J. of Plant Protection and Pathology, Mansoura Univ., Vol. 13 (6):123-127, 2022

Journal of Plant Protection and Pathology

Journal homepage & Available online at: www.jppp.journals.ekb.eg

Interaction between Certain Natural Enemies and some Stored-Grain Insect Pests

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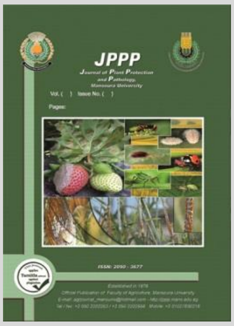

ABSTRACT

The relationship between certain natural enemies (the pteromalid parasitoid, *Anisopteromalus calandrae* Howard (Hymenoptera: Pteromalidae); the predatory ant, *Monomrium* sp. and the predacious mite, *Blattisocius* sp.) and some stored-grain insects was investigated under laboratory conditions. The parasitoid, *A. calandrae* exhibited a positive response to larval feces extracts of the cowpea beetle. *Callosobruchus maculatus* (F) and grain weevil, *Sitophilus granarius* (L)in acetone and ethanol solvents. Odors emitted by adult extracts of *C. maculatus* (in ether and acetone) or by *Bruchidius incarnatus* (in ethanol and acetone) significantly attracted the workers of the predatory ant, *Monomrium* sp. The searching rate and mutual interference values of the parasitoid, *A. calandrae*, and the predatory mite, *Blattisocius* sp. were estimated in response to different hosts. *A. calandrae* females showed a relatively higher searching rate and mutual interference value in response to *C. maculatus* reared on cowpea than those reared on chickpea grains. The predatory mite, *Blattisocius* sp. exhibited the highest searching rate with low mutual interference values on *C. maculatus* eggs in comparison with *B. incarnatus* eggs.

Keywords: Natural, enemies, some, stord, insects.

INTRODUCTION

Several parasitoids (Qumruzzaman & Islam, 2005; Ngamo *et al*., 2007 and Abd El-Gawad *et al*., 2009) and predators (Estrada & Fernandez, 1999; Riudavets & Quero 2003 and Pekar & Zdarkova, 2004) are associated with stored-grain insect pests and exert some level of natural control on most stored-grain insect pest populations. Hymenopteran parasitoids could serve as biological control agents of coleopteran insects (Lucas & Riudavets, 2002; Qumruzzaman & Islam, 2005 and Ngamo *et al*., 2007). The larval parasitoid, *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) could be used in the biological control of this grain pests, *Callosobruchus maculatus* (F.); *Callosobruchus chinensis* (L.), *Rhyzopertha dominica* (F.) and *Sitophilus oryzae* (L.) on faba bean seeds and wheat grains (Timokhov and Gokhman, 2003; Abd El-Gawad *et al*., 2009 and Hosamani *et al*., 2018). The use of predatory mites as naturally manipulate agents in grain stores has been nicely documented (Nielson 1998, 2003; Riudavets & Quero, 2003 & Pekar and Zdarkova, 2004). Numerous predatory mites are broadly utilized in augmentative biological control methodologies (Van Leneteren, 2012; Riudavets *et al*., 2020). *Blattisocius tarsalis*, can feed on several lepidopterous, and coleopterous species (Nielsen, 1999; Riudavets *et al*., (2002a, b); Stejskal *et al*., 2006; Gallego *et al*., 2020).

Predatory ants are generalist predators and have a marked effect on the terrestrial ecosystems wherein its life (Gonçalves *et al*., 2017). Many studies reported the importance of predatory ants in controlling pest populations (Symondson *et al*., 2002). They are very powerful biocontrol agents against several insect orders including Coleoptera (Aneni *et al*., 2018). Their colonies include massive numbers of individuals. Thus, they devour massive populations of prey. Formicidae is the maximum of the essential biotic components of most environments because of their immoderate species richness (Estrada and Fernandez, 1999). Due to the huge numbers of ants in terrestrial environments, as in Egypt, they have not been studied as predators of several insect pests.

Therefore, the present study aimed to shed light on the relationship between these natural enemies and some dangerous grain insect pests (*C. maculatus*, *S. granarius* and *B. incarnatus*) to work on maximizing the role of these natural enemies (*A. calandrae*, *Blattisocius* sp. and the formicid, *Monomrium* sp.) against these insects.

MATERIALS AND METHODS

All experiments were conducted in the laboratory of Economic Entomology Department, Faculty of Agriculture, Mansoura University, Mansoura, Egypt.

1.Influence of the larval frass (LFs) of cowpea beetle and grain weevil extracts on the parasitoid *Anisopteromalis calandrae* host location

Insect and legume seed sources.

laboratory cultures of the cowpea beetle, *Callosobruchus maculatus* (F.) and grain weevil, *Sitophilus granarius*(L) were established from the naturally infested cowpea and chickpea seeds and reared separately on cowpea and chickpea grains for two generations in an incubator maintained at 25 ± 2 °c. The beetles were sexed using the keys described by (Rees, 2004). Mated females (2day old) that emerged from a fresh culture were used for egg-laying.

The ectoparasitoid, *A. calandrae* was collected from its hosts, *C. maculatus* and *S. granarius* on cowpea and chickpea seeds, respectively. Two colonies of the collected parasitoid under the same laboratory conditions were reared

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DOI: 10.21608/jppp.2022.137671.1072

on cowpea and chickpea seeds. They were fed and reared for two generations to generate high numbers of individuals before being used in laboratory bioassays. Newly emerging parasitoid females were collected in glass tubes for kairomone tests and were provided with honey drops for feeding.

Kairomone extraction of *C. maculatus* and *S. granarius* larval frass

Devereau *et al.* (2003) reported that once the larva gets inside, the egg shell turns opaque as it gets filled with the larval feces (LFs). So, to have homogenous LFs in age, mature mated females of each stored grain pest (*C. maculatus* and *S. granarius*) were supplied with healthy cowpea and chickpea seeds for oviposition for 24hrs in glass vials. Mated females (2day old) that emerged from a fresh culture were used for egg-laying. After one week, the (LFs) was collected by cutting about $2.25m^2$ (1.5x1.5m) of the grain surface containing the eggshell with the larval frass (LFs). Then the LFs was extracted by immersing cut parts (10 LSs/ 1 ml) for 24 hr_s in three different solvents (acetone, ether, and ethanol). All extracts were stored at -4 ºC for laboratory bioassays.

Laboratory bioassay

To determine the behavioral reaction of *A. calandrae* females in response to the LS extracts, arena tests were carried out by using uncontaminated Petri-dishes, each containing one filter paper disc (12cm, diameter) under laboratory circumstances. Ten microliters of each extract (i.e. one LF equivalents) were distributed on a small part of one half of the Petri-dishe and on the other half put a similar quantity of pure solvent to represent the control. Five parasitoid females were released at the center of the disc, and data were recorded 15 min. after the introduction of parasitoids. The parasitoid females that showed searching behavior inside the treated disc-half was registered as positive. Each treatment had four replicates. All solvents were tested individually to determine the best solvent for extraction of *C. maculatus* and *S. granaries* LF Kairomone , and each parasitoid female used in the bioassays was used only once.

2. Estimating the relative attractiveness of the ant, *Monomrium* sp. workers to extracts of *C. maculatus* and *B. incarnatus* adults.

Source of insects: laboratory cultures of both *C. maculatus* and *Bruchidius incarnatus* (BRCIIN) were established from the naturally infected cowpea and horse bean as previously mentioned. Workers of *Monomrium* sp. were collected from naturally infested cowpea and horse bean seeds with *C. maculatus* and *B. incarnatus*, respectively.

Kairomone extraction: the kairomone of *C. maculatus* and *B. incarnatus* bodies was extracted by immersing their adults at approximately one week old. (15 adult bodies /1ml solvent) during 48 hours in the above-mentioned solvents and stored at -4 ºC for laboratory bioassays.

Laboratory bioassay: The response of *Monomrium* sp. to *C. maculatus* and *B. incarnatus* body extracts was evaluated by using the experimental Y-tube (Abd El-Kareim *et al*., 2011) that consists of three dark branches (3.5 cm diameter x 10 cm height) and an exposure chamber (6.0 cm in diameter x 5.0 cm height) and each branch was closed by the black plastic cover (internal side coated by Tanglefoot). One tube cover was coated with 0.1 ml of the extract, while the other two covers were coated with an equal amount of pure solvent (as control). The ants were placed inside the exposure chamber, which was immediately closed. Each trial was carried out four times with five individuals/time. Counts were done 15 min. after exposure of ants. After each trial, the Y-tube was cleaned with ethanol and distilled water.

Statistical analysis was fulfilled by using one-way ANOVA.

4. Influence of host species on the foraging behavior of some natural enemies

The parasitoid, *A. calandrae*

To have eggshells with homogenous larval frass of *C. maculatus*, mated females were supplied with healthy cowpea and chickpea grains for oviposition for 24hrs in glass vials. After one week, the infested grains of both cowpea and chickpea were collected for bioassay.

To estimate the searching rate and matual interference values of the above-mentioned parasitoid, five parasitoid densities 1, 3, 5, and 7 individuals were examined by confining 50 hosts of the previously in tested grains with *C.maculatus* on cowpea as well as on chickpea with each parasitoid density in-plastic cage (10 x 10x 5 cm high) for 24 hours. The plastic cage was covered with a fine-meshed screen. Each parasitoid density was replicated ten times. After 14 days, the numbers of living, dead insects, as well as those damaged by the parasitoid were recorded.

The searching rate (a_t) was calculated according to Varley *et al*., (1973) as follow: -

$$\mathbf{a_t = \frac{1}{p} \, Log_e \, \frac{N}{S}}$$

Where, P: the number of parasitoids, N: the initial number of hosts, and S: the number of hosts not parasitized.

The predatory mite, *Blattisocius* sp.

In order to have homogeneous eggs from *C.maculatus* and *B. incarnatus* beetles on cowpea and horse bean grains, respectively, the female insect was exposed to laying eggs within 24 hours. To assess the searching rate of the predatory mite in response to *C.maculatus* and *B. incarnatus* eggs, 10 one-day-old eggs of each species (one /grain) were offered to different predator densities (1, 3, 5, and 7 individuals) in a small cage (6 cm diameter). Female predatory mites were collected using a fine hairbrush and transferred into the experimental cages. After 24 hs, the number of damaged eggs was recorded by using a stereomicroscope (Iturralde-Garcı´a *et al*., 2020). Ten replicate experiments of each predator density were conducted.

RESULTS AND DISCUSSION

Results

1.Attractiveness of the parasitoid *A. calandrae* to *C. maculatus* and *S. granarius* LFs extracts.

Figure (1) shows the percentage of *A. calandrae* females attracted to kairomone extracts of larval frass of both *C. maculatus* and *S. granarius* in the three different solvents (acetone, ether, and ethanol). Ethanol and acetone extracts of both *C. maculatus* and *S. granarius* LFs attracted more *A.calandrae* famels (70±11.54%) each ,with non-significantly differences between the two solvent extracts. Ether extracts of *C. maculatus* or *S. granarius* LFs were less attractive (45±10 and 25±10, respectively. Differences between the ether extract and the two former ones were statistically significant. Generally, Ether proved to be insufficient solvent for kairomone extraction.

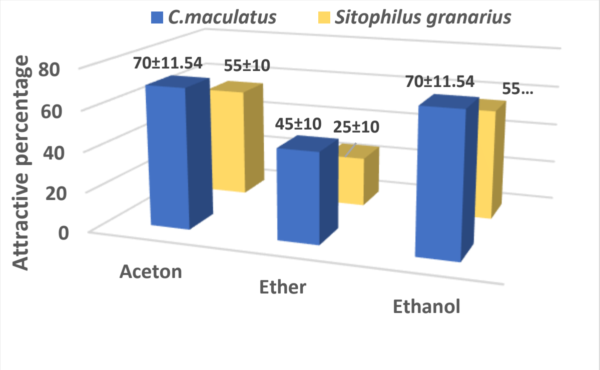

Figure 1. Percentage of attracted parasitoids *Anisopteromalis calandrae* to kairomone extracts from *C. maculatus and Sitophilus granarius* LFs with different solvents. (L.S.D. value was 17.68 and 15.99 for *C. maculatus* and *S. granarius*).

2. Attractiveness of the formicoid, *Monomorium* sp. to *C. maculatus* and *B. incarnatus* adult bodies extracts

Catches of olfactometry Y-tube covers baited with extracts of *C. maculatus* and *B. incarnatus* bodies in the different solvents (acetone, ethanol, and ether) are shown in Figure (2). Ether followed by acetone extracts of *C. maculatus*, significantly lured more percentage of *Monomorium* sp (75±10 and 70±11.54%), in assessment than with ethanol extract (45±19.14) in the experimental y- tube. On contrary, the predator showed the highest response to ethanol and acetone extracts of *B. incarnatus* bodies, where the attractiveness percentages were 70±11.54 and 65±10 %). As shown in Figure (2), the predator showed a very low response (35±19.14 %) to ether extract of *B. incarnatus*. However, statistical analysis revealed that there were significant differences between the attractiveness % of ether and the former two extracts to *Monomorium* sp. adults. Generally, ethanol and ether approved to be unsuitable for kairomone extraction of *C. maculatus* and *B. incarnatus*, respectively.

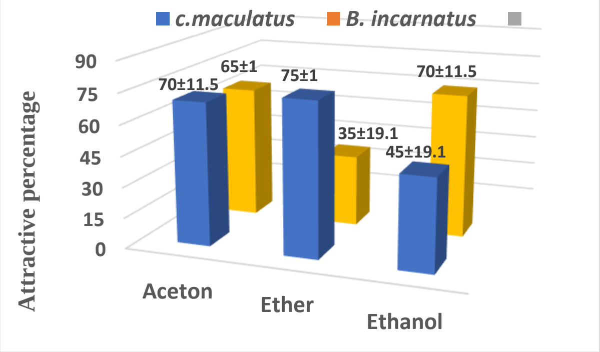

Figure 2. Percentage of attracted *Monomrium* sp. workers to kairomone extracts from *C. maculatus and B. incarnatus* adult bodies with different solvents. (L.S.D. value was 22.62 and 22.58 for *C. maculatus* and *B. incarnatus*).

3. Influence of different hosts on the foraging behavior of *A. calandrae* and *Blattisocius* sp.

The ectoparasitoid, *A. calandrae*

The searching rate (a_t)and matual interference values of the parasitoid at different host densities under laboratory conditions are illustrated in Fig. (3). *A. calandrae* females showed a relatively higher searching rate for *C. maculatus* reared on cowpea (0.487) in comparison with those reared on chickpea (0.3226). By increasing parasitoid density, the searching rate of *A. calandrae* was relatively higher less for cowpea than for chickpea. The mutual interference values of *A. calandrae* were (0.798), (0.8781) on chickpea and cowpea, respectively.

As illustrated in Figure (3), the relationship between parasitoid density (log p) and search rate (log a_t) on cowpea and chickpea could be represented by the following formula:
($\log a_t = -0.4913 - 0.798 \log_p$) on chickpea, and
($\log a_t = -0.3121 - 0.8781 \log_p$) on cowpea.

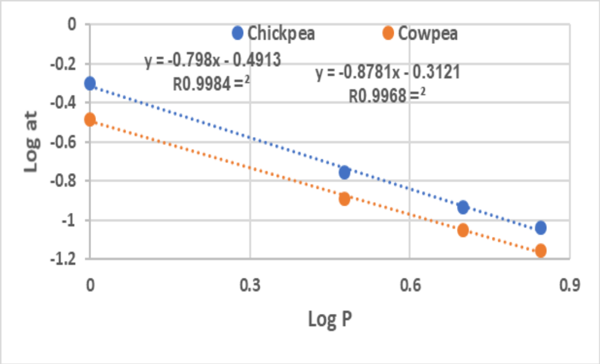

Fig. 3. The relation between parasitoid density (log p) and searching rate (a_t) of *Anisopteromalis calandrae* in response to *Callosobruchus maculatus* reared on cowpea and chickpea under laboratory conditions.

The predatory mite, *Blattisocius* sp.

The searching rate (a_t) of the predatory mite at different prey densities, under laboratory conditions, is illustrated in Fig. (4). *Blattisocius* sp. females reveated that higher searching rate for *B. incarnates* eggs (0.1674) than for *C. maculatus* eggs (0.1106). By increasing parasitoid density, the searching rate of *Blattisocius* sp. females was slightly less for cowpea beetle eggs (m =0.3931) than for *B. incarnatus* eggs (0.6862).

As illustrated in Figure (3) cleared that the relationship between the predatory mite density (log p) and search rate (log a_t) for *B. incarnatus* and *C. maculatus* eggs could be represented by the following formula: ($\log a_t = -0.6862 \log_p - 0.7763$) on *B. incarnatus* and on *C. maculatus* eggs was ($\log a_t = -0.3931 \log_p - 0.9564$).

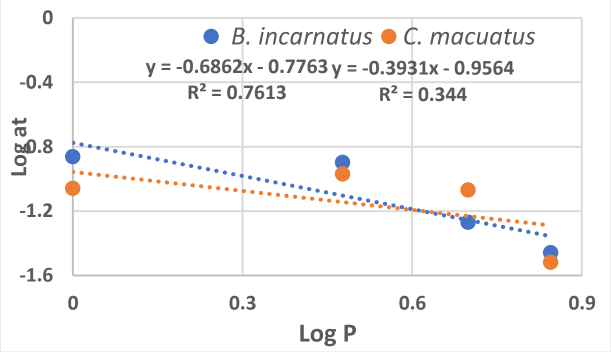

Fig. 4. The relation between predator density (log p) and searching rate (a_t) of *Blattisocius* sp. in response to eggs of *Callosobruchus maculatus* and *Bruchidius incarnatus* under laboratory conditions.

Discussion

Insect natural enemies exploit a variety of chemical signals from different trophic levels as kairomones to locate their herbivorous hosts. kairomones from materials such as cuticular extracts, and insect feces (Abd El-Kareim *et al.*,2011; Usha

Rani,2014 and Blomquist *et al*., 2019), lead natural enemies to their hosts which further results in successful parasitization. Chemical cues used in host-searching behavior were examined for the ecto-parasitoid (*A. calandrae*) of seed-feeding larvae of the cowpea beetle, *C. maculatus*, and the grain weevil, *S. granarius*. The parasitoid exhibited a positive response to feces extracts from *C. maculatus* and *S. granarius* (in ethanol and acetone). Similar results were obtained by Rogers and Potter (2002) that the hymenopterous parasitoids (*Tiphia vernalis* Rohwer and *Tiphia pygidialis* Allen) locate their coleopterous hosts (Japanese beetle, *Popillia japonica* Newman) using contact kairomones present in faces odor. With respect to the formicid predator, *Monomrium* sp. workers were significantly attracted to whole-body extracts of both *C. maculatus* (in ether and acetone) and *B. incarnatus* (in ethanol and acetone). The present study results are coupled with those of Srivastava *et al*. (2008), Maruthadurai *et al*. (2011), and Parthiban *et al*. (2015a and 2015b) repored that several hydrocarbon components of the whole-body extracts of some insect species significantly exerted a higher level of kairomone effect on some parasitoids and predators.

The ectoparasitoid, *A. calandrae* exhibited different search rates on its larval host (*C. maculatus*) according to parasitoid density and the grain host species. Where *A. calandrae* females showed a relatively higher searching rate on *C. maculatus* reared on cowpea in comparison with those reared on chickpea. Ngamo *et al*. (2007) reported that one mated *A. calandrae* female induced a reduction of 4.97% of the emergence of *C. maculatus* while 4 females performed more. They added that in suitable density, *A. calandrae* may play an important role in the biological control of *C. maculatus* on cowpea during storage. In the present study, *Blattisocius* sp females showed a preference for the eggs of *B. incarnatus* than *C. maculatus*. However, *Blattisocius* sp females showed a relatively higher searching rate on *B. incarnatus* in comparison with those on *C. maculatus* eggs. In addition, by increasing predator density, the searching rate of *Blattisocius* sp. females was slightly decreased on cowpea beetle eggs in comparison with *B. incarnatus* eggs. Iturralde-García *et al* (2020 a) reported that the predatory mites, *Amblyseius swirskii* (Acari: Phytoseiidae) and *Blattisocius tarsalis* (Acari: Ascidae) showed differences in the acceptance of their prey (*C. chinensis* eggs), where A. *swirskii* preyed on *C. chinensis* eggs (but did not consume a large amount); while *B. tarsalis* did not accept *C. chinensis* eggs. On contrary, *B. tarsalis* had a significant effect on other prey eggs (Thind and ford, 2006). The predatory mite *B. tarsalis* was able to prey on *Acanthoscelides obtectus* Say eggs (Coleoptera: Chrysomelidae). On contrary the predator was unable to prey on the chrysomelid, *Zabrotes subfasciatus* Boheman eggs. This is probably because the eggs have a protective coating that may impede the predator's perforation of the eggshell (Iturralde-Garcı´a etal 2020 b).

The larval parasitoid, *A. calandrae* was played a major role in eminently successful biological; control projects directed against serious stored grain insect pests (*C. maculates*, *C. chinensis*, *A. obtectus*, *R. dominica* and *S. oryzae*) (Ngamo *et al*. (2007); Abd El-Gawad *et al*,2009 and Iturralde-García *et al*, 2020 and b). It has some of the most important attributes of effective natural enemies (i.e., resulting in high mortality rates on host population, usually capable of inflicting an excellent searching rate in the host population, and it will probably be able to locate hosts at least 150 cm of distance in a storage facility (Iturralde-García *et al*, 2020 b). Therefore, the hymenopterous parasitoid *A. calandrae* combined with the predatory mite, *Blattisocius* sp. appears to offer good prospects for biological control of the stored grain insects. In addition, kairomone as host finding stimuli may be used to stimulate the parasitoid to greater activity in grain stores.

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العلاقة التفاعليه بين بعض الأعداء الحيوية وبعض أفات الحبوب المخزونة

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تم في هذا البحث دراسة العلاقة بين بعض الاعداء الحيوية بصفه خاصة (الطفيل ، والاكاروس المفترس *Monomrium* sp ونوع النمل المفترس ، *Anisopteromalus calandrae* وبعض الافات الحشرية التي تصيب الحبوب المخزونة .تحت الظروف المعملية .*Blattisocius* sp) تجاه مستخلص *A. calandrae* وقد أوضحت النتائج وجود استجابة موجبة للطفيل فضلات يرقات خنفساء اللوبيا وسوسة الحبوب وذلك باستخدام مذيبات الاسيتون والايثانول . كما أوضحت النتائج أن الروائح المنبعثة لمستخلصات الحشرات الكاملة لخنفساء اللوبيا في كل من الايثير والاسيتون أوخنفساء الفول الصغيرة في كل من الأيثانول والأسيتون كان لها أثر ايجابي واضح في جذب *Monomrium* sp شغالات النمل المفترس *Anisopteromalus calandrae* تم أيضا في هذا البحث تقدير المعدل البحثي وقيم التداخل التبادلي للطفيل. بالنسبة لاختلاف العائل *Blattisocius* sp والأكاروس المفترس قد حققت معدل بحث عالي تجاة *Anisopteromalus calandrae* قد أوضحت النتائج أن أناث الطفيل حشرة خنفساء اللوبيا التي تم تربيتها علي بذور اللوبيا أكثر من تلك التي تم تربيتها علي بذور الحمص. قد أبدي معدل بحث عال مع انخفاض قيم التداخل *Blattisocius* sp أما بالنسبة للأكاروس المفترس التبادلي بالنسبة لبيض خنفساء اللوبيا مقارنة ببيض خنفساء الفول الصغيرة.