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Predation and Parasitisation of Prostephanus Truncatus by Teretrius Nigrescens and Anisopteromalus Calandrae Respectively under Controlled Environmental Conditions

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Abstract
Laboratory studies were conducted at Kpeve Agricultural Station in the Volta Region of Ghana and Crop Science Department, University of Ghana, to evaluate and compare the potentials of a predator, *Teretrius nigrescens* Lewis (formerly *Teretriosoma nigrescens*) (Coleoptera: Histeridae) and a parasitoid, *Anisopteromalus calandrae* (Howard) (Hymenoptera: Pteromalidae) as biological control agents against the larger grain borer, *Prostephanus truncatus* (Hom) (Coleoptera: Bostrichidae). The study was carried out using shelled maize grains infested with *P. truncatus*. In the experimental set up, the predation and parasitic abilities of *T. nigrescens* and *A. calandrae* respectively were evaluated in a controlled temperature and humidity chamber. The relative humidities were produced using saturated salt solutions of sodium chloride at 25°C, 30°C, and 35°C. The number of *P. truncatus* larvae consumed in 24 hours by *T. nigrescens* was found to be 5.02 ± 0.1 LGB larvae at 30°C, 75% r.h. Daily consumption rate of LGB larvae by *T. nigrescens* at other conditions of temperature and relative humidities were lower. Parasitic effect of *A. calandrae* was also high at 30°C, 70% r.h. (3.76± 0.20 larvae). At 25°C, significantly lower rates of parasitism were observed at70% r.h., 75% r.h. and 80% r.h. These findings suggest that in a situation where there is inadequate number of *T. nigrescens* for the control of LGB, *A. calandrae* could be used to augment the number of *T. nigrescens* for the control of the pest.
Keywords: *Prostephanus truncatus, Anisopteropmalus calandrae, Teretrius nigrescens,* predation, parasitism.

INTRODUCTION

Maize and cassava are two of the most important staple crops for people living in sub-Saharan Africa. It is therefore of paramount importance that any quantities of these crops harvested should be properly stored in as secure a manner as possible, in order to minimize post harvest losses due to insects.

Insects are the major cause of losses in stored maize in the tropics. They infest and damage grain, resulting in direct and indirect losses of both quality and quantity of food stored (Kossou and Bosque-Perez, 1991).

Many insects of stored products, such as *Sitophilus spp, Rhyzopertha dominica,* (Fabricius) *Sitotroga cereallela* (Olivier), etc have already demonstrated their potentials to destroy food produce, but the arrival of the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) can best be described as a disaster. *Prostephanus truncatus* is a primary pest of farm-stored maize. Whole grains on the cob may be attacked both before and after harvesting (GASGA, 1993). *Prostephanus truncatus* is indigenous to Central America, tropical South America and the extreme South of the USA. It was first reported in Africa in 1980 in the Tabora Region of Tanzania where it was found attacking stored maize and dried cassava chips (Gilman, 1984). It is speculated that *P. truncatus* might have entered Tanzania through a consignment of Food Aid. Since its first report in Tanzania (Harnisch and Krall, 1994), the insect has progressively extended its range, such that it has been found and positively identified in Kenya (Kega and Warui, 1983), Togo (Harnisch and Krall,1984) Burundi (Gilman, 1984), Benin (Krall and Favi, 1986), Ghana (Dick et al., 1989), Guinea (Kalivogui and Muck, 1991), among others.

Scientists and research institutions have over the years been trying to control or manage this notorious pest in an economical and effective way. Consequently, various control options, such as chemical, physical, biological and integrated management methods have been tried.

In laboratory studies, many chemicals such as deltamethrin, permethrin, lindane, primiphos-methyl, malathion, etc, were used in attempts to control *P. truncatus*. The response of *P. truncatus* to some of these compounds in the laboratory by filter paper tests, topical application and exposure to small quantities of grain treated with the chemicals was promising (Golob et al., 1985). Even though chemicals are generally quick acting against pests, there are serious limitations to their use. Misuse or overuse of chemicals have serious consequences, such as the development of resistance, pollution of the environment, effect on non-target organisms, risk of handling, among others (Otto, 1992). Biological control makes use of natural agents (viruses, bacteria, fungi, parasitoids or predators) to combat organisms causing the damage. Biological control, unlike chemical control, does not always require repetitive application and so once established, it has a long-term effect on the pest. Interaction of two biological systems with variable features reduces the risk of resistance to a

minimum (Otto, 1992). It is in this light that the search for biological control agent(s) was initiated. The bioagents searched for included bacteria, pathogentic fungi, protozoans, and insects. Out of 80 bacteria strains screened, only two had a pathogenic effect on the pest. Hymenopterous wasps (Pteromalidae), *Anisopteromalus calandrae* and *Chaetospila elegans* (Brethes) were also observed to be good biological control agents of *P. truncatus* in Costa Rica (Boye, 1988; 1990). Unfortunately, in Africa no further investigations have been carried out intensively except recently in Togo (Leliveldt, 1990; Markham et al., 1991). The main antagonists of *P. truncatus* in Costa Rica were the predatory histerid beetle, *Teretrius nigrescens* (formerly *Teretriosoma nigrescens*) Lewis (Col. Histeridae), and a predatory bug, *Calliodis sp.* (Poschko, 1994). Under the auspices of the organization of African Unity (OAU), the histerid beetle, a native of Central America was recommended for introduction in LGB infested areas, to combat its activities.

Teretrius nigrescens has been identified as the most effective natural antagonist of *P. truncatus* (Rees, 1985, 1987; Boye, 1988; Leliveldt, 1990; poschko, 1994; Helbig, 1995), feeding on eggs, larvae and sometimes even on adults of *P. truncatus. Teretrius nigrescens* though effective, has not been able to entirely eradicate the grain borer. It is for this reason that the search for local parasitoids to complement *T. nigrescens* became necessary. Fortunately, preliminary research has shown that there are some native hymenopteran parasitiods associated with *P. truncatus* in Africa (Helbig, 1988). These include *Anisopteromalus calandrae* (Howard), *Dinarmus basalis* (Rondani) (*Dinarmus laticeps* (Ashmead), *Choetospila elegans* (Westwood) and *Holepyris sylvanidis* (Brethes) (*Rhabdepyris zeae* (Turner &Waterston) (Murphy and Cross, 1992; Seini, 1998).

The larvae of *Teretrius nigrescens* are predators. They, however, resort to cannibalism among themselves when there is shortage of food (Poschko, 1994). Nevertheless *P. truncatus* is the preferred food of *T. nigrescens* (Poschko, 1994; Ayertey et al., 1999). Laboratory investigations conducted by Rees (1985) and Boye (1988) showed that *T. nigrescens* larvae are more voracious than the adults. There is, however, some evidence that *T. nigrescens* imagines can also feed on plants with high starch content, such as maize, wheat, sorghum and cassava (Poschko, 1994).

Most of the parasitoids that attack primary beetle pests of stored products are in the family Pteromalidae and Bethylidae (Hagstrum and Flinn, 1992). These hymenopteran parasitoids are very small and do not feed on grain. They will normally die within 5 – 10 days if no beetles are present in the grain. They occur naturally in grain infested with *Sitophilus or Callosobruchus* species, which suggests that once released they may continue to suppress pests for many years (Sinha et al., 1979). Parella et al. (1992), however, contended that because of the disruptive, seasonal nature of grain storage and the widespread occurrence of *A. calandrae*, it is thought that successful use of this parasitoid will depend on innoculative releases.

Biological control is an important component of integrated pest management of stored grain. *Anisopteromalus calandrae* is one of the many promising parasitoids of some coleopteran pests that are native to Africa and which can serve as a biocontrol agent. *A. calandrae* is a cosmopolitan parasitoid of *Sitophilus* weevil larvae that develop inside the kernels of maize. It is found associated with other coleopterans including *P. truncatus* and so if its parasitisation can exert an impact on *P. truncatus*, it will augment the effect of the other natural enemies of *P. truncatus,* especially *T. nigrescens.*

Murphy and Cross (1992) showed that the parasitoid *A. calandrae*, has the potential to contribute to the control of the LGB. They also contended that *A. calandrae* could co-exist with *T. nigrescens* and compliment the impact of *T. nigresceens* as a biocontrol agent. Against this background, the aim of this work was also to investigate the possible complementary role of *A. calandrae* as a biocontrol agent against *P. truncatus.* This work was done to assess the predatory and parasitic abilities of *T. nigrescens* and *A. calandrae,* respectively under controlled temperature and relative humidity conditions.

MATERIALS AND METHODS

Rearing of *Prostephanus truncatus*

The rearing of *P. truncatus* requires maize because it is the most preferred substrate of the insect (Haines, 1991). The maize was sterilized by putting it in a freezer for two weeks to kill any insects or organisms that might be in it. After the two weeks the maize was then transferred into an oven which was set at a temperature of 70^{o}C for heat sterilization for three hours. When cooled, 400g of it was measured into four one-litre Kilner jars. Fifty *P. truncatus* adults were then introduced into each jar of maize. The whole set up was then placed in trays containing frytol oil to prevent undesirable insects from crawling into the culture (see plate 2b). This was to serve as a starter culture.

Plate 2a
Anisopteromalus calandrae culture (Parasitoids)

Plate 2 b
P. truncatus culture

Rearing of *Teretrius nigrescence*

Teretrius nigrescence was reared on *P. truncatus* culture. One hundred unsexed *P. truncatus* adults were put into each of one-litre Kilner jars containing 400g of maize and left for two weeks before introducing 10 unsexed adults of the predator, *Teretrius nigrescence*. This was done to ensure that by the second week there was food available for the predator in the form of eggs and larvae.

To get the larvae of *P. truncatus* for the experiment, maize biscuits were prepared using a technique developed by Adda (pers.comm.) (see plates 1a and 1b).

Five sexed female *P. truncatus* adults were introduced into the biscuits contained in glass petri-dishes and the tops covered with nylon mesh. After 10 days the biscuits were cracked along the lengths of the tunnels in order not to damage the eggs. The eggs were brushed into containers and put in a controlled temperature and humidity chamber at 30 ± 1ºC, 70% ± 5% r.h to hatch. The hatched larvae were used for determination and parasitisation of *P. truncatus larvae* by *T. nigrescence* and *A. calandrae* (see plate 3).

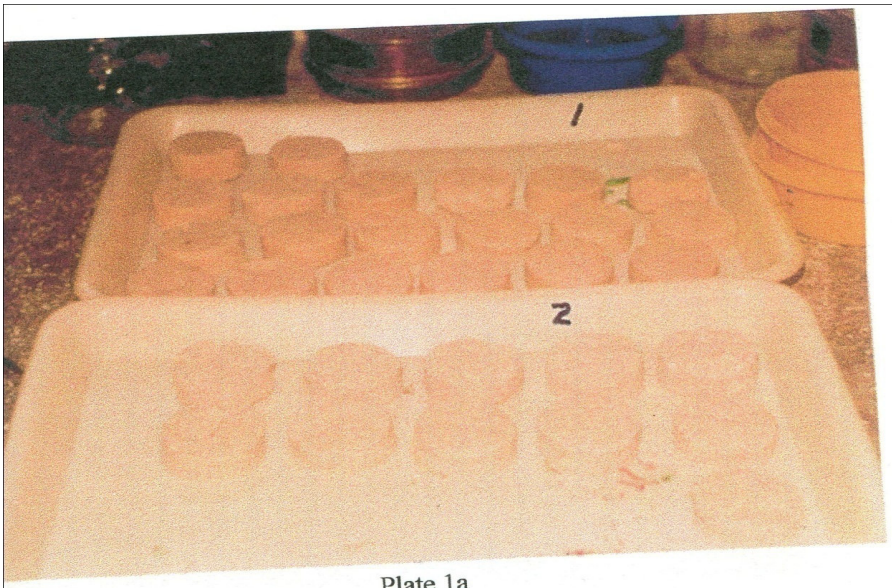

Plate 1a
Freshly prepared maize *biscuits*

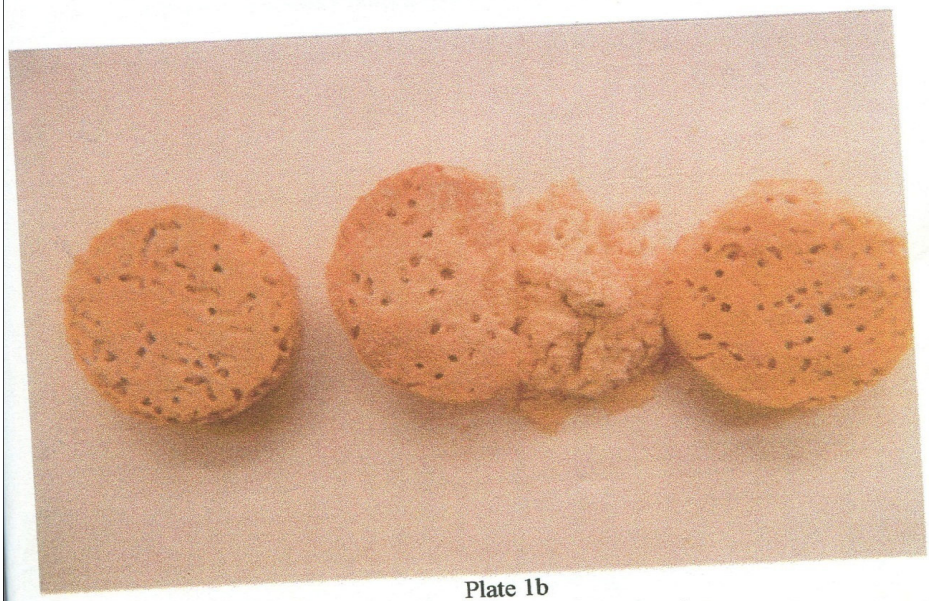

Plate 1b
Damaged maize biscuits by P. truncatus.
Biscuits used for collection of LGB eggs.

Success of Predation

The success of predation of *T. nigrescens* on the larvae of *P. truncatus* was determined as follows. Two, four, six, eight and ten third instar larvae of *P. truncatus* were placed in separate petri dishes. *Teretrius nigrescens* adults were taken out of the culture and placed in a container without food for 24 hours so as to starve them. One unsexed adult of *T. nigrescens* was then introduced into each of the petri-dishes containing the larval instars. There were eight replicates. The experiments were left for 24 hours in a climatic chamber set at 30˚C and 70± 1% r.h. created by using saturated salt solution. The experiments were placed in the upper chamber of the desiccators whilst the saturated salt solution was in the lower chamber of the desiccator. After the period of 24 hours the *T. nigrescens* were removed and the larvae of LGB were left in the temperature controlled chamber to develop to adults (Plate 4). The number of adults that developed in each container gave an indication of the number of larvae that were not preyed on by *T. nigrescens*. Predation rate was determine at the following condition of temperature and relative humidity: 25˚C, 70% r.h.; 25˚C, 75% r.h.; 25˚C, 80% r.h.; 30˚C, 75% r.h.; 30˚C, 80% r.h.; 35˚C70% r.h.; 35˚C, 75% r.h.; 35˚C, 80% r.h.

Since parasitisation in *A. calandrae* is exhibited by only the females that oviposit in the host, there was the need to sex this insect. *A. calandrae* were sexed with the help of Dr. Rich Hodges and Ms Lucy Birkinsshaw, both of Greenwich University. According to them, the male has a pale abdominal background while the entire

body of the female is dark black. The male is also smaller than a female of the same age.

Similar to the method used to estimate the success of predation on *P. truncatus* by *T. nigrescence*, two, four, eight and ten third instar larvae of *P. truncatus* were put in separate perti dishes. One female *A. calandrae* was introduced into each of the perti dishes containing *T. nigrescence* larvae for 24 hours.

The experiment was replicated eight times. After this, the parasitoids were removed, leaving the *P. truncatus* larvae in the controlled temperature and humidity chamber to develop to adults. The number of *P. truncatus* adults that emerged was noted. The number of mummies were also countered. The number of mummies indicated the number of larvae parasitised.

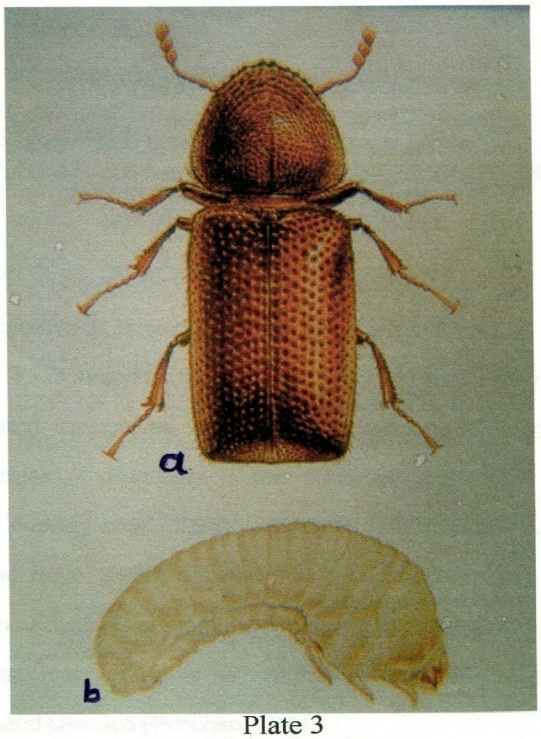

Plate 3
a. Adult *P. truncatus*
b. Larva of *P. truncatus*

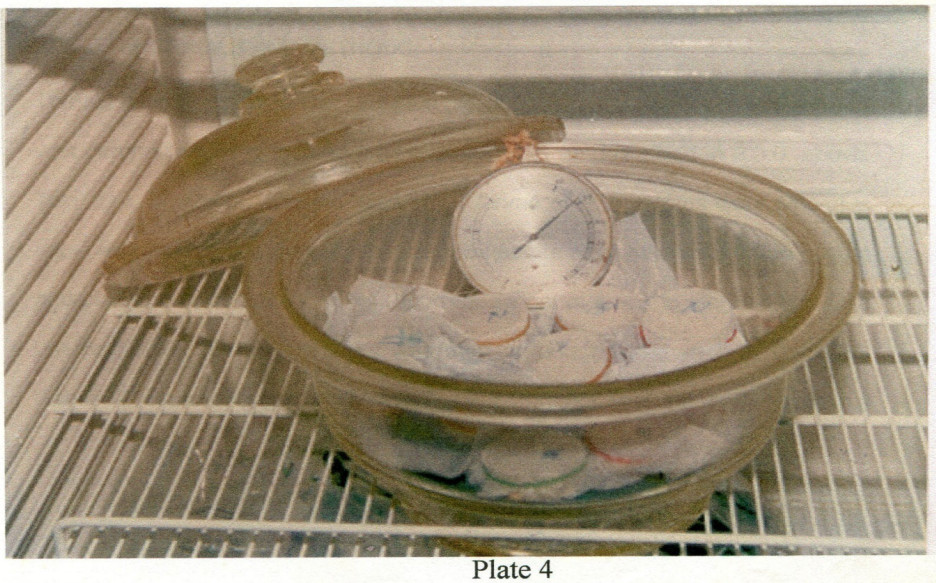

Plate 4
Experimental set up in a climatic chamber

ESULTS AND DISCUSSION

The mean number of *P. truncatus* larvae consumed by *T. nigrescens* is showed in Table 1.

Table 1. Mean number of *P. truncatus* larvae (±S.E) consumed by *T. nigrescens* in 24 hours under controlled condition of temperature and relative humidity.

Mean Level of Predation of LGB larvae by *T. nigrescens* under controlled conditions of temperature and relative condition

	Relative humidity (%)			
	70	75	80	Mean (˚C)
Temp. (˚C)				
25	2.05 ± 0.8 [a]	2.24 ± 1.1 [c]	2.19 ± 1.1 [b]	2.16 ± 0.10 [x]
30	4.33 ± 1.00 [a]	5.02 ± 0.10 [c]	4.49 ± 0.30 [b]	4.61 ± 0.47 [y]
35	2.17 ± 0.01 [a]	2.28 ± 0.90 [b]	2.16 ± 1.20 [a]	2.20 ± 0.70 [x]
Mean (r.h.)	2.85 ± 0.60 [e]	3.18 ± 0.70 [f]	2.95 ± 2.87 [e]	

*Means ± S.E followed by the same letter(s) in a row (and means / T ˚ in the column) are not significantly different (P > 0.05).
Fisher's Protected least Significant Difference (LSD)
Temperature = 0.28
Relative humidity = 0.22
Temperature* Relative Humidity = 0.32

(Temperature-humidity interaction)
The consumption rate of *T. nigrescens* is high. The results indicated that *T. nigrescens* optimum consumption was 5.02 ± 0.10 *P. truncatus* larvae during a 24 hour period at 30˚C, 75% r.h. Generally, *T. nigrescens* predation was highest at 30˚C and lowest at 25ºC and 35˚C. The most effective condition at which *T. nigrescens* preyed on *P. truncatus* were apparently at 30˚C, 75% r.h. (see Table 1). These findings agree with that of Leliveldt and Laborius (1990) who observed that *T. nigrescens* predation on LGB larvae was 4.9 ± 1.5 per 24 hour at 30 ˚C, 75% r.h.

In this work, predation at 35˚C, 70% r.h was higher than predation at 25˚C, 70% r.h. Earlier works by Rees (1985) and Boye (1988) established that *T. nigrescens* has an average consumption rate of 1.7 and 1.1 larvae of *P. truncatus* respectively when the test were performed in 6-hour daily light and dark cycle.

Probably, a lower temperature did not enhance voracious feeding. Higher temperature (e.g. 35˚C) was generally higher under all conditions of relative humidities tested. This may be due to attainment of optimum activity and generally high metabolic activity at 30˚C, 75% r.h. that probably enhanced more food consumption.

Comparison of data (arc sine, $P^{1/2}$, transformation) obtained using the two-way ANOVA, shows there was a significant difference between temperatures (F pr = 0.04; p < 0.01) but no significant difference between humidities (F pr = 0.02; p > 0.05). The analysis of variance also indicated that a temperature-humidity interaction influenced predation (p < 0.002). The results therefore showed that temperature and relative humidity had a marked effect on predation of LGB by *T. nigrescens.* The predation, however, varied considerably and was greatest at 30˚C, 75% r.h. and least at 25˚C, 70% r.h.

The number of *P. truncatus* larvae parasitised by *T. nigrescens* with 24 hour light is shown in Table 2.

Table 2. Mean number of *P. truncatus* larvae parasitised by *A. calandrae* in 24 hour light under controlled condition of temperature and relative humidity.

Mean Level of Parasitization of LGB larvae by *T. nigrescens* under controlled conditions of temperature and relative condition

	Relative humidity (%)			
	70	75	80	Mean (˚C)
Temp. (˚C)				
25	2.60 ± 1.00 [c]	2.2 ± 0.12 [a]	2.30 ± 0.50 [b]	2.30 ± 0.5 [a]
30	3.76 ± 0.20 [c]	3.08 ± 0.06 [a]	3.28 ± 0.03 [b]	3.37 ± 0.09 [c]
35	3.20 ± 0.11 [b]	2.28 ± 0.20 [a]	2.40 ± 0.01 [a]	2.63 ± 0.11 [b]
Mean (r.h.)	3.19 ± 0.44 [f]	2.52 ± 0.13 [e]	2.66 ± 0.18 [d]	

**Mean ± S.E followed by the same letter(s) in a row (and means / T˚ in the column) are not significantly different (P > 0.05).
Fisher's Protected LSD; Tempreature = 0.09 Relative humidity = 0.06
Tempreature*Relative Humidity = 0.16
Anisopteromalus calandrae is well known parasitoid of *Sitophilus spp* (Williams and Floyd, 1971; Press, 1992; Wen et al, 19940). The female are able to detect the larvae of the pest in the grain, and pierce through the grain, paralyse it and kill it (Cotton, 1923; van der Assem and Kuenen, 1958).

The number of *P. truncatus* parasitised by *A. calandrae* was found to be within the range of 2.20 ± 0.12 to 3.76 ± 0.20 at temperature range of 25˚C to 35˚C and relative humidity range of 70% r.h. to 80% r.h. The number of larvae parasitised was higher at 30˚C (3.76 ± 0.200 than at 25˚C or 35˚C. Also, more *P.truncatus* larvae were parasitised at 70% r.h. (3.19 ± 0.44) than at 75% r.h. or 80% r.h. (Table 2). Generally there were variation in parasitisation between relative humidities at 25˚C. At 30˚C there were high significant differences in parasitism between the relative humidities (Table 2).

Analysis of Variance shows that *A. calandrae* suppressed the population development of *P. truncatus*. This confirmed the results of Boye (1988) who observed that *A. calandrae* caused higher parasitisation on *P. truncatus* larvae. The analysis of variance further revealed that parasitisation was influenced by both temperature ($P < 0.001$) and humidity ($P < 0.002$). The effect of both temperature and relative humidity (temperature-humidity interaction) was also significant ($P < 0.01$). The results therefore show that parasitisation of *P. truncatus* by *A. calandrae* were affected by both temperature and humidity. This suggest that certain temperatures and relative humidities could affect the activity of the insect, and hence its parasitisation. The results reported here are therefore encouraging for *P. truncatus* control.

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