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ORIGINAL RESEARCH ARTICLE

Temperature and density dependent efficacy of *Dinarmus basalis* (Hymenoptera: Pteromalidae) against *Callosobruchus chinensis*

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Abstract

The pulse beetle, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) is a serious pest of stored chickpea seed and distributed worldwide. Synthetic insecticides and fumigants are random practices against this pest but with serious drawbacks. The larval-pupal parasitoid *Dinarmus basalis* Rondani (Hymenoptera: Pteromalidae) has been successfully used to control *C. chinensis* as an alternative. Hosts (*C. chinensis*) were cultured in the laboratory in confined conditions (30 ± 1 °C and 80 ± 10% RH) till (12–15 days) to get optimal size for parasitism. The bio-control efficacy was studied at 20, 25, 30 and 35 °C with three parasitoid density pairs i.e. 2, 4 and 6. The number of emerged parasitoids, parasitism and suppression rates were studied. The parasitism and suppression rate of the parasitoid increased with increasing temperature and parasitoid densities except 35 °C. The highest parasitism (98.55%) and suppression (98.43%) by *D. basalis* on *C. chinensis* populations were observed at 30 °C with six pairs of the released parasitoids. Conversely, the lowest parasitism (77.34%) and suppression (74.35%) were observed for two pair parasitoid densities at 25 °C. Therefore, 30 °C temperature and maximum *D. basalis* density have potential to suppress *C. chinensis* populations.

Keywords Pulse beetle · Chickpea · Temperatures · Bio-control potential

Introduction

Pulses are economically important crops and play a pivotal role in the diet of common people of third world countries, including Bangladesh (Darmadi-Blackberry et al. 2004). Globally, they are the fifth most important legume (Aslam et al. 2002). But pulses are attacked in storage by several bruchids worldwide (Titouhi et al. 2017; Mssillou et al. 2022). The bruchid, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) is commonly known as the pulse beetle in Bangladesh. It is the most destructive insect of pulses both in the field and in storage (Bhalla et al. 2008). It attacks all kinds of pulses but is considered a major pest of stored chickpea, *Cicer arietinum* L. (Ahmed et al. 2003; Aslam 2004). Pulse seeds were completely destroyed due to pulse beetle infestation after 3 months of storage (Jat et al. 2013). Control of pulse beetle mostly relies on synthetic insecticides and fumigants in Bangladesh.

But chemical protection measures have many serious drawbacks (Lee et al. 2001; Mahmud et al. 2002; Hossain et al. 2014). Hence, researchers are relentlessly trying to develop alternative control measures(s) of *C. chinensis* using bio-control agents. The use of biotic agents as a tool of biological suppression of stored product pests is now recognized as an accepted sustainable strategy for insect pest management. Among the biotic agents, parasitoids get maximum attention because of their host specificity and good compatibility with the agricultural ecosystems (Xie 1984). Bruchids are attacked by several parasitoids in storage (Soundararajan et al. 2012; Fung et al. 2023).

Among them, *Dinarmus basalis* (Rondani) is an important pteromalid ectoparasitoid attacking the pulse beetle, especially *C. chinensis* (Dugravot et al. 2002; Rojas et al. 2005). The success or failure of a bio-control program in storage systems may depend on the application time and the parasitoid density that are essential factors (Tazerouni et al. 2019; Akinbuluma and Chinaka 2023). But information

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is scanty on the number and time of application of *D. basalis* that could most effectively manage the bruchid population. Besides, the potential of natural enemies significantly relies on environmental conditions. Among these, temperature plays a pivotal role in the physiology, behaviour and biological attributes of insects (Ratte 1985). It is one of the most important abiotic factors that influences the distribution, abundance, development, fecundity, longevity, and the fitness of parasitoids as well (Maceda et al. 2003; Liu et al. 2012). Therefore, this study was conducted to assess the effect of temperatures on the bio-control potential of *D. basalis*, against *C. chinensis* in the laboratory conditions.

Materials and methods

The biotic materials used

The pulse beetle, *C. chinensis* was used as host insect in the present study. The insect was collected from the storehouse of Chalk Bazar at Dinajpur town of Bangladesh. The parasitoids were collected from the warehouse of *C. chinensis* infested chickpea seeds and reared in the laboratory for experiment. Chickpea (*Cicer arietinum*) seeds (var. BARI Chola-10) were used in the experiment as the host of *C. chinensis*. Healthy seeds of *C. arietinum* were collected from the same storehouse and separated from other crop seeds and other foreign materials. The seeds were free from insect infestations and disease symptoms. The seeds were properly dried (12 ± 2% RH) and stored in airtight plastic containers (5 kg) for experimental use.

Mass culture of *C. chinensis*

Collected *C. chinensis* were introduced and allowed to oviposit in glass jars (47 H × 14 D cm) having approximately 200 g of chickpea seeds. Each container was covered with nylon cloth and kept in an incubator (BD 53 / 0581505, Germany) at 30 ± 1 °C, 80 ± 10% RH in the laboratory.

Then the insects were randomly allowed for free mating and oviposition for seven days. After oviposition, live and dead *C. chinensis* were removed from the glass containers. The seeds with beetle eggs were kept in a container (8 cm L x 6 cm W x 14 cm H) for the emergence of adults. Newly emerged adults were again allowed for mating and egg-laying on fresh new 200 g seeds added in different (60) glass containers (Hossain et al. 2014). The rearing procedure was repeated in different batches to ensure a continuous supply of the test insect.

Mass culture of *D. basalis*

Chickpea seeds infested with *C. chinensis* were collected from a godown of 'Chalk Bazar' in Dinajpur town for one month from June to July and kept in the laboratory for development. After a few days, *C. chinensis* and parasitoids emerged simultaneously from the chickpea seeds. The parasitoids were identified properly by the expert Zoologist as *D. basalis*. The collected parasitoid was cultured for experimental use. Thirty mated one-day-old females of *D. basalis* from stock culture were introduced into the plastic containers (14 cm H × 10 cm D) containing 12–15 days old host of *C. chinensis* infesting chickpea seeds for parasitization. After five-days, the parasitoids were removed from the containers and left undisturbed in the laboratory for further development. After 10–12 days, adult parasitoid progenies emerged from the larva containing parasitized host in chickpea seed. The newly emerged parasitoids (1-day-old) were again introduced in a plastic container along with 12–15 days old *C. chinensis* infested chickpea seeds. Both hosts and parasitoids containers were maintained in the incubator (30 ± 1 °C and 80 ± 10% RH) for mass rearing.

Culture of test *D. basalis*

Approximately 100 mated females of *C. chinensis* were released in an individual glass jar (47 H × 14 D cm) containing fresh chickpea seeds for oviposition. After three hours, the *C. chinensis* were removed and the seeds with eggs were kept in the incubator (BD 53 / 05–81505) at 30 ± 1 °C temperature and 80 ± 10% RH for rearing. When the developing larva of *C. chinensis* became 12–15 days old, they were used for the culture of *D. basalis*.

Preparation of *C. chinensis* for parasitization

24-h old 50 mated females of *C. chinensis* were released in a Petri dish (120 D × 20 H mm) containing clean fresh chickpea seeds for oviposition. After 3–4 h, the *C. chinensis* were removed and the seeds with eggs were kept in an incubator (30 ± 1 °C and 80 ± 10% RH) for further development. When the developing larvae of *C. chinensis* became 12–15-days old, they were used to parasitize *D. basalis*.

Parasitism and suppression of the parasitoid

Twelve to fifteen-days old 300 single hosted seeds infested by *C. chinensis* were collected from the stock culture and placed in plastic containers. From the stock culture 2, 4, 6 pairs of newly hatched parasitoids were introduced in each

Table 1 Number of parasitoid progeny emerged at three released levels of *Dinarmus basalis* against *Callosobruchus chinensis* at different temperatures levels

Parasitoid (pairs)	Parasitoid progeny emerged at different temperature levels			
	20 °C Mean ± SE	25 °C Mean ± SE	30 °C Mean ± SE	35 °C Mean ± SE
2	45.20 ± 1.39 cC	48.80 ± 0.86 bcC	55.80 ± 2.13 abB	53.40 ± 0.87 aB
4	51.20 ± 1.07 bB	60.20 ± 1.53 aB	64.00 ± 1.55 aA	60.60 ± 1.69 aA
6	62.40 ± 0.43 cA	67.40 ± 1.03abA	68.40 ± 0.98 aA	63.40 ± 0.81 bcA

Column wise capital but row wise having small same letter are not significantly different among the treatments at $P < 0.01$ level of probability

SE Standard error of the means

container with 70 single hosted seeds infested by *C. chinensis* for parasitization at 20 ± 1 °C with 80 ± 10% RH in an incubator. After the release of parasitoids, the containers were closed with a fine cloth. In the control treatment, no parasitoid was introduced. All the treatments were replicated five times. After seven days, the parasitoids were removed from the containers and parasitized host seeds were left undisturbed for the development of the parasitoids. After emergence, both *D. basalis* and *C. chinensis* were removed daily with an aspirator, counted and recorded. Efficacy of *D. basalis* was determined between the exposed population and non-exposed population (control). The experiments were conducted at 20, 25, 30 and 35 °C and 80 ± 10% RH. All the tests were replicated five times. The parasitism rate was estimated by calculating the ratio between the total numbers of parasitoids emerged and the total number of parasitoids and bruchids that emerged. While suppression rate was calculated by subtraction of emerged treatment *C. chinensis* from control divided by emerged *C. chinensis* (Qumruzzaman 2003).

Statistical analyses

The number of parasitoids and hosts emerged were analyzed with analysis of variance (ANOVA) using Statistix 10 program. Parasitism and suppression rates of the emerged parasitoids were analyzed by a Chi-square test of a contingency table. If a significant difference was detected, a Tukey-type multiple comparison test for post-hoc analysis was run (Zar 2010). Correlation coefficient of parasitism and suppression were established between temperatures and pairs of parasitoid (Excel 365).

Results

Effect of temperature and introduced *D. basalis* density on progeny emergence

Emerged parasitoid progeny increased significantly (20 °C: $F = 44.48$, $df = 2$, $P < 0.001$; 25 °C: $F = 63.74$, $df = 2$, $P < 0.001$; 30 °C: $F = 15.53$, $df = 2$, $P < 0.001$ and 35 °C: $F = 18.65$, $df = 2$, $P < 0.001$) with increasing temperature levels except 35 °C and pairs of parasitoid (2 pair: $F = 11.21$, $df = 3$, $P < 0.001$; 4 pair: $F = 13.71$, $df = 3$, $P < 0.001$ and 6 pair: $F = 7.31$, $df = 3$, $P < 0.001$) introduced with all levels of parasitoid introduction (Table 1). The highest (68.40) parasitoids emerged were found while released six pair of parasitoids at 30 °C. Conversely, the lowest (45.20) emerged parasitoids were recorded at 20 °C when allowed two pairs of parasitoids.

Table 2 Number of *Callosobruchus chinensis* emerged from different introduction levels of *Dinarmus basalis* at different temperature levels

Parasitoids (Pairs)	Number of *C. chinensis* emerged at different temperature levels			
	20 °C Mean ± SE	25 °C Mean ± SE	30 °C Mean ± SE	35 °C Mean ± SE
2	13.20 ± 0.03 abB (1.11)	16.00 ± 0.30 aB (1.19)	10.40 ± 0.10 bB (0.98)	11.20 ± 0.01 bB (1.04)
4	8.00 ± 0.90 aC (0.90)	4.00 ± 0.56 bC (0.59)	2.40 ± 0.24 bC (0.31)	3.60 ± 0.49 bC (0.53)
6	4.00 ± 0.56 aD (0.57)	1.40 ± 0.10 bD (0.12)	1.00 ± 0.00 bC (0.00)	3.60 ± 0.52 aC (0.53)
Control	57.00 ± 0.10 bA (1.75)	62.40 ± 0.00 aA (1.79)	64.00 ± 1.05 aA (1.80)	62.80 ± 0.01 aA (1.79)

In a column capital while in row having the small same letter are not significantly different among the treatments at $P < 0.01$ level of probability. Values within parenthesis are arcsine transformed

SE 1Standard error of the means

Table 3 Parasitism on *Callosobruchus chinensis* at treated density of *Dinarmus basalis* at different temperature levels

Parasitoids (Pairs)	Hosts used (n)	Parasitism (%) on *C. chinensis* at different temperature levels			
		20 °C Mean ± SE	25 °C Mean ± SE	30 °C Mean ± SE	35 °C Mean ± SE
2	70	77.39 ± 1.09 bC	75.30 ± 1.38 bC	84.29 ± 1.09 aB	82.66 ± 1.41 bB
4	70	86.48 ± 1.45 bB	93.76 ± 1.26 aB	96.38 ± 0.89 aA	94.39 ± 1.13 aA
6	70	93.97 ± 1.02 bA	97.96 ± 0.81 aA	98.55 ± 0.37 aA	94.62 ± 1.14 bA

Column wise capital but row wise having small same letter are not significantly different among the treatments at $P < 0.01$ level of probability

SE Standard error of the means

Effect of temperatures and parasitoid density on the *C. chinensis* emergence

Significant differences of host emergence were recorded among the different temperature levels (20 °C: $F = 118.25$, $df = 2$, $P < 0.001$; 25 °C: $F = 233.54$, $df = 2$, $P < 0.001$; 30 °C: $F = 97.60$, $df = 2$, $P < 0.001$; and 35 °C: $F = 165.90$, $df = 2$, $P < 0.001$) and densities of parasitoid (2 pair: $F = 8.85$, $df = 3$, $P < 0.01$; 4 pair $F = 21.10$, $df = 3$, $P < 0.00$ and 6 pair: $F = 11.25$, $df = 3$, $P < 0.001$) (Table 2). The number of host emergence was the lowest (1.00) at 30 °C but highest (16.00) at 25 °C with 2 pairs of parasitoid densities.

Parasitism and host suppression potentiality

Parasitism potentiality (20 °C: $\chi^2 = 36.138$, $df = 2$, $P < 0.001$: 25 °C: $\chi^2 = 99.137$, $df = 2$, $P < 0.001$; 30 °C: $\chi^2 = 62.232$, $df = 2$, $P < 0.001$ and 35 °C: $\chi^2 = 35.704$, $df = 2$, $P < 0.001$) and parasitoid densities (2 pair: $\chi^2 = 10.931$, $df = 3$, $P = 0.012$; 4 pair: $\chi^2 = 26.071$, $df = 3$, $P < 0.001$ and 6 pair: $\chi^2 = 13.382$, $df = 3$, $P < 0.001$) while host suppression (20 °C: $\chi^2 = 29.711$, $df = 2$, $P < 0.001$; 25 °C: $\chi^2 = 96.002$, $df = 2$, $P < 0.001$; 30 °C: $\chi^2 = 60.243$, $df = 2$, $P < 0.001$; and 35 °C: $\chi^2 = 34.789$, $df = 2$, $P < 0.001$) and parasitoid densities (2 pair: $\chi^2 = 11.143$, $df = 3$, $P = 0.010$; 4 pair: $\chi^2 = 26.531$, $df = 3$, $P < 0.001$ and 6 pair: $\chi^2 = 16.415$, $df = 3$, $P = 0.009$) differed statistically among 20, 25, 30 and 35 °C at all levels of parasitoid introduced (Tables 3 and 4). Parasitism and suppression potentiality increased with increasing temperature levels except 35 °C with all levels of parasitoid introduction. The highest parasitism and suppression were estimated as 98.55 and 98.43% but the lowest as 75.30 and 74.35% at 30 and 25 °C at introduced 6 and 2 pairs of parasitoids, respectively (Tables 3 and 4). The parasitism and suppression rates increased with increasing parasitoid pairs at all temperature levels.

The parasitism and suppression rates were found positive on the four levels of temperatures and the pairs of *D. basalis* introduced on the *C. chinensis* population. The regression lines were positive and the slope and intercept of the equations for parasitism's fitted; $y = 0.1493x + 91.357$, $R^2 = 0.1094$; $y = 0.3907x + 79.705$, $R^2 = 0.3412$; $y = 0.5767x + 61.858$, $R^2 = 0.9805$ (Fig. 1) while suppressions; $y = 0.4777x + 82.938$, $R^2 = 0.7646$; $y = 0.4556x + 77.622$, $R^2 = 0.3777$ and $y = 0.5795x + 60.756$, $R^2 = 0.929$ (Fig. 2) at four temperature levels and the pairs of parasitoids.

Discussion

The suppression of pests using bio-control agents indicates fundamental aspects of ecological fitness ability. The adverse environmental conditions negatively affected the potentiality of diverse biological control programs. Development of a parasitoid control through determination of optimal thermal requirement is a focal point of any bio-control program against the pests particularly for its mass rearing, laboratory production, field release and efficiency. Since the potentiality of laboratory

Table 4 Suppression on *Callosobruchus chinensis* at treated density of *Dinarmus basalis* at different temperatures levels

Parasitoids (Pairs)	Suppression (%) on *C. chinensis* at different temperature levels			
	20 °C Mean ± SE	25 °C Mean ± SE	30 °C Mean ± SE	35 °C Mean ± SE
2	76.84 ± 1.51 abC	74.35 ± 1.68 bC	83.75 ± 2.85 aB	82.16 ± 1.03 aB
4	85.96 ± 0.90 bB	93.58 ± 0.74 aB	96.25 ± 0.95 aA	94.26 ± 0.88 aA
6	92.98 ± 1.34 bA	97.75 ± 0.39 aA	98.43 ± 0.02 aA	94.26 ± 1.01 aA

In a column capital while in a row having small same letter are not significantly different among the treatments at $P < 0.01$ level of probability

SE Standard error of the means

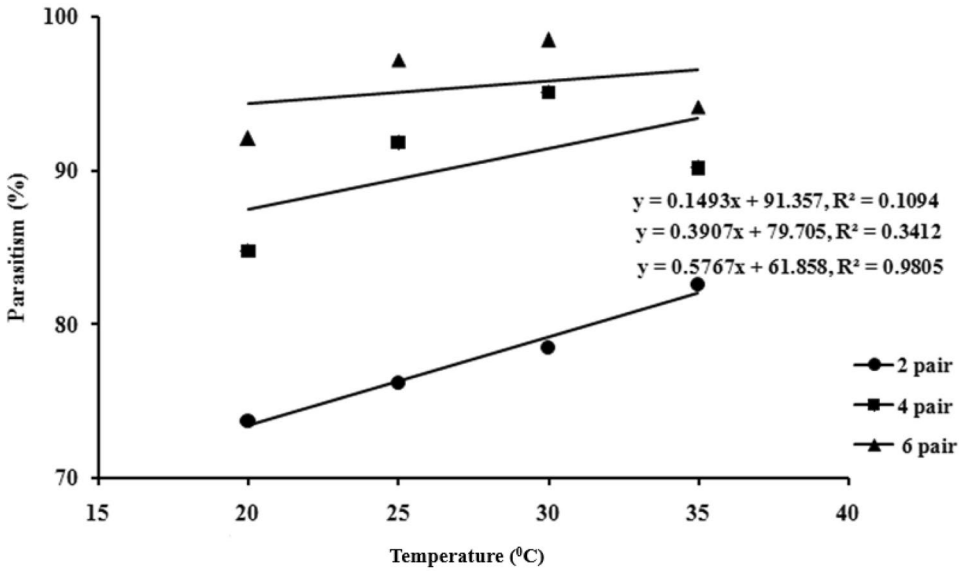

Fig. 1 Relationship between percent parasitism of *C. chinensis* and temperatures following introduction of 2, 4 and 6 pairs of *D. basalis*

strains of parasitoids might be affected after releasing due to fluctuations of temperature as they were cultured in confined conditions. Firake and Khan (2014) showed that the variation of temperature strongly affects the performance of parasitoids cultured in the laboratory.

The outcomes of the temperature levels and parasitoid density potentially influenced the parasitoid progeny and host emergence, parasitism and suppression rates (Islam et al. 2003; Qumruzzaman and Islam 2005). The introduction of parasitoid progeny production increased significantly with increasing temperatures and density at a certain levels of parasitoids introduction (Table 1). The parasitoid progeny production was the lowest at 20 °C but the highest at 30 °C with different pairs of parasitoid introduced. The decreased number of parasitoid progenies emerged at 35 °C compared to 30 °C. The results of the present study are similar with Qumruzzaman and Islam (2005), who cited that progeny production of *D. basalis* increased with increasing parasitoid density at a certain level. Again, Hossain et al. (2014) confirmed that the potentiality of the parasitoid was consequently changed with the change of the density of the parasitoid. The emergence of *D. basalis* was maximum at the highest parasitoid introduction density. Islam et al. (2005) noted that the number of progenies produced by *D. basalis*

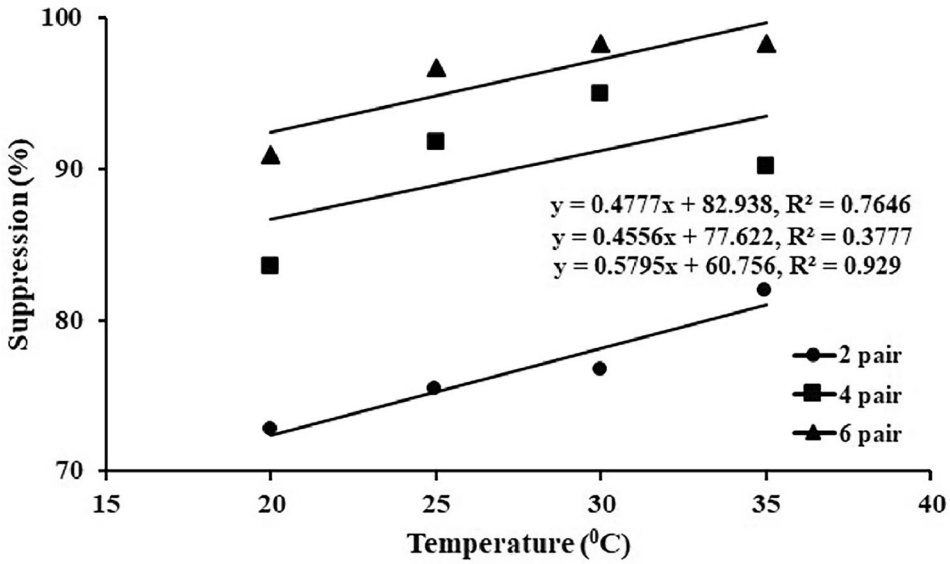

Fig. 2 Relationship between percent suppression of *C. chinensis* and temperatures following introduction of 2, 4 and 6 pairs of *D. basalis*

increased with increasing temperatures. The intrinsic rate of increases was the lowest at 15 °C while the highest at 35 °C.

Emerged hosts allied to the number of parasitoids and levels of temperature (Table 2). Islam et al. (2003) and Hassell (1986) cited that the reduction of the host population was directly related to the number of parasitoids introduction. Press et al. (1984) noted that the F_1 progeny produced was the lowest at the higher introduction levels, but it was the highest at lower introduction level of *D. basalis*. Again, Qumruzzaman and Islam (2005) observed that the host emergence decreased with the increasing densities of *D. basalis*. The present results indicated that the highest densities of parasitoid reduced host emergence.

Parasitism and suppression exerted by the parasitoid on its hosts indicated that it concurrently changed with subsequent change in density of the parasitoid introduced. Variation of temperatures played a great role on the efficiency of a parasitoid. The conclusions of parasitism and suppression trends of the existing study are parallel with Hossain et al. (2014). They experienced that the percentage of parasitism reached the highest 88.46 and 99.25% at the 25 pairs of parasitoids introduced while 62.83 and 77.05% at five pairs exploited on the same parasitoid, *D. basalis* hosted on *C. chinensis*. The suppression by the parasitoid of the hosts was 74.11 and 82.86% at five pairs whereas 92.37 and 99.21% at 25 pairs of parasitoids introduced on the same seasons, respectively. Similarly, the subsequent incidence was also indicated by Qumruzzaman (2003) as *D. basalis* suppressed 85.09–96.67% and 57.50% of *C. chinensis* population at 50-pair and 5-pair levels of application, respectively. Outcomes of the present findings are also comparable with the results of Islam et al. (2003) who found that the reduction of the bruchid, *C. maculatus* population was directly related to the number of *D. basalis* introduced. Again, Iloba et al. (2007) stated that application of *D. basalis* increased 25.52 to 99.48% mortality of *C. maculatus*.

Our results indicate that the relationship between parasitism and suppression was positively correlated (Figs. 1 and 2). However, parasitism's are considered as independent while host suppression analyzed as a dependent variable in the regression analysis. The coefficient of determination on four temperature levels and the pairs of parasitoids is comparatively low. The low combination value of coefficient of determination (R^2) indicates that the parasitism is correlated with the suppression, but they do not explain much of the inconsistency of the parasitism. These findings are in partial agreement to Qumruzzaman (2003) with *D. basalis* hosted on *C. chinensis*. Hossain et al. (2014) experienced a similar pattern with a larger coefficient of determination.

The parasitism and suppression as a bio-control agent of the cosmopolitan pteromalid ectoparasitoid *D. basalis* may be explored on *C. chinensis* (L.), a notorious pest of stored chickpea. Potential of this species is now found in many countries of the world like Bangladesh especially northern region. The parasitism and suppression efficiency of the parasitoid was consequently changed with the change of the parasitoid density and corresponding temperatures. Parasitoid *D. basalis* may be released shortly to reduce the infestation against notorious pulse beetles to ensure biosafety and food security as a vital part of integrated pest management tactics.

The study revealed that the potential of such parasitoid against bruchid pests would be dependent on the prevailing temperature and parasitoid density. The use of such parasitoids against stored product pest insects of bruchids has often been suggested but also rarely been demonstrated. In order to achieve management of *C. chinensis*, suitable temperature and *D. basalis* density appear to the most critical factors under laboratory conditions. The mass culturing techniques of the parasitoid and the feasibility for large scale release to control bruchids to be studied in future.

Abbreviations H: Height; D: Diameter; L: Length; cm: Centimeter; g: Gram; RH: Relative humidity; °C: Degree Celsius; d: Day; r^2: Regression coefficient; df: Degree of freedom; *P*: Probability; F: F-statistic; R^2: Coefficient of determination

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Authors' contributions MAH and MAA conceived and designed the experiments. MAA, and MAH analyzed the data. JRK performed the experiment, collected the data and wrote the paper. MAH and MAA revised the early and final draft of manuscript. All authors read and approved the final manuscript.

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Availability of data and materials All data and materials are mentioned in the manuscript.

Declarations

Ethics approval and consent to participate Not Applicable.

Consent for publication Not Applicable.

Competing interests The authors declare that they have no competing interests.

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