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Development of the Parasitoid Fly *Drino inconspicuoides* (Diptera: Tachinidae) in the Host *Mythimna separata*: Effect of Temperature and Clutch Size

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Authors' contributions

This work was carried out in collaboration between both authors. Author AK designed the study, wrote the protocol, analyzed the data, managed the literature searches and wrote the first draft of the manuscript. Author SN managed the experimental process. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

The development of the tachinid fly, *Drino inconspicuoides*, a polyphagous and gregarious ovolarviparous parasitoid of lepidopteran larvae was investigated in the laboratory, under different regimes of temperature and varying clutch sizes, using the host, *Mythimna separata*. The objective of this work was to determine the effect of clutch size (number of maggots per host) and varying constant temperatures on development and survival of *D. inconpicuoides* in order to optimize rearing for this species. Development was studied at seven temperature regimes 15, 17.5, 20, 22.5, 25, 27.5 and 30ºC at 60-70% RH and a photoperiod of 16L: 8D. Larval developmental time decreased from 18 days for males, 16 days for females at 15ºC to about 6 days for both male and female at 30ºC. Regardless of the number of maggots laid per host, not more than 8 puparia egressed and pupariated per host. Increasing clutch size resulted in decreased developmental time, reduced puparial weight and reduced emergence/survival of parasitoids. The study provides key information relevant in optimizing rearing procedures for a tachinid parasitoid.

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1. INTRODUCTION

Tachinid flies (Diptera) are a diverse group of insects which live as endoparasitoids of insects and other arthropods. They are abundant in many areas and although not well studied compared to hymenopteran parasitoid wasps, they are suitable for use to control phytophagous insect pests [1]. Tachinids have a wide diversity of host attack strategies and because of their predominance as parasitoids of the larval stage of lepidoptera pests; they are likely to have a wide influence in regulating herbivore populations in natural and artificial ecosystems [2,3]. If their use can be enhanced, they are good alternatives to chemical insecticides in agriculture.

The tachinid, *Drino inconspicuoides* (Baranov), is a polyphagous gregarious parasitoid attacking several economically important lepidopteran larvae. Its host range includes the cabbage cluster caterpillar *Crocidolomia pavonana* [4], the chestnut tiger *Parantica sita niphonica* and the fall web worm, *Hyphantria cunea* [5]. Females lay incubated macrotype eggs directly on the host, which hatch into larvae within two to three seconds and burrow into the host body [6].

Temperature is one of the major biotic factors that influence survival, abundance, distribution, life history and fitness of insects [7,8]. Insect development occurs only within a specific temperature range [9] and also bears a strong influence on growth rate of insects in general [10]. It is therefore essential to understand how temperature influences parasitoid characteristics particularly since this varies from species to species. Such information is also relevant in optimizing conditions during rearing and mass production of biological control agents.

Superparasitism is another big problem in rearing of beneficial insects and is detrimental to a rearing programme. It is important to consider how many eggs a parasitoid especially a gregarious one like *D. inconspicuoides* can allocate to a host since this may have a bearing on the quality (fitness) of the emergent offspring and the parasitoids reproductive success [11,12,13].

In order to develop a rearing programme for any beneficial insects, it is important to understand the key factors essential for each species. Such factors may be reproductive, behavioral, environmental, physiological, nutritional and genetic factors [14]. For *D. inconspicuoides,* some biological characteristics (reproductive) such as mating, oviposition and fecundity have been described [6].

To improve our knowledge on this important beneficial insect, the effect of clutch size and temperature on the developmental time and survival of this gregarious tachinid parasitoid was investigated.

2. MATERIALS AND METHODS

To investigate the effect of temperature and number of maggots per host (clutch size) on the development and survival of *D. inconspicuoides*, a 1-day old (last instar larvae) of the host *Mythimna separata* (Lepidoptera: Noctuidae) was used. *M. separata* is an occasional and usually serious pest of gramineous crops and pastures in Asia, with outbreaks recorded every few years and it is a potential host species for *D. inconspicuoides*. Parasitoids were provided hosts of *M. separata* in cups in a cage (35 x 21 x 28 cm, width x diameter x height). Each larva was enclosed with a single parasitoid fly in a cup. Several groups of host larvae containing 75-200 larvae were placed in fly rearing cages for parasitization. Exposure times ranged from 30 min to 6 h to ensure a large variation in the number of maggots per host. After parasitization, the host larvae were removed from the cages and placed in separate vials (30 ml, plastic), provided with food [15] and incubated under different temperature regimes (15, 17.5, 20, 22.5, 25, 27.5 and 30^{o}C) at 60-70% RH and 16L: 8D photoperiod. At each temperature, the hosts were monitored until the larvae aggressed from the host carcass to pupate. The number of puparia/host (hereby referred to as maggots/host and also known as clutch size) was thereby established.

The puparia were individually weighed (6 days after egression), put in separate plastic vials and returned to the temperature chambers until parasitoid emergence. The sex of emerging parasitoids was also determined. The times from parasitization to egression (larval developmental time), pupation to emergence (pupal developmental time) and parasitization to emergence (total developmental time) were thereby determined.

2.1 Data Analysis

Analysis of variance (ANOVA) procedure mixed (Proc Mixed, [16]) was used to examine main effects (temperature, clutch size) and their interactions on developmental time. Data on numbers of eggs parasitized and adults emerged were log (x+1) transformed to stabilize the variance before being subjected to analysis [17].

3. RESULTS

3.1 Temperature and Developmental Time

Temperature significantly affected larval developmental time (F = 249.52; df = 6, 1700; P<0.0001). Larval developmental time decreased from 18 days for males, 16 days for females at 15ºC to about 6 days for both male and female at 30^{o}C Fig. 1a. Larval developmental time at the different temperatures did not differ significantly between the sexes (F = 0.0; df = 6, 1700; P<0.955) although males aggressed earlier than females.

Pupal developmental time similarly decreased from 34 days for females and 31 days for males at 15^{o}C to about 8 days for both male and female at 30^{o}C (F = 3407.5; df = 6, 1700; P<0.0001) Fig. 1b.

The total developmental period similarly decreased from 50 days for females and 49 days for males at 15^{o}C to about 14 days for male and female at 30^{o}C (F = 104.45; df = 6, 1700; P<0.0001). Males emerged significantly earlier than females at the different temperatures (F = 27.98; df = 6, 1700; P<0.0001) Fig. 1c.

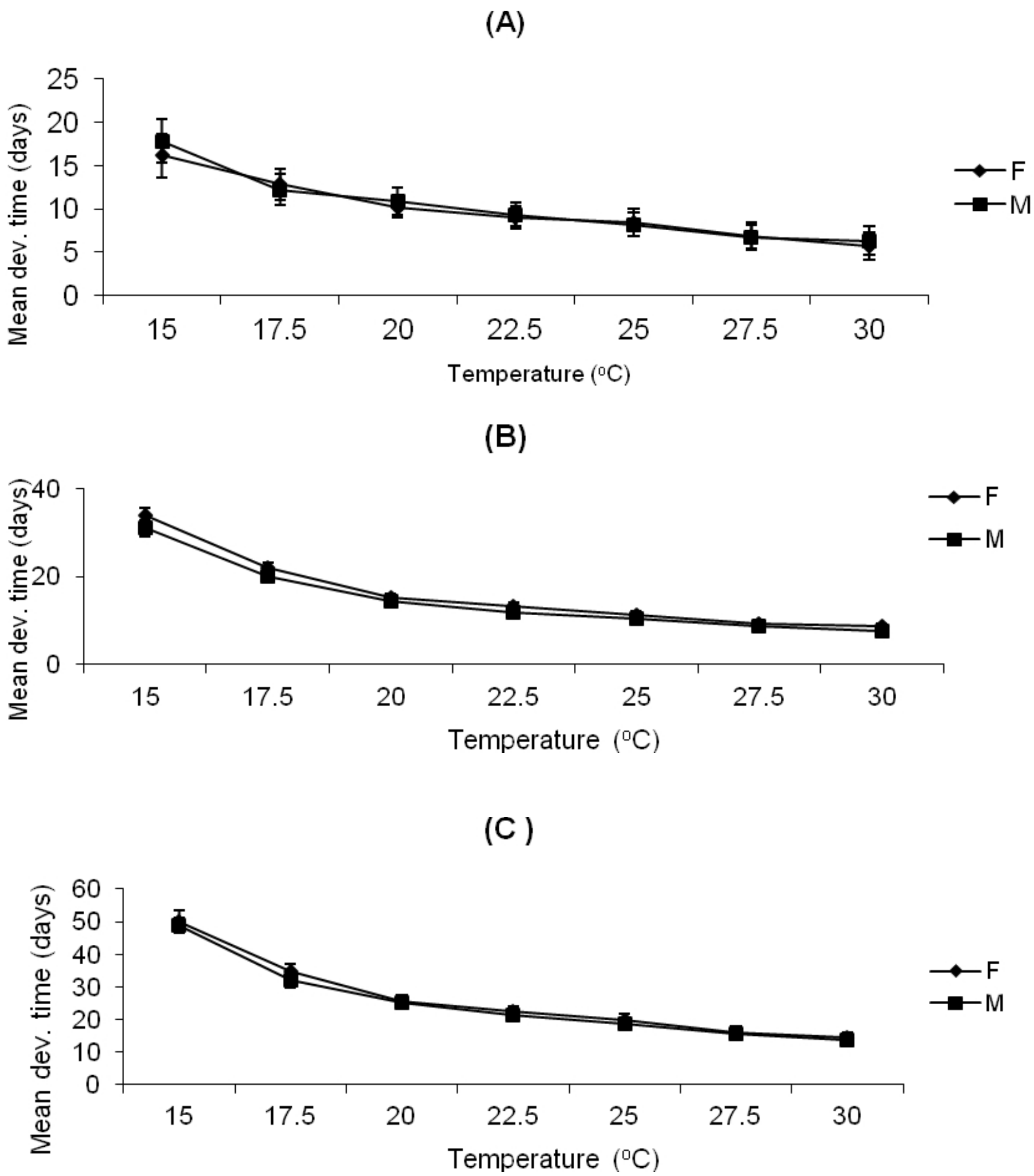

Fig. 1. Relationship between temperature and development time of *Drino inconspicuoides*. Changes in developmental times for male and female parasitoid flies are reflected for three stages (A) larval development time (B) pupal development time (C) total (adult) development time, at different temperatures

3.2 Oviposition Behavior/Pattern

Out of the total number of hosts (n=2582) parasitized during the 6 hour interval, the distribution of puparia (maggots/host) that developed per host ranged from 1 to 8 with a mode of 3 followed by 2 and 4, respectively Fig. 2. Regardless of the size of the host and number of maggots laid /host, not more than 8 puparia successfully egressed and pupariated per host. Relatively very few hosts were in clutch size clusters 7 and 8.

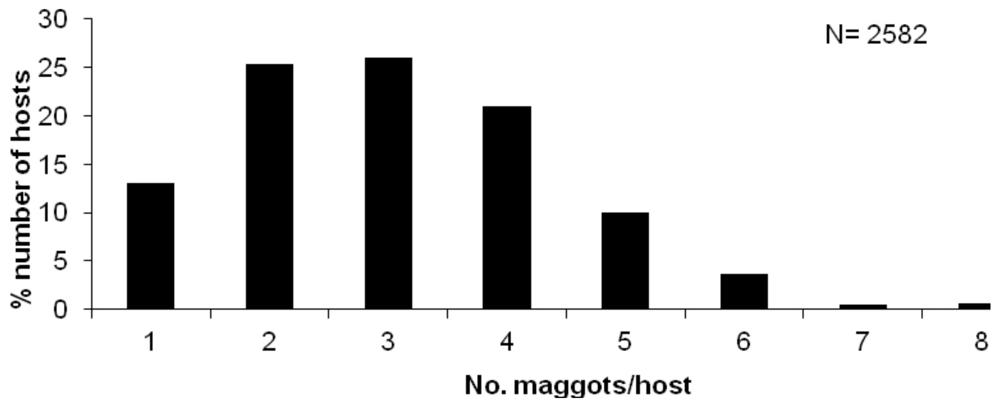

Fig. 2. Relationship between % number of hosts and the distribution of clutch sizes (number of maggots per host) as recovered from the different hosts provided for parasitization (N = 2582)

3.3 Clutch Size, Developmental Time and Puparial Weight

The developmental time of the parasitoid fly was greatly influenced by clutch size (F = 28.68; df = 7, 1700; P<0.0001 for larval development time; F = 3.09; df = 7, 1700; P<0.003 for pupal development time and F = 23.75.45; df = 7, 1700; P<0.0001 for total development periods). The trends /patterns of change of development time for the three stages (larval, pupal and adult) were similar all decreasing with increasing clutch size Fig. 3. For example, total development time decreased from 14 days when only 1 maggot developed per host to 11 days when 6 maggots developed per host and 9 days when 8 maggots developed per host Fig. 3.

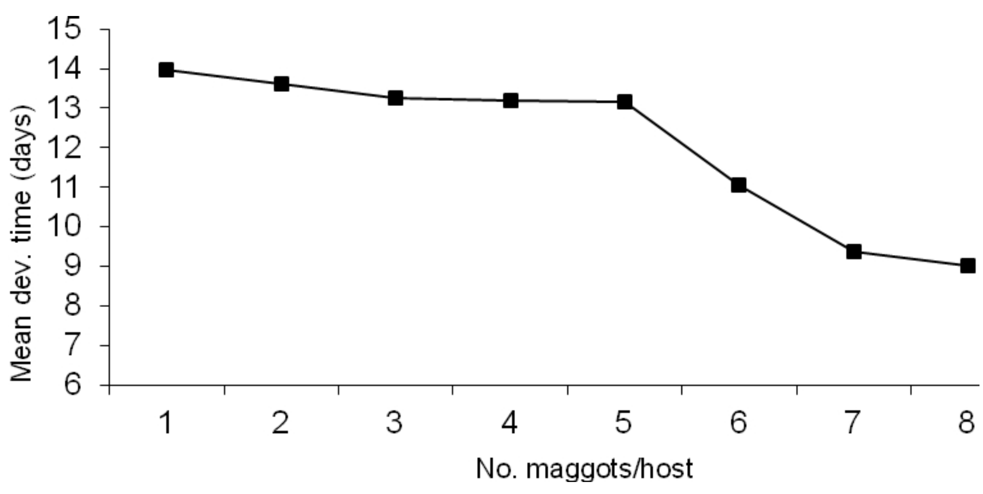

Fig. 3. Relationship between developmental time of *D. inconspicuoides* and clutch size (number of maggots/host)

While increasing number of maggots/host resulted in decreased developmental time, it had a similar effect on the puparial weight and hence emergence of parasitoids. As the number of maggots/host increased, the puparial weight decreased significantly and emergence rate also decreased Fig. 4. For example, puparial weight decreased from 38 mg when one maggot developed per host to 15 mg when 6 maggots developed per host and 10 mg when 8 developed maggots per host Fig. 4. The decrease in puparial weight corresponded to a concomitant reduction in developmental time as the number of maggots/host increased.

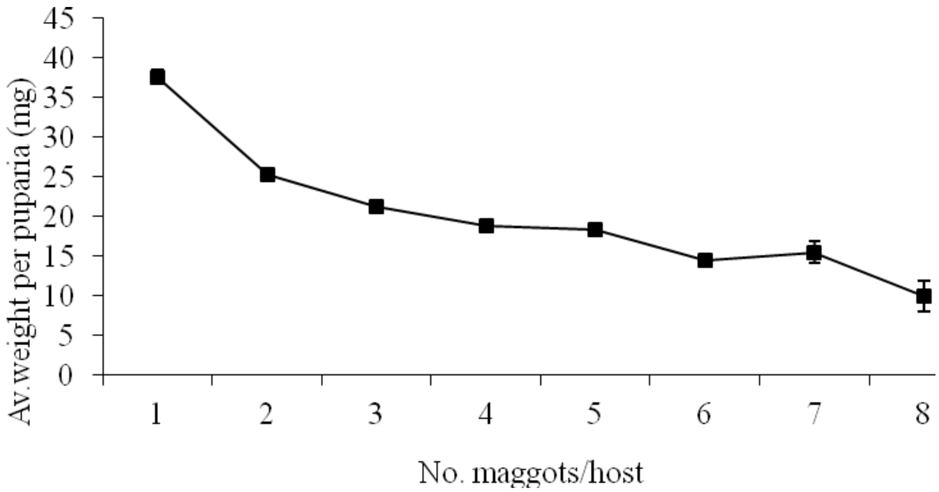

Fig. 4. Relationship between puparial weight *D. inconspicuoides* and clutch size (number of maggots/host)

The increasing number of maggots/host greatly affected the parasitoid's puparial weight and resulted in lower parasitoid survival and emergence Fig. 5. For example, emergence/survival decreased from 83% when 1 maggot developed/host to 33% when 6 maggots developed per host, a decrease of about 40%. Temperature similarly influenced parasitoid survival and emergence. Whereas percentage survival and emergence of parasitoids was 83% at 25 °C, it was 75% at 15 °C. Emergence at 22.5 and 27.5 °C was 80% and 75.8%, respectively while that at 30 °C was 40%, the lowest recorded of all temperatures evaluated Fig. 6.

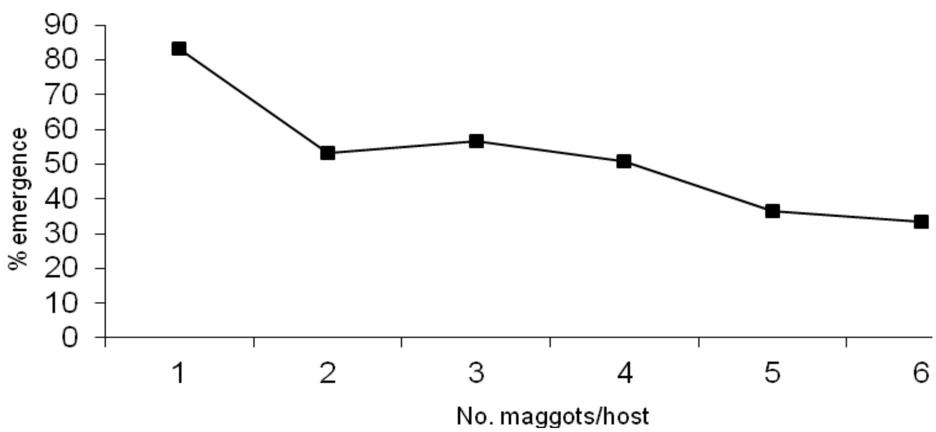

Fig. 5. Relationship between clutch size (number of maggots/host) of *D. inconspicuoides* and number of parasitoids emerged

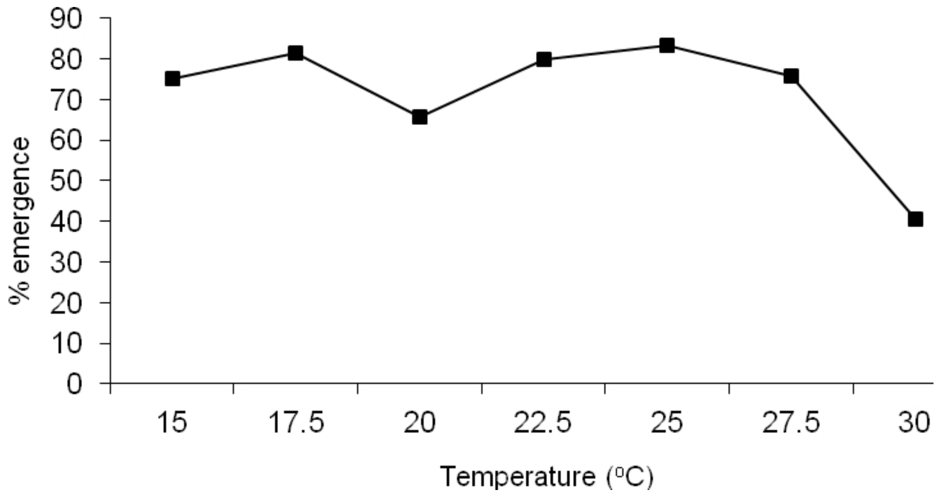

Fig. 6. Relationship between temperature and adult parasitoid emergence for *D. inconspicuoides*

There was an interactive effect of temperature and clutch size on development (F = 2.01; df = 30, 1700; P < 0.0001) Fig. 7. Regardless of the clutch size, developmental time decreased with increasing temperature and the trends were similar for the different clutch sizes Fig. 7. Both factors interactively strongly influenced parasitoid survival and development.

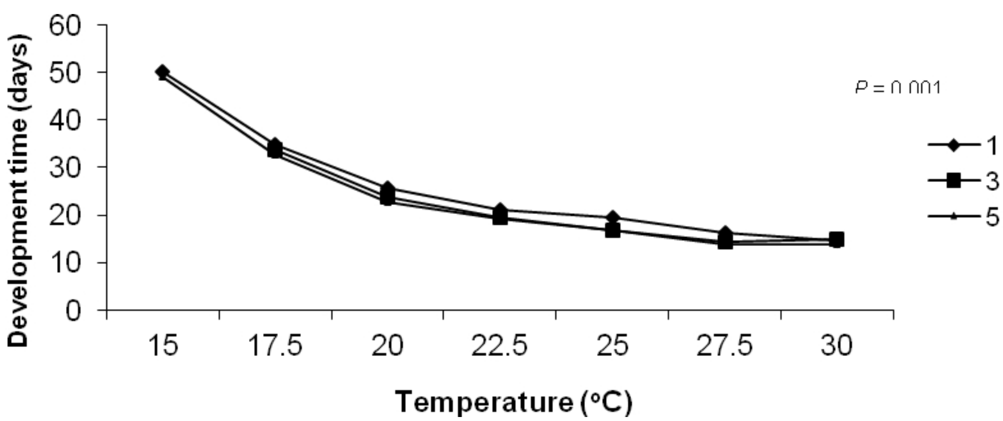

Fig. 7. Relationship between developmental time of *D. inconspicuoides* regarding temperature and clutch size

4. DISCUSSION

Rearing of beneficial insects is a specific operation in which optimum conditions of the environment have to be worked out for each species [14]. In this study, both temperature and clutch size were important factors that affected the development, survival and emergence of *D. inconspicuoides*. Both increasing temperatures and clutch sizes decreased developmental time and affected survival and emergence of the parasitoid. The effect of temperature on arthropods has been well studied. Several authors [18,19] have shown that among the physical factors, temperature exerts the strongest influence. In general, insects develop faster at higher temperatures [20] but optima, maxima and minima differ among species and this has important consequences for parasitoid performance. Because maximum survival and emergence of *D. inconspicuoides* occurred between 22.5 and 25°C decreasing from about 80% to 40% at 30°C, it appears the optimum for this species is about 25°C and therefore temperatures below and above this may not be conducive for rearing.

Increased clutch sizes increased the rate of development of *D. inconspicuoides* and negatively affected parasitoid survival and emergence. Clutch sizes higher than 6 resulted in no parasitoid emergence. A number of studies support findings in this study, for example; the tachinid *Lixophaga diatreae* (Townsend) was found to develop rapidly within the host *Diatraea saccharalis* (F) when density of parasites/host increased [21]. Additionally, the tachinid *Eucelatoria* spp developed faster when 17 puparia/host were present in *Heliothis virescens* [22]

One of the major decisions that a gregarious parasitoid must make is how many eggs to allocate on an encountered host [23,24]. It is hypothesized that a female parasitoid should lay the number of eggs that maximize her gain in fitness from a single host [11,12,13]. The oviposition behavior of *D. inconspicuoides* regarding distribution of clutch size is such that regardless of the number of eggs laid per host, not more than 8 can pupariate in a single host. Because increased clutch size results in decreased puparial weight and survival; it appears that *D. inconspicuoides* is able to discriminate between parasitized and un-parasitized hosts when allocating eggs. While the maximum clutch size for *D. inconspicuoides* was 8 in this study, the optimum may be between 1 and 4 since this ensures at least 50% survival and emergence Fig. 5. Clutch sizes that can support survival and successful emergence vary from species to species. *Exorista japonica* emergence increased until clutch size 15 and then decreased [25]. For the tachinid *Archytas marmoratus*, all parasitoids died before emergence when maximum clutch size was reached [26]. Some authors agree that clutch sizes are selected to be small because of a negative relationship between clutch size and progeny fitness [27]. Accordingly, some tachinid species rather than increase clutch size are capable of regulating oviposition to avoid superparasitism [28]. Empirical observations have shown that oviposition decisions vary according to a number of factors such as physiological status [29,30], biotic factors and biotic quality of its environment [25,31,32,33,34].

5. CONCLUSION

For effective rearing of *D. inconspicuoides*, a clutch size of less than 4 puparia/host ensures over 50% survival and emergence. Increasing clutch sizes result in reduced puparial weight and reduced emergence/survival of parasitoids. The results of this study provide insights into the developmental requirements necessary for successfully rearing and maintaining the parasitoid fly *Drino inconspicuoides*.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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