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**Article**

# Optimization of microencapsulated artificial diets for mass rearing of the predacious big eyed bug, *Geocoris pallidipennis*

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With 4 figures and 2 tables

**Abstract:** *Geocoris pallidipennis* (Costa, 1843) (Heteroptera: Lygaeidae) is a generalist predator of insect pests and it is used in insect pest biological control. However, there has yet to be established a mass rearing protocol for this predator that using artificial diets, and therefore its field applications are limited. This study was undertaken to optimize the mass rearing of *G. pallidipennis* employing a microencapsulated artificial diet. We selected four types of ingredients for the diet recipes: insect body, homogenate of *Rhopalosiphum maidis* (Fitch, 1856); micronutrient (fish egg) homogenates; nectar source (pure honey & corn pollen); and nutritional supplements, namely soluble L-Tyrosine & yeast extract. We tested 25 combinations of the ingredients using an orthogonal test to establish the most effective factors in the ingredients, and we screened the best five combinations. The results showed that the different ingredients and their combinations significantly influenced various biological traits of *G. pallidipennis*. Development to sexual maturity was achieved only with five ingredient combinations.

**Keywords:** Biological Control, generalist predator, Heteroptera, Lygaeidae, mass rearing

## 1 Introduction

True bugs are characterized by piercing-sucking mouthparts which defines their feeding mechanisms (Hori 2000; Cohen 2000a, 2000b). The predatory bugs use a feeding method termed "solid-to-liquid" feeding, i.e., they inject digestive enzymes into the prey's body and then suck digested and liquefied tissues (Cohen 1990, 2000b). *Geocoris pallidipennis* (Costa, 1843) (Heteroptera: Lygaeidae) is distributed in China and in several European countries (Protić 2011, Jong et al. 2014). This predator is an important biological control agent (BCA) of several arthropod pests, such as the whitefly *Bemisia tabaci*, the maize aphid *Rhopalosiphum maidis* and the green peach aphid *Myzus persicae* (Tong et al., 2011).

Numerous researchers have succeeded in rearing a variety of predatory stinkbugs on prey analogs or artificial diets (Riddick 2009, Mollà et al. 2014). In North America, species belonging to the *Geocoris* genus, such as *G. punctipes* (Say, 1832), have been reared continuously for over six years (60 generations) on an artificial diet (Cohen 2000a, 2000b). De Clercq et al. (1998) demonstrated that a cylindrical stretched Parafilm-made artificial diet could increase the efficiency of the rearing of the spined solider bug *Podisus maculiventris* (Say, 1832). Cohen (1985a, 1985b) proposed a meat-based diet in stretched Parafilm for the rearing of the big eyed bug, *G. punctipes*. Meridic diets have been developed for rearing several hemipteran predators such as *G. punctipes*, which was reared for more than 90 generations on this diet (Cohen


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1985a, Cohen & Staten 1993, Cohen & Urias 1986). Greany & Carpenter (1998) reported that artificial diets containing ground liver and fresh egg yolk encapsulated in Parafilm allow egg-to-adult development of several predatory heteropterans. Wittmeyer & Coudron (2001) reported that an artificial diet for *P. maculiventris* based on beef liver and whole egg encapsulated in Mylar–Parafilm domes was less effective than coddled cabbage looper larvae.

In spite of these progresses, previous artificial diets have not met the requirements for commercial storage and transportation. To overcome these issues, the microencapsulation method was introduced for increasing the stability and the activity of the product. This is an advanced technique widely used for packaging microbial agents, chemical or food products, and for containing, storing and transporting artificial diet for biological control agents (Kondo 1979, Clancy et al. 1992, Gharsallaoui et al. 2007). Thus, the artificial diet microcapsule (ADM) technique has been recently proposed for the mass rearing of predators for augmentative biological control (Tan et al. 2013, 2014). Tan et al. (2010) found that ADM increases acceptance of the artificial diet for *Orius sauteri* (Poppius, 1909). Our previous work also indicated a higher consistency in diet produce using this method, and that ADM was sufficient for successful development and reproduction of *O. sauteri* (Tan et al. 2010, 2013, 2014). ADM can eliminate the negative effects of a simple solid or liquid artificial diet for *Geocoris* spp., as the microcapsules were found to be more easily accessed by the mouthparts of members of this genus.

The aim of this study was to optimize the ADM in order to improve the development and increase the survival rate of *G. pallidipennis*. We optimized the combination of the basic ingredients used in ADM based on their effects on the development and reproductive capacity of the predator. We also investigated the effects of the optimized ADM on *G. pallidipennis* mating preference to determine whether there are differences between wild females and females reared on optimized ADMs, which may be indicative of successful field release of reared populations.

## 2 Materials and methods

### 2.1 Insects, formulation and production of ADMs

*Geocoris pallidipennis* adults (total of 1553: 863 males 690 females) were collected from a maize field located at Beijing Academy of Agriculture and Forestry Sciences (BAAFS), Haidian District, Beijing (116°16'47.21" E; 39°56'30.64" N) in June and July, 2015. Several specimens were kindly examined by Dr. Cuiqing Gao (College of Forestry, Nanjing Forestry University P. R. China) for specific taxonomic identification. Additional identification and verification was conducted by a taxonomist at BAAFS. The predator was fed on eggs of *Corcyra cephalonica* (Stainton, 1866) (Lepidoptera: Pyralidae) at the Institute of Plant & Environmental Protection of BAAFS under the following environmental conditions: 25 ± 5°C, 14:10-h (L:D) at 3000–4000 lx, and 65% RH (Tan et al. 2013).

The *G. pallidipennis* colony was maintained in plastic jars (10 cm of diameter × 20 cm high) with 25 predator pairs in each. The jars were covered with black muslin cloth held by rubber bands. Small pieces of wet cotton were used for moisture retention, and one maize leaf was provided daily as oviposition substrate. During the peak rearing period (high daily oviposition on maize leaves, which begins in September) the eggs were removed by a moist soft-camel-hair brush and kept in Petri dishes (85 cm of diameter × 10 mm high) to avoid cannibalism. Newly hatched individual nymphs were reared on the eggs of *C. cephalonica* in plastic vials (2.5 cm of diameter × 6.5 cm high) covered with 170 mesh iron sieve, provided with a soaked cotton ball near the cover for moisture retention and isolated for 24 h, prior to artificial diet treatment. This rearing cycle was repeated for at least two generations before carrying out the experiments.

Building on previous work by ourselves and others (Cohen 1985a, 1985b, Cohen & Urias 1986, Cohen & Staten 1993, De Clercq et al. 1998, Greany & Carpenter 1998, Wittmeyer & Coudron 2001, Tan et al. 2010, 2013, 2014), we developed several integrated artificial diet recipes by using microencapsulation, to facilitate mass rearing of *G. pallidipennis*. The experiment mainly involved four types (including six ingredients) of diet at different proportions: i) insect body; homogenates of *Rhopalosiphum maidis* (Fitch, 1856) (Hemiptera: Aphididae); ii) micronutrient (fish eggs) homogenates; iii) nectar source (pure honey and corn pollen); iv) nutritional supplements (soluble L-Tyrosine & yeast extract). *Rhopalosiphum maidis* was chosen because during *G. pallidipennis* samplings in maize fields, these predators were attracted to maize aphids and preyed upon them both in the field and in the laboratory (Wang Su unpublished data). We thus assumed that some volatile substance in the maize aphids attracted *G. pallidipennis*.

An orthogonal $L_{25}$ ($_{5(\text{levels})}{}^{6(\text{factors})}$) test, which is an orthogonal array of six factors (ingredients) and five levels (concentrations) (Table S1), was used to investigate the optimal artificial recipes according to the developmental (juvenile) and reproductive performances of *G. pallidipennis*. The optimization studies were performed using six ingredients at five concentrations (0.1, 0.2, 0.3, 0.4, 0.5gram 100ml$^{-1}$): the 3$^{rd}$ instars nymphs of *R. maidis*, the eggs of big head carp fish, pure honey, corn pollen, soluble L-Tyrosine and yeast extract. One-liter stock solutions for each of these ingredients were prepared. The concentration ranges of each ingredient were measured during preliminary tests. Overall, 25 recipes of artificial diet were tested (Table S1).

Five grams of living maize aphids or fish eggs were added to 100 ml distilled water and grounded separately for about 15 minutes. This allowed getting a homogenous solu-

tion. Ten grams of Pure honey, 10 grams of corn pollen, 2 grams of soluble L-Tyrosine and 10 grams of yeast extract were added in 200 ml distilled water and were mixed using a magnetic stirrer for about 5 min at 3000 rpm to ensure a uniform mixing, and one-liter stock solution of all ingredients was made to the final concentrations (Table S1). From these 25 stock solutions the required concentrations were taken and used in the microencapsulation process.

For all artificial diet microencapsulations we used chemicals as follows: sodium alginate 2%, chitosan 1% and a 13:1 ratio of core material to wall-forming material. Production of the ADMs involved different steps shown in detail in our previous publication (Tan et al. 2013). We produced 25 co-allocated groups of ADMs based on the orthogonal setup detailed in Table 1. In total, 3000 microcapsules were prepared for each ADM group.

## 2.2 Assessments of ADMs for the development and reproduction of *Geocoris pallidipennis*

To select the best ingredient combinations for the artificial diets, the 25 different ADMs were screened by supplying them to newly hatched nymphs under the conditions described above in an environmental chamber (MLR- Sanyo, Japan). Basing on preliminary experiments, six microcapsules were offered to all nymph stages and eight microcapsules to the adults. Each nymph was placed in a Petri dish (8.5 cm of diameter) with a soaked cotton ball and a maize leaf. The Petri dish was covered with Parafilm with holes for ventilation. Every 12 hours we supplied fresh ADM microcapsules, maize leaf and newly soaked cotton. For controls we used all-stage *G. pallidipennis* with a continuous feeding on 3rd and 4th instar of *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae). Then we recorded the total num-

**Table 1.** Instar-specific development time (Mean ± SE) of *G. pallidipennis* feeding on ADMs and control diet (3rd and 4th instar *B. tabaci*).

| Recipe | First Instar | Second Instar | Third Instar | Forth Instar | Fifth Instar | Total Nymphal | Male | Female | Total Adult |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 5.90±0.10ab | 5.40±0.16b | 4.90±0.10b | 4.80±0.13ab | 5.60±0.16b | 26.6±0.21 | 6.20±0.13b | 6.80±0.13ab | 13.0 ±0.25 |
| 2 | 5.50±0.17bcd | 4.80±0.20bcde | 4.30±0.15bcdefg | 4.10±0.18bcde | 5.30±0.15bc | 24±0.27 | 5.60±0.16bcdef | 6.20±0.13bcd | 11.8±0.1 |
| 3 | 6.30±0.15a | 6.40±0.27a | 6.10±0.10a | 5.30±0.15a | 6.80±0.25a | 30.±0.25 | 7.30±0.15a | 7.50±0.27a | 14.8±0.15 |
| 4 | 4.90±0.10cde | 4.70±0.15bcde | 3.80±0.13efgh | 3.60±0.16def | 4.90±0.10bcd | 21.9±0.28 | 5.30±0.15defg | 6.10±0.10bcd | 11.40±0.15 |
| 5 | 5.80±0.13ab | 5.40±0.16b | 4.70±0.15bcd | 4.80±0.13ab | 5.50±0.17bc | 26.2±0.21 | 6.20±0.13b | 6.60±0.16bcd | 12.8±0.1 |
| 6 | 4.50±0.17e | 4.20±0.13ef | 3.60±0.16gh | 3.50±0.17ef | 4.70±0.15cd | 20.5±0.24 | 4.90±0.10fg | 5.80±0.13de | 10.7±0.05 |
| 7 | 4.60±0.16e | 4.40±0.16def | 3.70±0.15fgh | 3.50±0.17ef | 4.80±0.13cd | 21±0.25 | 5.10±0.18efg | 5.80±0.13de | 10.9±0.15 |
| 8 | 5.60±0.16abc | 4.90±0.10bcde | 4.10±0.10cdefg | 4.30±0.15bcd | 5.30±0.15bc | 24.2±0.29 | 5.60±0.16bcdef | 6.30±0.15bcd | 11.9±0.1 |
| 9 | 5.70±0.15ab | 5.00±0.15bcde | 4.10±0.10cdefg | 4.30±0.15bcd | 5.30±0.15bc | 24.4±0.30 | 5.60±0.16bcdef | 6.40±0.16bcd | 12.0±0.05 |
| 10 | 5.40±0.16bcd | 4.70±0.15bcde | 3.90±0.10efgh | 3.90±0.10cdef | 5.20±0.13bcd | 23.1±0.32 | 5.40±0.16bcdef | 6.10±0.18bcd | 11.50±0.2 |
| 11 | 5.70±0.15ab | 5.10±0.18bcd | 4.20±0.13bcdefg | 4.30±0.15bcd | 5.30±0.15bc | 24.6±0.29 | 5.70±0.15bcde | 6.30±0.15bcd | 12.0±0.05 |
| 12 | 5.90±0.10ab | 5.30±0.15bc | 4.70±0.15bcd | 4.80±0.13ab | 5.50±0.17bc | 26.2±0.22 | 6.10±0.10bc | 6.60±0.16bcd | 12.7±0.15 |
| 13 | 5.80±0.13ab | 4.70±0.15bcde | 3.90±0.10efgh | 3.90±0.10cdef | 5.20±0.13bc | 23.5±0.37 | 5.50±0.17bcdef | 6.30±0.15bcd | 11.8±0.05 |
| 14 | 5.60±0.16abc | 4.70±0.15bcde | 4.00±0.15defgh | 4.10±0.10bcde | 5.20±0.13bc | 23.6±0.31 | 5.50±0.17bcdef | 6.30±0.15bcd | 11.8±0.2 |
| 15 | 5.70±0.15ab | 5.20±0.13bcd | 4.20±0.13bcdefg | 4.50±0.17bc | 5.40±0.16bc | 25±0.28 | 5.80±0.13bcde | 6.40±0.16bcd | 12.0±0.3 |
| 16 | 5.70±0.15ab | 4.70±0.15bcde | 4.10±0.10cdefg | 4.20±0.13bcde | 5.30±0.15bc | 24±0.31 | 5.60±0.16bcdef | 6.30±0.15bcd | 11.9±0.25 |
| 17 | 5.80±0.13ab | 5.30±0.15bc | 4.70±0.15bcd | 4.80±0.13ab | 5.50±0.17bc | 26.1±0.21 | 5.90±0.10bcd | 6.50±0.17bcd | 11.4±0.5 |
| 18 | 5.80±0.13ab | 4.90±0.10bcde | 4.30±0.15bcdefg | 4.40±0.16bc | 5.50±0.17bc | 24.9±0.30 | 5.80±0.13bcde | 6.40±0.16bcd | 11.2±0.2 |
| 19 | 4.80±0.13de | 4.50±0.17cdef | 3.70±0.15 | 3.60±0.16def | 4.90±0.10bcd | 21.5±0.27 | 5.20±0.13defg | 5.90±0.10cde | 11.1±0.1 |
| 20 | 5.80±0.13ab | 5.30±0.15bc | 4.80±0.13bc | 4.80±0.13ab | 5.60±0.16b | 26.3±0.20 | 6.10±0.10bc | 6.70±0.15abc | 12.8±0.25 |
| 21 | 5.80±0.13ab | 5.40±0.16b | 4.50±0.17bcde | 4.60±0.16abc | 5.40±0.16bc | 25.7±0.25 | 5.80±0.13bcde | 6.60±0.16bcd | 12.4±01 |
| 22 | 5.70±0.15ab | 5.10±0.10bcd | 4.40±0.16bcdef | 4.50±0.17bc | 5.40±0.16bc | 25.1±0.25 | 5.70±0.15bcde | 6.40±0.16bcd | 12.1±0.15 |
| 23 | 5.80±0.13ab | 5.20±0.13bcd | 4.50±0.17bcde | 4.60±0.16abc | 5.40±0.16bc | 25.5±0.24 | 5.90±0.10bcd | 6.50±0.17bcd | 12.4±0.2 |
| 24 | 5.60±0.16abc | 5.30±0.15bc | 4.50±0.17bcde | 4.70±0.15ab | 5.60±0.16b | 25.7±0.23 | 5.90±0.10bcd | 6.50±0.17bcd | 12.4±0.2 |
| 25 | 5.90±0.10ab | 5.10±0.10bcd | 4.70±0.15bcd | 4.70±0.15ab | 5.50±0.17bc | 25.9±0.23 | 5.90±0.10bcd | 6.50±0.17bcd | 12.4±0.2 |
| Control | 4.30±0.15e | 3.80±0.13f | 3.30±0.15h | 3.20±0.13f | 4.30±0.15d | 18.9±0.24 | 4.60±0.16g | 5.20±0.13e | 9.8±0.1 |

The different letters within each column indicate significant differences based on the LSD test (P< 0.05).($F_{Nymph\ 1}$ = 11.790, df = 25, 234, $P < 0.001$; $F_{Nymph\ 2}$ = 10.215, df = 25, 234, $P < 0.001$; $F_{Nymph\ 3}$ = 15.558, df = 25, 234, $P < 0.001$; $F_{Nymph\ 4}$ = 12.237, df = 25, 234, $P < 0.001$; $F_{Nymph\ 5}$ = 7.621, df = 25, 234, $P < 0.001$; $F_{Adult\ Male}$ = 12.855, df = 25, 234, $P < 0.001$; $F_{Adult\ Female}$ = 6.748, df = 25, 234, $P < 0.001$)

ber of ADMs consumed, development time, survival rate, sex ratio and weight of all stages of *G. pallidipennis*, which required Mettler Toledo's XS Analytical Balances (http://www.mt.com/) (± 0.1 mg). Next we introduced newly mated females (< 1 hours old) to the Petri dish and we recorded the pre-oviposition, oviposition, post-oviposition times and fertility (newly hatched 1st instar nymph offspring) until the female died. For each orthogonal group we performed 10 replications, each with 5 nymphs.

To optimize the ADM recipes, we evaluated *G. pallidipennis* according to selected biological traits: total nymphal development time (ADM-D: recipe 6), feeding of all stages (ADM-F: recipe 7), weight of all stages (ADM-W: recipe 10), survival rate (ADM-S: recipe 4) and egg laying (ADM-E: recipe 19). The effect of ADMs on the mating behaviour of *G. pallidipennis* was tested as follows: 150 pairs of healthy adults of *G. pallidipennis* were collected in the field for use as the wild experimental population in mating experiments, as per Ramirez-Romero et al. (2014) and Tan et al. (2014). Four copulatory groups were established: ADM reared male (ADMM) + wild female (WF), wild male (WM) + ADM reared female (ADMF), ADM-reared male (ADMM) + ADM-reared female (ADMF) and wild male (WM) + wild female (WF). A pair of *G. pallidipennis* adults was then placed into a plastic vial (2.5 cm of diameter × 6.5 cm of high) covered with 170 mesh (0.088 mm) iron sieve. The time of mating was recorded as the start of the mate selection. When the sexual organs of the male and female came into a contact and did not separate for a period of time, the mate selection process was recorded at the end of the mating (60 minute's observation for each pair). This 'time to copula' was used in the assessment of mating preference differences among all four copulatory groups. The observations were replicated 25 times for each copulatory group.

Based on the above experiments, we made four copulatory groups, supplied with five different types of the best ADMs and checked egg laying performance, egg to adult development time and survival rate. The mated females of different copulatory groups were placed in the jars as described above, and 8 ADM microcapsules were supplied. The microcapsules, cotton and the maize leaf were replaced after 12 hours. The jar was checked carefully after 24 hours to record eggs laid until the death of the female. For the development time, at least 20 eggs were selected for each copulatory group. Newly hatched nymphs were placed in the Petri dish (same setup as above) with the best six ADM microcapsules as food for all nymphal stages and eight for the adults. The microcapsules, cotton and maize leaf were replaced every 12 hours. The first instars of *G. pallidipennis* were fed continuously on ADMs until reaching adulthood, then the development time and survival rate was recorded. This experiment was repeated 10 times. For control, 3rd and 4th instar *B. tabaci* were used.

For testing the predatory potential, ten newly emerged adult females of *G. pallidipennis* were selected from each optimal ADM-reared experimental population. The females were placed in a plastic Petri dish (8.5 cm of diameter) and maintained for 12 h without food. A plastic jar (10 cm of diameter ×20 cm of high) was set up with a soaked cotton ball and the opening covered with 140-mesh muslin cloth. Five cotton leaves infested with 300 insect pests, 3rd and 4th instar nymphs of *Frankliniella occidentalis* Pergande 1895 (Thysanoptera: Thripidae), *Aphis gossypii* Glover 1877 (Hemiptera: Aphididae) and *Bemisia tabaci* (Gennadius, 1889) (B biotype), were placed in each jar. The predation rate of *G. pallidipennis* was monitored by counting the residual number of the prey every 10 h over 48 h. Each optimal ADM-reared treatment and control group was replicated 20 times.

### 2.3 Statistical analyses

One-way analyses of variance (ANOVA) was used to test the effects of the ADMs with different ingredient combinations as independent factors and biological parameters such as development time, body weight of all stages of *G. pallidipennis*, survival ratio, reproductive parameters, mating preference, feeding and predatory potential as dependent factors. Shapiro-Wilk and Levene tests were carried out for checking the normality of distribution and homogeneity of variance. When significant differences were found we used the least significant difference (LSD) *post hoc* tests for multiple mean comparisons at P = 0.05. SPSS 16.0 software (SPSS 16.0, SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

## 3 Results

### 3.1 *Geocoris pallidipennis* fitness feeding on alternative ADMs

The ADMs consumed and development time of *G. pallidipennis* are reported in Table S2 and Table 1, respectively. The maximum number of ADMs fed by *G. pallidipennis* juveniles was using recipe 7 (Table S2). The optimal ADM combination was: 3rd instar aphid nymphs 0.5 g/100ml, corn pollen 0.5 g/100ml, fish eggs 0.4 g/100ml, pure honey 0.3 g/100ml, yeast extract 0.2 g/100ml and soluble L-Tyrosine 0.1 g/100ml.

The development time from newly hatched first instar nymphs to adult of *G. pallidipennis* showed significant differences among ADM recipes (Table 1). The ideal ADM combination was the 6 containing 3rd instar aphid nymphs 0.4 g/100ml, fish eggs 0.3 g/100ml, pure honey 0.3 g/100ml, corn pollen 0.3 g/100ml, soluble L-Tyrosine 0.2 g/100ml and yeast extract 0.1 g/100ml.

The body weights of all stages of *G. pallidipennis* in the treatments were significantly different (Table S3). ADM

diet recipe 10 showed the highest body weight in all stages, and the ideal combination was: fish eggs 0.5 g/100ml, yeast extract 0.4 g/100ml, 3rd instar aphid nymphs 0.3 g/100ml, corn pollen 0.3 g/100ml, pure honey 0.2 g/100ml and soluble L-Tyrosine 0.1 g/100ml.

The orthogonal test results indicated that not all nymphs fed on ADMs developed into adults. The survival rate of the first instar nymphs to adult and the sex ratio were significantly different. The total maximum life time survival (Table 2) was observed under recipe 4 and minimum was recorded in recipe 3. The highest survival rate was recorded as 70.00% in the control. Different recipe combinations significantly influenced the mortality of *G. pallidipennis* (Table 2). The most female based sex ratio was observed for recipe 4, and the lowest for recipe 23 (Fig. 1. $F_{Male\ sex}$ = 26.963, d.f. = 25, 234, $P < 0.001$; $F_{Female\ sex}$ = 34.944, d.f. = 25, 234, $P < 0.001$).

These results indicate that the longevity, pre-oviposition, oviposition, post-oviposition periods and fecundity of males and females of *G. pallidipennis* were significantly affected by the different ingredients of the ADM diets. The highest adult longevity and female oviposition time and total fecundity were observed in ADM recipe 7 (Table 2): 3rd instar aphid nymphs 0.5 g/100ml, corn pollen 0.5 g/100ml, fish eggs 0.4 g/100ml, pure honey 0.3 g/100ml, yeast extract 0.2 g/100ml and soluble L-Tyrosine 0.1 g/100ml. The ADM diets also affected the pre-oviposition time. The highest pre-oviposition time was recorded in ADM recipe 3 (Table 2). The highest post-oviposition period was recorded in ADM recipe 4, which was 7.20 (Table 2). We also confirmed that recipe 4 was optimal for survival rate, with an ingredient combination as follows: fish eggs 0.5 g/100ml, and corn pollen 0.5 g/100ml, pure honey 0.5 g/100ml, soluble L-Tyrosine

**Table 2.** Reproductive Parameters (Mean ± SE) of *G. pallidipennis* feeding on ADMs and control diet (3rd and 4th instar *B. tabaci*).

| Recipe | Male Longevity | Female Longevity | Pre-Oviposition | Oviposition | Post-Oviposition | Fecundity | Survival rate of male & female |
|---|---|---|---|---|---|---|---|
| 1 | 18.20±0.291h | 15.70±0.153p | 8.40±0.163b | 5.20±0.814hij | 3.40±0.221f | 9.50±0.563l | 17.30±1.055j |
| 2 | 23.00±0.211b | 24.30±0.153cd | 7.40±0.163cdef | 10.50±0.167bc | 6.70±0.153abcd | 36.10±2.698bcd | 43.50±1.778cde |
| 3 | 13.90±0.314i | 13.40±0.306q | 9.40±0.221a | 1.40±0.163k | 2.40±0.267g | 1.10±0.100m | 8.10±0.781k |
| 4 | 23.30±0.213b | 24.80±0.249bc | 7.00±0.211ef | 10.50±0.224bc | 7.20±0.249a | 40.80±0.646c | 56.00±0.907b |
| 5 | 18.70±0.213h | 16.40±0.163op | 8.00±0.211bc | 4.50±0.167ijk | 4.00±0.149f | 12.90±0.657jkl | 32.80±1.541hi |
| 6 | 23.20±0.200b | 24.40±0.163cd | 7.20±0.200def | 11.10±0.888b | 7.10±0.233ab | 39.70±0.651c | 38.40±1.536efgh |
| 7 | 23.40±0.427b | 25.70±0.153b | 6.50±0.224g | 12.10±0.348b | 7.00±0.211abc | 50.90±1.643b | 50.60±1.558bc |
| 8 | 20.90±0.233efg | 21.90±0.233fg | 7.70±0.153bcde | 7.50±0.307efg | 6.80±0.133abcd | 31.50±0.637de | 39.60±1.514efg |
| 9 | 21.10±0.180efg | 21.30±0.153gh | 7.60±0.163bcde | 7.30±0.153efgh | 6.40±0.163abcde | 29.60±0.670ef | 37.50±1.708fghi |
| 10 | 22.80±0.249bc | 23.60±0.163de | 7.60±0.163bcde | 9.30±0.213cd | 6.80±0.133abcd | 36.50±1.098bcd | 41.20±1.731def |
| 11 | 20.70±0.260efg | 20.60±0.221hi | 7.60±0.163bcde | 6.00±0.298fghi | 6.80±0.133abcd | 24.50±0.833fg | 36.60±1.213efgh |
| 12 | 20.20±0.133g | 16.90±0.180no | 7.80±0.133bcd | 3.50±0.167ijk | 5.60±0.163e | 9.40±0.7331l | 36.50±1.067efgh |
| 13 | 21.70±0.213cde | 23.30±0.153de | 7.30±0.213cdef | 9.00±0.258cde | 6.90±0.100abc | 37.50±1.025bc | 40.00±1.291efg |
| 14 | 21.50±0.167def | 22.90±0.180ef | 7.40±0.163cdef | 8.50±0.307de | 6.90±0.100abc | 36.50±0.969bcd | 39.50±2.167efg |
| 15 | 20.50±0.167fg | 20.30±0.153hij | 7.60±0.163bcde | 6.30±0.213efgh | 6.60±0.163abcd | 24.10±0.862g | 35.50±1.572efgh |
| 16 | 21.00±0.211efg | 22.50±0.167ef | 7.60±0.163bcde | 7.90±0.233def | 6.90±0.100abc | 33.90±0.983cde | 40.30±1.909efg |
| 17 | 20.10±0.100g | 17.70±0.213mn | 7.70±0.153bcde | 3.80±0.200ijk | 6.20±0.133cde | 11.80±1.031kl | 37.00±1.333efgh |
| 18 | 20.40±0.163g | 20.10±0.180ijk | 7.60±0.163bcde | 6.10±0.180fghi | 6.30±0.153cde | 23.30±0.633gh | 34.00±2.963hi |
| 19 | 22.50±0.453bcd | 25.30±0.260bc | 6.80±0.200f | 11.50±0.342b | 6.90±0.100abc | 51.80±1.133b | 49.10±1.269bcd |
| 20 | 20.10±0.100g | 17.30±0.153mno | 7.70±0.153bcde | 3.30±0.153j | 6.30±0.153cde | 9.30±0.633l | 42.10±1.629def |
| 21 | 20.40±0.163fg | 19.20±0.200jkl | 7.60±0.163bcde | 5.20±0.200hij | 6.40±0.163abcde | 20.50±0.778ghi | 32.30±1.571hi |
| 22 | 20.50±0.167efg | 19.70±0.213ijk | 7.60±0.163bcde | 5.60±0.221ghi | 6.50±0.167abcd | 21.40±0.670ghi | 34.50±1.572hi |
| 23 | 20.50±0.167efg | 19.00±0.211kl | 7.60±0.163bcde | 4.80±0.200hijk | 6.60±0.163abcd | 18.10±0.862hij | 32.80±1.227hi |
| 24 | 20.40±0.163fg | 18.20±0.249lm | 7.70±0.153bcde | 4.40±0.163ijk | 6.20±0.133cde | 16.10±0.781ijk | 31.30±1.165i |
| 25 | 20.20±0.133g | 18.30±0.260lm | 7.70±0.153bcde | 4.50±0.269ijk | 6.00±0.149de | 14.20±0.757jkl | 30.50±1.384i |
| Control | 25.10±0.233a | 29.90±0.407a | 5.60±0.163g | 18.30±0.396a | 6.00±0.149de | 87.30±01.399a | 71.00±1.422a |

The different letters within each column indicate significant differences based on the LSD test ($P < 0.05$). $F_{Male\ Longevity}$ = 85.383, d.f. = 25, 234, $P < 0.001$; $F_{Female\ Longevity}$ = 311.397, d.f. = 25,234, $P < 0.001$; $F_{Female\ Pre\text{-}oviposition}$ = 13.786, d.f. = 25, 234, $P < 0.001$; $F_{Female\ Oviposition}$ = 120.395, d.f. = 25,234, $P < 0.001$; $F_{Female\ Post\ oviposition}$ = 49.675, d.f. = 25, 234, $P < 0.001$; $F_{Female\ Fecundity}$ 315.659, d.f. = 25, 234, $P < 0.001$; $F$ Survival rate of male & female = 54.994, d.f. =25, 234, $P < 0.001$).

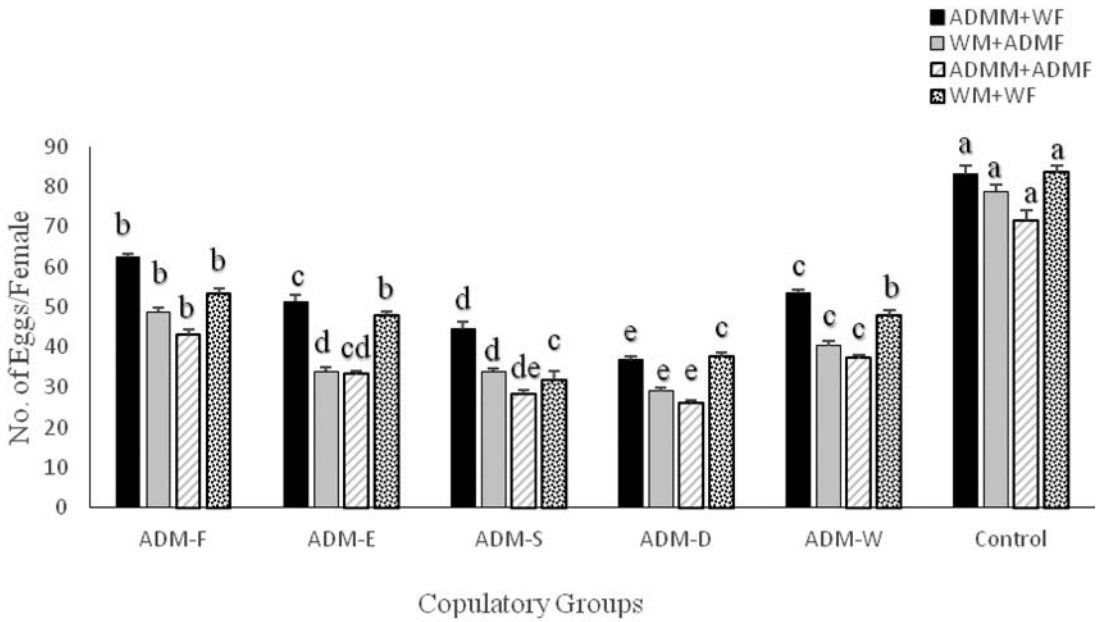


**Fig. 1.** Mean (± SE) values of male and female progenies of *G. pallidipennis* fed on ADMs and control diet (3rd and 4th instar *B. tabaci*).

0.3 g/100ml, 3rd instar aphid nymphs 0.2 g/100ml and yeast extract 0.1 g/100ml.

### 3.2 Performances of *Geocoris pallidipennis* fed on the best five ADMs

The time for copula in all five optimum ADM treatments were significantly different (Fig. 2, $F_{ADMM+WF}$ = 45.934, d.f. = 5, 144, $P < 0.001$; $_{FWM+ADMF}$ = 15.046, d.f. = 5, 144, $P < 0.001$; $F_{ADMM+ADMF}$ = 15.949, d.f. = 5, 144, $P < 0.001$; $F_{WM+WF}$ = 10.438, d.f. = 5, 144, $P < 0.001$). Moreover, the adults reared under overall optimum ADM and the controls (3rd and 4th instar *B. tabaci*) prefer to copulate with wild insects. The wild female preference to copulate with wild males is greater than for ADM-reared male and female. The time for copula in all optimal ADM-treatment groups was found to be less than the time in which the wild insects were involved.

The egg laying performance of the four copulatory groups of *G. pallidipennis* fed on the five optimum ADMs (along with control 3rd and 4th instar *B. tabaci*) differed significantly (Fig. S1, $F_{ADMM+WF}$ = 125.854, d.f. = 5, 54, $P < 0.001$; $F_{WM+ADMF}$ = 258.543, d.f. = 5, 54, $P < 0.001$; $F_{ADMM+ADMF}$ = 148.568, d.f. = 5, 54, $P < 0.001$; $F_{WM+WF}$ = 129.679, d.f. = 5, 54, $P < 0.001$). In the copulatory groups of female feeding on the optimal ADMs, the egg hatching time was not significantly different, except for the group WM+WF (Fig. S2, $F_{ADMM+WF}$ = 1.260, d.f. = 5, 54, $P$ = 0.295; $F_{WM+ADMF}$ = 1.039, d.f. = 5, 54, $P$ = 0.404; $F_{ADMM+ADMF}$ = 2.727, d.f. = 5, 54, $P$ = 0.29; $F_{WM+WF}$ = 5.300, d.f. = 5, 54, $P < 0.001$).

In all copulatory groups fed on optimal ADMs, the 1st instar nymphal development time was not significantly different (Fig. S3, $F_{1^{st}\ Instar\ ADMM+WF}$ = 6.671, d.f. = 5, 54, $P < 0.001$; $F_{1^{st}\ Instar\ WM+ADMF}$ = 8.612, d.f. = 5, 54, $P < 0.001$; $F_{1^{st}\ Instar\ ADMM+ADMF}$ = 10.059, d.f. = 5, 54, $P < 0.001$; $F_{1^{st}\ Instar\ WM+WF}$ = 23.666, d.f. = 5, 54, $P < 0.001$). In the 2nd instar nymph, developmental time in all copulatory groups were significantly different, except for the group ADMM+ADMF (Fig. S4, $F_{2^{nd}\ Instar\ ADMM+WF}$ = 5.166, d.f. = 5,54, $P < 0.001$; $F_{2^{nd}\ Instar\ WM+ADMF}$ = 4.093, d.f. = 5, 54, $P$ = 0.003; $F_{2^{nd}\ Instar\ ADMM+ADMF}$ = 0.110, d.f. = 5, 54, $P$ = 0.851; $F_{2^{nd}\ Instar\ WM+WF}$ = 8.247, d.f. = 5, 54, $P < 0.001$). The development time of the 3rd instar nymph in all copulatory groups was not significantly different, except for the groups ADMM+WF and ADMM+ADMF (Fig. S5, $F_{3^{rd}\ Instar\ ADMM+WF}$ = 2.057, d.f. = 5, 54, $P$ = 0.085; $F_{3^{rd}\ Instar\ WM+ADMF}$ = 6.240, d.f. = 5, 54, $P < 0.001$; $F_{3^{rd}\ Instar\ ADMM+ADMF}$ = 1.042, d.f. = 5, 54, $P$ = 0.403; $F_{3^{rd}\ Instar\ WM+WF}$ = 20.891, d.f. = 5, 54, $P < 0.001$). The development

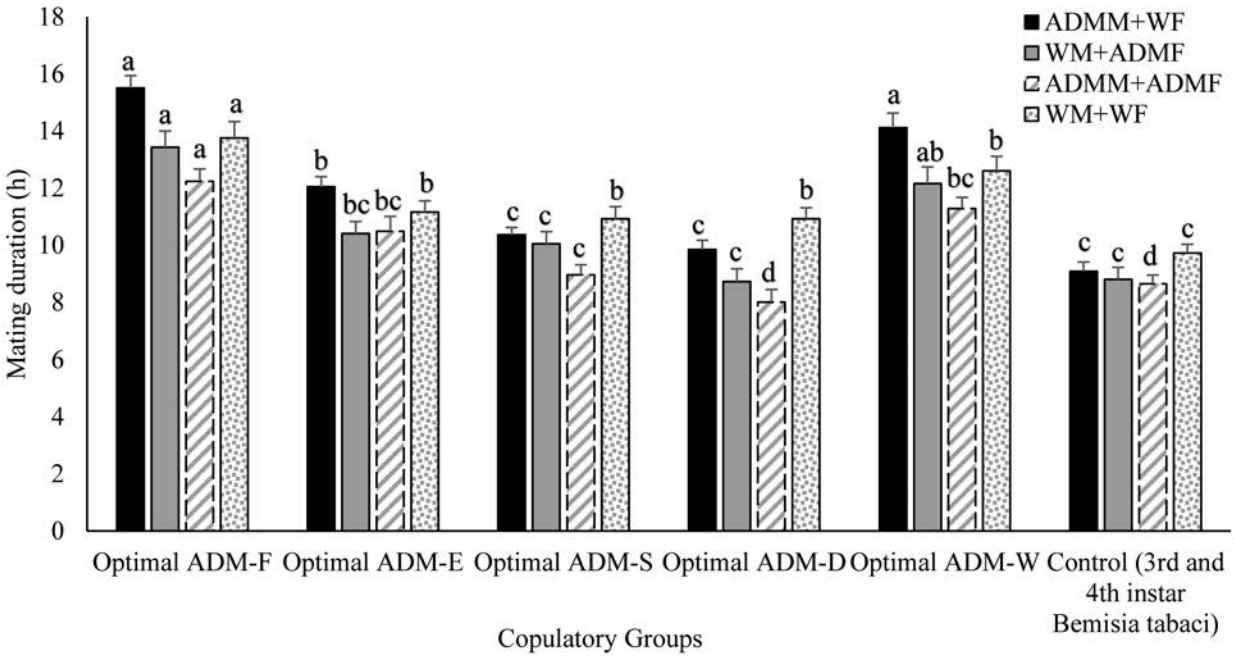


**Fig. 2.** Mean (± SE) values of 'time for copula' of different copulatory groups (based on the five best ADMs and control (3rd and 4th instar *B. tabaci*) including optimal ADM-reared and wild-collected *G. pallidipennis* adults (ADM reared male and female: ADMM, ADMF; wild male and female: WM and WF). The different letters at the top of the columns indicate significant differences based on the LSD test (P < 0.05)

time of the 4th instar nymph in all copulatory groups was significantly different except for the two groups ADMM+WF and ADMM+ADMF (Fig. S6, $F_{4^{th}\ Instar\ ADMM+WF} = 2.239$, d.f. = 5, 54, $P = 0.063$; $F_{4^{th}\ Instar\ WM+ADMF} = 8.524$, d.f. = 5, 54, $P < 0.001$; $F_{4^{th}\ Instar\ ADMM+ADMF} = 1.892$, d.f. = 5, 54, $P = 0.111$; $F_{4^{th}\ Instar\ WM+WF} = 15.253$, d.f. = 5, 54, $P < 0.001$). The development time of the 5th instar nymph in all copulatory groups was significantly different (Fig. S7, $F_{5^{th}\ Instar\ ADMM+WF} = 5165$, d.f.=5, 54, $P < 0.001$; $F_{5^{th}\ Instar\ WM+ADMF} = 4.050$, d.f. = 5, 54, $P = 0.003$; $F_{\text{Fifth Instar ADMM+ADMF}} = 2.749$, d.f. = 5, 54, $P = 0.028$; $F_{5^{th}\ Instar\ WM+WF} = 10.379$, d.f. = 5, 54, $P < 0.001$).

The development time of the male adult in all copulatory groups was significantly different (Fig. 3, $F_{Adult\ Male\ ADMM+WF} = 84.190$, d.f. = 5, 54, $P < 0.001$; $F_{Adult\ Male\ WM+ADMF} = 268.361$, d.f. = 5, 54, $P < 0.001$; $F_{Adult\ Male\ ADMM+ADMF} = 206.691$, d.f. = 5, 54, $P < 0.001$; $F_{Adult\ Male\ WM+WF} = 624.669$, d.f. = 5, 54, $P < 0.001$). The development time of the female adult in all copulatory groups were significantly different (Fig. 3, $F_{Adult\ Female\ ADMM+WF} = 211.318$, d.f. = 5,54, $P < 0.001$; $F_{Adult\ Female\ WM+ADMF} = 277.349$, d.f. = 5, 54, $P < 0.001$; $F_{Adult\ Female\ ADMM+ADMF} = 429.310$, d.f. = 5, 54, $P < 0.001$; $F_{Adult\ Female\ WM+WF} = 612.428$, d.f. = 5,54, $P < 0.001$). In all copulatory groups fed on optimal ADMs (and control 3rd and 4th instar *B. tabaci*), the survival rate was significantly different (Fig. S8, $F_{ADMM+WF} = 87.834$, d.f. = 5, 54, $P < 0.001$; $F_{WM+ADMF} = 31.260$, d.f. = 5, 54, $P < 0.001$; $F_{ADMM+ADMF} = 12.278$, d.f. = 5, 54, $P < 0.001$; $F_{WM+WF} = 37.204$, d.f. = 5, 54, $P < 0.001$).

The results showed variable predatory ability of *G. pallidipennis* fed on ADMs for different prey species (thrips, whiteflies and maize aphids) and at different time intervals. After 24 hours, in all optimal ADM groups, the maximum prey feeding was recorded for ADM-F as follows: thrips 188.20, whiteflies 143.00, and aphids 240.90. The minimum feeding on thrips and aphids was recorded for ADM-D, as 154.50 and 220.50, respectively. In contrast, the maximum number of thrips and aphids consumed in the control group was 181.30 and 239.40, respectively. The minimum feeding on whiteflies was recorded in ADM-S as 124.30 (Fig. 4, $F_{Thrips\ 24h} = 15.731$, d.f. = 5, 54, $P < 0.001$; $F_{whitefly\ 24h} = 4.948$, d.f. = 5,54, $P < 0.001$; $F_{M.\ aphids\ 24h} = 3.766$, d.f. = 5,54, $P < 0.001$). Moreover, after 48 hours in all optimal ADM groups, the number of the prey feeding was less, and not significantly different across groups (Figure 4, $F_{Thrips\ 48h} = 0.420$, d.f. = 5, 54, $P = 0.833$; $F_{whitefly\ 48h} = 1.658$, d.f. = 5, 54, $P = 0.161$; $F_{M.\ aphids\ 48h} = 0.336$, d.f. = 5, 54, $P = 0.889$).

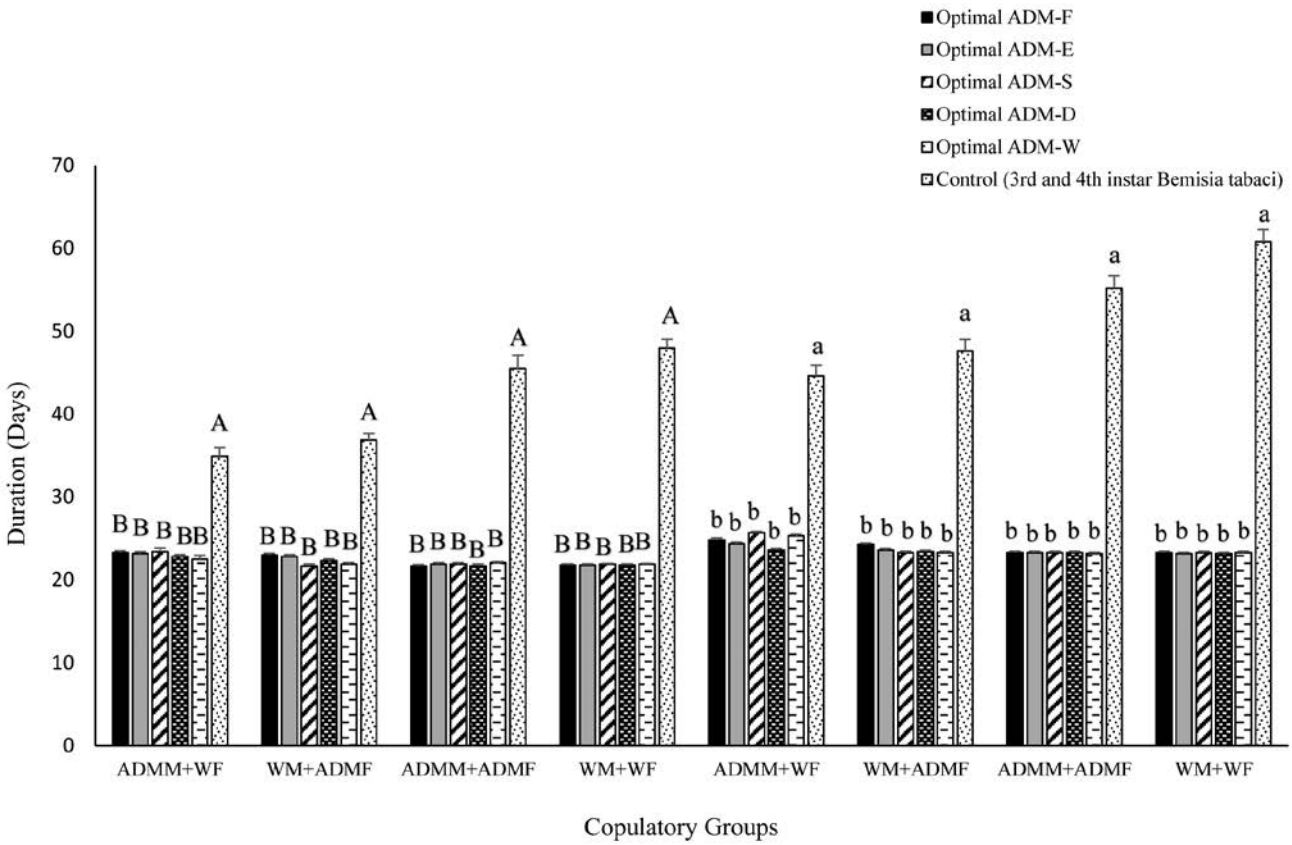


**Fig. 3.** Mean (± SE) values of adult male and female development time of different copulatory groups of *G. pallidipennis* feeding on the five best ADMs and control (3rd and 4th instar *B. tabaci*) (ADM reared male and female: ADMM, ADMF; wild male and female: WM and WF). The different letters at the top of the columns indicate significant differences based on the LSD test (P<0.05)

## 4 Discussion

The successful development of biological control needs more efficient methods of artificial mass rearing (Thompson & Hagen 1999). Early studies on mass rearing using *Geocoris varius* (Uhler, 1860) suggested that artificial diets could reduce rearing costs (Igarashi & Nomura 2013). Since then, artificial diet has become a key component in automation of rearing and produces capable predators (Cohen et al. 1999, Smith & Nordlund 2000, De Clercq 2008). For example, a tailored artificial diet was essential for widespread adoption of the predatory big-eyed bug, *Geocoris* (Cohen 1993, Zheng et al. 2013).

Our results for *Geocoris* showed that ADMs reduced undesirable features of a simple solid or liquid artificial diet. The ADM developed in our previous project can be stored at 10 °C for over 110 days, and the appearance of the stored AMD in terms of surface flexibility and glossiness, the quality of the liquid diet contained, and the shape and size, did not show any significant deterioration from storage (Tan et al. 2013). Currently we are able to store ADM at 5 °C for over 180 days (Wang Su unpublished results). The dome size of the microcapsules of our previous work was on average 0.65 mm (Tan et al. 2010), whereas in the current study the dome size averaged 0.36 mm, which appears to be a factor in the increased attraction for *Geocoris*, but specific trials need to be carried out in order to confirm this hypothesis. Indeed, it has been observed that a consistent dome size is probably responsible for mitigating reduced oviposition during mass rearing on AMDs for *O. sauteri* (Ferkovich et al. 2007; Tan et al. 2010 & 2013).

Penn et al. (1998) discovered that modification of recipe components and their proportions is a key process in meeting demands for mass rearing of biological control agents. In this study, we optimized AMD recipes for feeding *G. pallidipennis* according to desirable biological outcomes, such as copulatory behaviour and egg laying. Tan et al. (2013) focused on the copulatory behaviour of *O. sauteri* fed on optimal ADM diets, assessed by copulation time and predatory ability, but not juvenile survival rate. Our results showed that the body weight of all stages of *G. pallidipennis* was higher when fed on whitefly nymphs compared to when feeding on ADMs. Similar results were found on *G. varius* feeding on the eggs of *Ephestia kuehniella* Zeller 1879 (Igarashi &

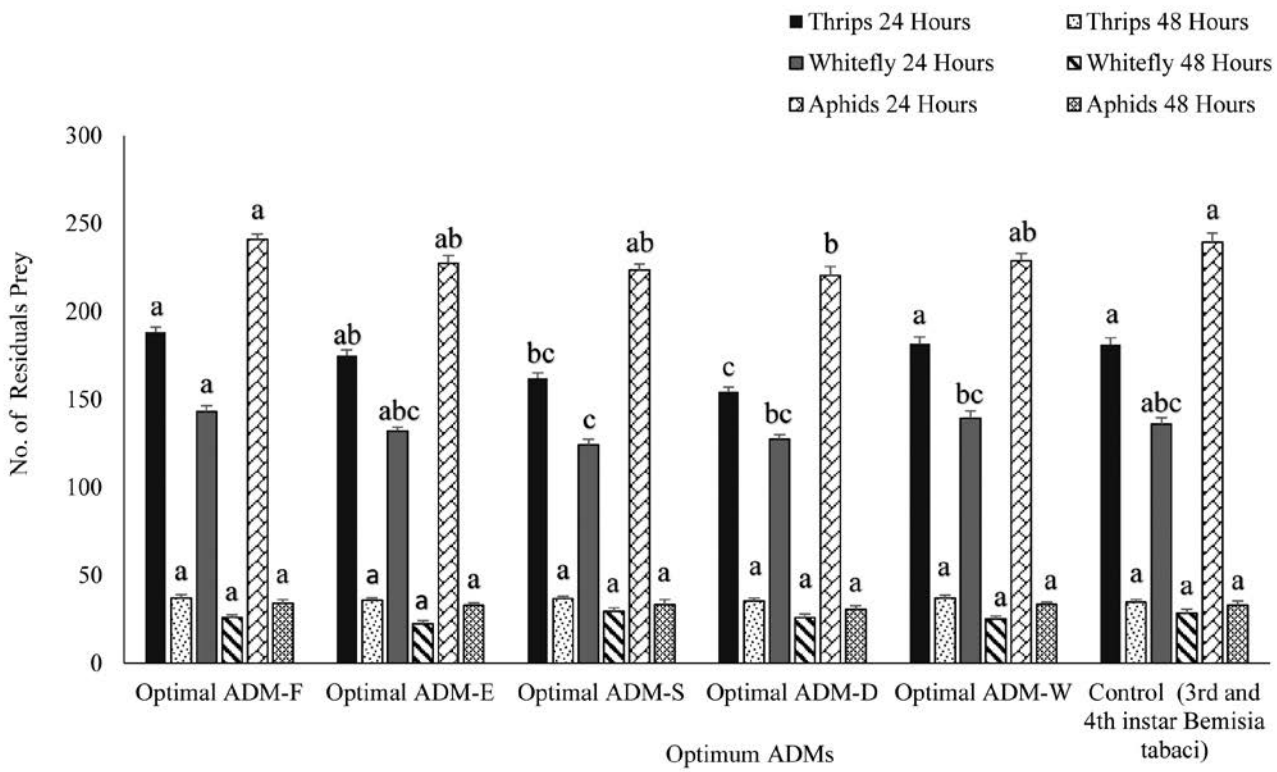


**Fig. 4.** Mean (± SE) values of predatory potential of *G. pallidipennis* fed on the five best ADMs and control (3rd and 4th instar *B. tabaci*). Within the same prey and time, different letters at the top of columns indicate significant differences based on the LSD test ($P < 0.05$).

Nomura 2013). Although such lowering of body weight may reduce predation, the concurrent reduced cost of rearing for ADM should be taken into account.

The ADM recipes shown herein to be suitable for the *G. pallidicornis* egg production and overall fertility, thus suggesting to be potentially employed on any further rearing and/or experimental trial (Thompson & Hagen 1999). Fertility and development have been long seen as key criteria in the arthropod mass-rearing industry (Ferkovich & Shapiro 2005, Ferkovich et al. 2007). In this study, we focused on dietary factors that had an effect on the biological and physiological characteristics of *G. pallidipennis*. Our previous work which used reproductive characteristics showed that there were no differences in mating performance between wild females and female *O. sauteri* reared on either of two optimal ADMs, for ADM-O (oviposition duration) and ADM-F (female fertility) (Tan et al. 2013). The results of the present study showed that there were significant differences in mating performance between wild females and female *G. pallidipennis* reared on the five optimal ADMs (ADM-F, ADM-E, ADM-S, ADM-D and ADM-W). The maximum egg laying was recorded in the ADM-W copulatory groups when fed on optimal ADM-F and ADM-E. The maximum survival rate was found in ADMM+WF when fed on optimal ADM-F and ADM-S. These results suggest ADM-F and ADM-S are prime candidates for mass rearing of *G. pallidipennis*, in the context of inoculative releases in biological control, because of the resulting reproductive and copulatory efficiencies.

Our results suggest that in terms of reproduction, the optimal ADMs benefit mass rearing of this predator to a degree that should improve efficiency in BCA production. More importantly, *G. pallidipennis* that have been reared on optimal ADMs appear efficient in suppressing pests in the field, although further field experiments are needed to confirm this finding.

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Table S1, S2, S3, Fig. S1, S2, S3, S4, S5, S6, S7, S8