين البارد في تطور مراحل *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) تحت الظروف المعملية بمحافظة الفيوم.

مرفت عبد المنعم الجنيدي[1] وأماني نادي صيام[2]

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تم تصميم تجربة معملية بمحافظة الفيوم موسم شتاء 2023/2022 لتقييم فترات الحضانة المختلفة لتطور *Bactrocera zonata*. تم إخضاع يرقات نفس الطور لفترات حضانة باردة لمدة 1-15 يومًا كمعاملات عند درجة حرارة 5 ± 1 درجة مئوية ونسبة رطوبة نسبية 50%. وتم حساب فترات استكمال دورة حياتها بعد المعالجات الباردة للكشف عن الفترة الباردة التي تؤثر على بقاء *B. zonata*. أظهرت النتائج أنه بالمقارنة مع الشاهد الذي أكمل دورة حياته بشكل طبيعي، فإن جميع فترات المعاملات لم تؤثر على دورة حياة الحشرات المعاملة بالبرد باستثناء معاملة اليوم التاسع التي أنتجت حوالي نصف العدد، في حين أن المعاملات من اليوم الثالث عشر- وأدى اليوم الخامس عشر إلى بقاء أكبر عدد من الحشرات مكملة دورة حياتها وقد تبين نشاط للحشرة شهر يناير من خلال تعليق المصائد الجنسية والغذائية للحشرة تحت ظروف الحقل بالفيوم.

الكلمات المفتاحية: ذبابة فاكهة الخوخ *B. zonata* - الحضانة الباردة- التطور- دورة الحياة – محافظة الفيوم.