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Scientific Note

Potentiality of the parasitoid *Anisopteromalus calandrae* (How.) (Pteromalidae) on control of the rice weevil *Sitophilus oryzae* (L.) in laboratory condition

M. M. Rahman[1] and W. Islam*
Institute of Biological Sciences, Rajshahi University, Rajshahi, Bangladesh

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The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), is one of the most destructive pest of stored cereals in the warmer parts of the World. Adult weevils attack whole grain and deposit their eggs on the endosperm; the newly hatched larvae cryptically feed and develop within the grains (Ileleji *et al.,* 2007). Infestation by this weevil begins in the field but most damage occurs during storage (Oudejans, 1991). Damage to grain caused by this weevil includes reductions in nutritional value, germination, weight and commercial value (Krishnamurthy, 1975; Watters & Shuyler, 1977). The rice weevil can be conventionally controlled by residual pesticides and fumigation (Vijayanna, 2006). Methyl bromide fumigation, however, has been banned in developed countries since 2005 and in developing countries since 2015 because it destroys ozone layer and endangers for human health (Nishi *et al.,* 2004). One of the most promising alternatives to pesticides and fumigants for postharvest pest management is biological control (Lucas & Riudavets, 2002) which should be particularly effective in the closed environment where grain stored (Hansen & Steenberg, 2007). Berger *et al.* (2017) highlights that the timing of the releases of natural enemies is crucial and needs to be determined specifically for every single system of pest, showing the importance of knowing the period of survival of the parasitoid, to assist in control measures.

Stored product companies used parasitoids as effective biological control agents (Belda & Riudavets, 2010). Due to damage caused by these insects, the difficulty of controlling them, and development of resistance to insecticides by many species, such as *Lesioderma serricorne* (Arthur *et al.,* 2017), new alternatives aimed at minimizing economic losses and controlling pests efficiently, biological control with the use of parasitoids is the most promising method (Jaworski & Hilszczański, 2013).

Among natural enemies that could act as biological control agents of the rice weevil, the wasp *Anisopteromalus calandrae* (How.) (Hymenoptera: Pteromalidae) is a dominant parasitoid naturally found in granaries (William & Floyd, 1971, Arbogast & Mullen, 1990). It is a solitary ectoparasitoid that parasitizes numerous stored-product beetles including *S. oryzae* (Lucas & Riudavets, 2002), *S. zeamais* (Williams & Floyd, 1971, Arbogast & Mullen, 1990), *S. granarius* (Hansen & Steenberg, 2007), *Callosobruchus maculatus* (Coleoptera: Bruchidae) (Ngamo *et al.,* 2007) and *Rhyzopertha dominica* (Coleoptera: Bostrichidae) (Menon *et al.,* 2002). The female wasp parasitizes coleopteran larvae that are feeding inside the grain kernels. In the laboratory conditions, Helbig (1998) found that *A. calandrae* suppressed *S. oryzae* populations on maize 23% relative to the untreated control. Wen & Brower (1995) found that both single and multiple releases of *A. calandrae* substantially reduced the population increase of rice weevils in drum of rice; suppression was > 90% with all rates of both single and multiple releases.

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*Corresponding author: mwislam2001@yahoo.com

The present research has evaluated *A. calandrae* introduction rates on the control of *S. oryzae* in milled rice. A short-term experiment determined the optimum host-parasitoid ratio for mass rearing of the parasitoid. A long-term experiment was carried out to estimate the suitable release rate for repeated, monthly parasitoid releases. The baseline data generated from this study can be used to develop an efficient method for control *S. oryzae* in rice granaries.

The rice weevil, *S. oryzae* and its parasitoid *A. calandrae* were obtained from the CT room, Entomology and Insect Biotechnology Laboratory, Institute of Biological Sciences, University of Rajshahi, Bangladesh. From culture of *S. oryzae*, 1500 adults were added to 2 kg of uninfested wheat grains in a glass container (1′×1′×1′). The grains had been disinfested by freezing at -10ºC>4 wks, shifted over a≠10 sieve to remove dockage, and equilibrated to room temperature. Twenty-four hours later, adults of *S. oryzae* were removed. For stock culture of *A. calandrae*, 100 adult *A. calandrae* was introduced into clear glass jar (10×25×6 cm) containing wheat grains infested with 4th instar of *S. oryzae*. The rearing of insects and experiments were conducted at 30±0.5ºC and 70±0.5% RH (CT room). Under these conditions, the generation times for the weevil and parasitoid were 32 and 13 days respectively. The parasitoid produces one progeny per parasitized weevil larva.

Short-term experiment

This experiment examined the optimum host-parasitoid ratio for mass rearing of the parasitoid. For this experiment, 20 unsexed adult weevils, less than two weeks old, were introduced into each of five 140ml beakers (12cm×5cm dia.) containing 50g of wheat grains. The beakers were then covered with markin cloth. The adult *S. oryzae* were allowed to oviposit for 7 days and then were removed. *S. oryzae* infested wheat was then returned to the beakers at CT room. After 25 days, the infested wheat transferred to small (12cm width ×10cm length) markin cloth bags (50 g of infested wheat per bag), which were sown shut with thread. Adult *A. calandrae* that had emerged within 1 day from the constant rearing colony at CT room were used in the experiment.

In the short-term experiment 35 glass beakers (17cm×25 cm×9 cm) were used. Each beaker contained a pile of four markin bags filled with *S. oryzae* infested wheat grains. The beaker had tight fitting lids. Each lid had two 3-cm diameter holes which were covered with a markin cloth to allow ventilation but prevent insect escape. Different numbers of mated females of *A. calandrae* were introduced into each beaker (0, 2, 4, 6, 8,12, 16, or 20 per beaker) separately. There were five replicate/beakers per parasitoid level. After 1 month, the beakers were frozen at 0ºC for 2 days. The number of *S. oryzae* and *A. calandrae* in each beaker were determined.

Long-term experiment

This experiment examined the long-term interaction of weevil and parasitoid as affected by numbers of parasitoids released at monthly intervals. Unsexed 10 adult weevils were introduced into each beakers, each containing 500 g of fresh wheat. After 25 days, different numbers of presumably mated female *A. calandrae* were added to the beakers (0, 2, 4, 6, 8, or 10 per beaker). There were 30 beakers per parasitoid level. One month after addition of *A. calandrae* to the beakers, 5 beaker of each parasitoid density were randomly selected and sampled; the numbers of emerged *S. oryzae* and *A. calandrae* were counted, and the sex of the parasitoids was determined.

The data on parasitoid emergence (PE), the weevil emergence (WE) and parasitoid-induced mortality (PIM) were analysed using the following formulas (Wen *et al.,* 1994, and Ryoo *et al*., 1996):

$$\text{PE}=\frac{\text{the number of parasitoids that emerged in each replicate}}{\text{the average number of rice weevils that emerged when no parasitoids were added}}$$

$$\text{WE}=\frac{\text{the number of rice weevils that emerged in each replicate}}{\text{the average number of rice weevils that emerged when no parasitoids were added}}$$

$$\text{PIM}=\frac{\text{the average number of rice weevils that emerged when no parasitoids were added the number of all insects including parasitoids that emerged in each replicate}}{\text{the average number of rice weevils that emerged when no parasitoids were added}}$$

In short-term experiment, the number of *S. oryzae* (mean±SE) that emerged from the experiment without parasitoids was 652.65±55.23 per beaker. The emergence of *A. calandrae*, *S. oryzae*, parasitoid induced mortality (PIM) and sex ratio of parasitoids progeny are presented in Table 1. At the introduction levels of 2 pairs of *A. calandrae* on *S. oryzae* recorded highest percentage of 60.80%. The percentage gradually decreased at the introduction levels of 8 and 12 pairs and finally it was 23.80% at the introduction levels of 20 pairs. Only 31.86% of *A. calandrae* were emerged at the introduction level of 2 pairs, it was gradually increased up to 57.55% at the introduction level of 12 pairs and finally 63.69% at the introduction level of 20 pairs. Parasitoid density significantly affected the PE (*F* =2765.71, *P*<0.001), and the PIM = WE (*F* = 54.08, *P* <0.001).

Table 1. Effect of parasitoid density on PE, WE, PIM, and sex ratio of parasitoid progeny in the short-term experiment

Parasitoid density	PE (%)	WE (%)	PIM (%)	Sex ratio parasitoid progeny
2	31.86±2.96^{d}	60.80±1.98^{a}	6.34±0.93^{a}	0.65 ± 0.12
4	43.66±2.09^{cd}	45.20±1.04^{ab}	7.20±0.93^{a}	0.69 ± 0.09
8	49.61±3.17^{bc}	40.20 ±0.79^{b}	8.72±1.43^{a}	0.63 ± 0.08
12	57.55±2.01^{abc}	31.80±1.49^{bc}	9.46±1.21^{a}	0.61 ± 0.03
16	65.92 ±2.84^{a}	22.80± 2.38^{c}	14.01±0.86^{a}	0.59 ± 0.11
20	63.69±1.84^{ab}	23.80± 1.70^{c}	11.41±1.58^{a}	0.57 ± 0.04

Note: Means with the same letter in each column are not significantly differ (Tukey's Test)

The weevil emergence (WE) decreased as parasitoid density increased while the PIM values tended to increase positively with parasitoid density (Table 1). The PIM was significantly higher with 20 female parasitoids per beaker than with smaller numbers per beaker. These results are consistent with those of Wen & Brower (1995) and Ryoo *et al*. (1996) Similarly, Mahal *et al.* (2005) who studied the effect of *A. calandrae* on the control of *R. dominica* in wheat grain, found that the PIM was positively correlated with the parasitoid density.

The parasitoid emergence (PE) tended to be lower and it was 63.69 at the introduction level of 20 females of *A. calandrae* (Table 1). This apparent decline in PE at 20 females per beaker was probably due to superparasitism, as suggested by Wen & Brower (1995). At this density, the PIM was nearly twice as high as the PIM with other densities. This was probably due to intra specific competition among female parasitoids when the available hosts were relatively limiting (Ryoo *et al.,* 1996). For example, although the *A. calandrae* female usually avoids ovipositing on previously parasitized hosts, it is likely to lay more than one egg per host when the number of available hosts becomes insufficient. The supernumerary larvae may be eliminated, leading to a lower percentage of emerged parasitoids.

The sex ratio of parasitoid progeny was not significantly affected by parasitoid density. The progeny sex ratio tended to decrease as parasitoid density increased (Table 1). This reduction in the number of female versus male offspring was probably due to the deficiency of larval hosts rather than to the high parasitoid density. However, the effect of parasitoid density on progeny sex ratio was inconsistent. Our results agree with those of (Wen & Brower, 1995) who reported that the sex ratio of *A. calandrae* progeny decreased as the number of parasitoids added per beaker increased.

In Long-term experiment the numbers of rice weevils that emerged in the beakers without parasitoid (n =5) at the end of 1- 6 months after the release of parasitoid averaged 207 ± 17, 475 ± 21, 594 ± 78, 667 ± 81, 831 ± 97, and 1247 ± 105 respectively.

Table 2. Percentage emergence of F_1 progeny of *S. oryzae* after introduction of *A. calandrae* at different densities and periods

Parasitoid density	**Emergence period of *S. oryzae***							
	M_1	**M_2**	**M_3**	**M_4**	**M_5**	**M_6**	**Average**	**F-values**
2	60.5	71.45	69.12	72.67	65.5	45.5	64.12±3.20	^{a}39.91*** ^{b}6.85 **
4	45.45	65.65	55.5	52.5	41.65	32.23	48.83±2.22	
6	41.66	61.78	51.5	55.52	39.45	30.34	46.70±1.98	
8	36.78	28.31	39.44	31.32	28.78	25.67	31.71±1.75	
10	30.2	23.66	29.34	28.12	21.79	17.45	25.09±0.95	

Note: a= beween density, b= between months, **P<0.01, ***P<0.001

The percentage emergence of rice weevil, *S. oryzae* at five densities of *A. calandrae* after six months are tabulated in Table 2. Out of five densities, two pairs always show the maximum emergence of pest but highest (72.67%) occurred after four months. Minimum pest emergence (17.45%) was recorded at 10 pair of densities after six months. There is significance difference between densities of parasitoid pairs (P<0.001, F=39.91) (Table 2). The statistically significant interaction between density and time indicated that the difference among the 5 densities was not constant at all times after the release of the parasitoid (Zar, 1999, Wen & Brower, 1995).

The percentage emergence of *A. calandrae* at different levels of introduction on *S. oryzae* is presented in Table 3. *A. calandrae* exhibited a significant emergence at all levels of introduction in different months. At 2 pairs level of introduction 55.50% emergence was observed after one month and it was gradually declined after month five (15.20%) and six (8.45%) months respectively. Minimum 4.96% of emergence was recorded after six months at 8 pairs of introduction. The percentage emergence of *A. calandrae* was significantly affected by parasitoid density (P<0.001, F = 59.39) (Table 3).

At each parasitoid density, the parasitoid emergence decreased as the time after the release of parasitoid increased. The decline in parasitoid emergence at each parasitoid density was probably due to superparsitism, which led to high mortality during larval development, rather than to reduced rates of oviposition. Similar effects of parasitoid density were reported for *Muscidifurax zarapter* Kogan and Legener (Hymenoptera: Pteromalidae), which is a solitary pupal parasitoid of the house fly *Musca domestica*; the decline in *M. zarapter* emergence at higher parasitoid-host ratios was due to superparasitism.

As demonstrated in the present experiments, the number of parasitoids released can greatly control the weevil and establishment of parasitoid. Similarly, Ngamo *et al.* (2007) reported that control of cowpea weevils depended on the number of *A. calandrae* females introduced into the infested cowpea. In granaries, host-parasitoid ratio is also likely to affect the control of maize weevil. If maximum number of parasitoids are released, superparasitism may cause the decline in *A. calandrae* emergence. As a result, the parasitoid progeny could disappear before maize weevils are adequately controlled.

Table 3. Percentage emergence of F_1 progeny of *A. calandrae* on *S. oryzae* after introduction of *A. calandrae* at different parasitoid densities and periods

Parasitoid density	Emergence period of *S. oryzae*							
	M_1	M_2	M_3	M_4	M_5	M_6	Average	F-values
2	55.5	30.45	22.56	20.74	15.2	8.45	25.48±2.96	$^{a}2.61^{NS}$ $^{b}59.39^{***}$
4	45.45	28.5	18.78	13.45	9.98	5.5	20.27±2.09	
6	41.25	26.55	20.5	16.63	10.23	7.12	20.38±3.17	
8	39.65	24.45	23.55	14.65	8.12	4.96	19.23±2.01	
10	33.78	22.2	19.23	14.12	18.88	7.78	19.33±2.84	

Note: a= beween months, b= between density, ***P<0.001, NS = Not Significant

Based on the present investigations, the pteromalid parasitoids *A. calandrae* may be an effective biological control agent if it is introduced in sufficient numbers at the beginning of the storage period so as to suppress the initial increase of *S. oryzae* populations. Host-parasitoid ratio greatly affects biological control in this and other systems, the pest population size in the field should be estimated and the optimum number of the parasitoids should be calculated before release. For long storage periods, the parasitoids may need to be added to prevent *S. oryzae* numbers from increasing when the parasitoid numbers decrease.

The present experiment advocates the suitability and efficacy of *A. calandrae* to control the population of *S. oryzae*. The present findings indicate that *A. calandrae* as a good biological control agent can suppress the population of *S. oryzae* and be a component of the Integrated Pest Management (IPM) system.

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