Parasitic capabilities of *Trichogrammatoidea* sp. and *Habrobracon hebetor* for biological control of *Noorda blitealis*, a defoliating caterpillar of *Moringa oleifera*

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Abstract

The damage caused by *Noorda blitealis* Walker (Lepidoptera: Crambidae) on Moringa, *Moringa oleifera* (Capparales: Moringaceae), a very important leafy vegetable in Niger, pushes producers to excessively use synthetic chemical pesticides. The present study was carried out in the laboratory for the parasitism test and at the station for the evaluation of the dynamics of predatory arthropods. The work aims on the one hand to test the parasitic capacities of two *Trichogrammatoidea* parasitoids sp. and *Habrobracon hebetor* respectively on the eggs and on the caterpillar of *N. blitealis* and on the other hand to evaluate the dynamics of the abundance of predator colonies in a Moringa plot, from a biological control perspective. At the end of these activities, the results obtained made it possible to determine that the average rate of emergence of individuals from a female of *Trichogrammatoidea* sp., on *C. cephalonica* eggs is 82.3% per day of infestation, or 13.6 individuals emerged per day of infestation. While the number of adults emerged from *N. blitealis* eggs is 4.1 individuals per day of infestation (with a rate of 62.2% per day), significantly lower compared to the emergence rate obtained eggs of *C. cephalonica*. We observed that a total of 32.8 individuals on average emerged from a batch of ten larvae of *C. cephalonica*, from a female of *H. bracon* significantly higher than the nine individuals emerged from a batch of ten larvae of *N. blitealis*. In short, *H. hebetor* and *Trichogrammatoidea* sp. do not appear to be good candidates for biological control agents against *N. blitealis*. The study of the dynamics of insect colonies predatory on the larvae and eggs of *N. blitealis*, namely, ants, spiders and mantises, shows that their populations are present on the plants of two varieties of *M. oleifera* almost during all year, with abundances varying greatly from one season to another.

Keywords: *Noorda blitealis*, *Trichogrammatoidea* sp, *Habrobracon hebetor*, *Moringa oleifera*, biological control, Niger

INTRODUCTION

Moringa (Capparales: Moringaceae) is becoming increasingly important in the Sahel and particularly in Niger. It is a source of income but also a food supplement, available almost all year round (Garba, 2000; Halilou, 2022). However, damage caused by the defoliating caterpillar, *Noorda blitealis* Walker (Lepidoptera: Crambidae), considered the main pest of Moringa, made producers to react by using more and more polluting chemical pesticides (pops), of dubious quality, often unapproved and banned by the Stockholm Convention (Abdoulaye *et al*., 2018; Ratnadass *et al*., 2011; Halilou *et al*., 2021). Indeed, in Niger, farmers are generally poor and are therefore deprived of the means enabling them to acquire good quality pesticides. In addition, these pesticides pose a contamination problem in the short or long term, depending on the nature of the molecule used in the treatments and the way in which they are applied (Bafada, 2019; Halilou *et al*., 2021).

Faced with the scale of this phenomenon linked to the abusive use of synthetic insecticides and the risks of contamination, significant efforts must be made to develop reasoned strategies to combat this pest. With this in mind, the use of biological control and varietal resistance can be interesting alternatives.

Biological control, considered one of the best alternative methods to chemical control, consists of using populations of parasitoids, predators, pathogens, antagonists or competitors to reduce a pest population, thus making it less abundant and therefore less damaging than it would otherwise be (Van Driesche and Bellows, 2001).

Indeed, the conservation of habitats and its management, at the scale of the plot or landscape, focused on agricultural practices, such as the non-use of synthetic insecticides, tending to promote the abundance of natural enemies, constitutes what is called a conservation biological control method (Van Driesche and Bellows, 2001). Thus, arthropods, particularly predatory insects and arachnids from the Formicidae, Mantidae and Spider families, constitute an enormous source of predation on *N. blitealis* populations in India, Sudan and Ethiopia (Nagusu, 2005; Satti *et al*., 2013 and Saha *et al*. (2014).

Furthermore, hymenopterous, oophagous and larval parasitoids are the most used in the method of augmentative biological control in the fields to fight against pests (Van Driesche and Bellows , 2001).

However, tests of the parasitic capacities (parasitism, emergence) of augmentative biological control agents must be carried out beforehand in the laboratory to determine their real potential (Amadou, 2019; Saidou *et al*., 2020).

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Based on the fact that, in general, parasitoid hymenoptera such as poachers and trichogramma are considered generalist parasitoids, especially with respect to insects of the Lepidoptera family. In addition, because all the results obtained by Karimoune (2018) showing that *Trichogrammatoidea armigera* (Hymenoptera: Trichogrammatidae), an oophagous parasitoid, was a potential candidate for the biological control against the millet ear miner caterpillar, *Heliocheilus albipuctella* de Jaons (Lepidoptera: Noctuidae) in the conditions of the Sahel, Niger. Several research studies have also demonstrated the effectiveness of another category of parasitoid, ecto -larval, namely *Habrobracon hebetor* (Hymenoptera: Braconidae) against this same pest (Ba *et al*., 2014; Amadou, 2019).

The objectives of this study are, on the one hand, to test the parasitic capacities of two *Trichogrammatoidea parasitoids* sp. and *H. hebetor*, respectively on the eggs and on the caterpillar of *N. blitealis* in the laboratory. On the other hand, to evaluate the dynamics of the abundance of predator colonies in the Moringa field with a view to develop an integrated management approach that is sustainable and respectful of the environment.

METHODOLOGY

Mass breeding of insects

Insects in cultivation include the Moringa defoliating caterpillar *N. blitealis*, the rice borer *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidde) and the egg parasitoid *Trichogrammatoïdea* sp. and *Habrobracon hebetor* implemented at the ICRISAT laboratory, Sadoré in Niger in 2020.

Mass breeding of *Noorda blitealis* was set up using larvae collected from Moringa plants within the Sadoré research station. The latter are kept in plastic boxes (12 cm x 14.5 cm height) and provided every morning with fresh leaves of *Moringa oleifera* collected from healthy plants not treated with insecticide according to the method described by Halilou (2023).

Mass production of *Corcyra cephalonica* was established from wild individuals collected in farmers' granaries. The insects are raised on a mixture of millet grains (1/3) and flour (2/3) in plastic buckets according to the method described by Ba *et al.* (2014).

Mass production of *Habrobracon hebetor* was carried out on *C. cephalonica* larvae in petri dishes according to the method also described by Ba *et al.* (2014).

The colony of *Trichogrammatoïdea* sp. was maintained using the method described by Karimoune *et al.* (2018) used for mass breeding of *Trichogrammatoidea armigera Nagaraja* (Hymenoptera : Trichogrammatidae). The colony was initially collected from sorghum fields from fall armyworm eggs on the ICRISAT station, Sadore in 2018.

Testing the parasitic capacity of *Trichogrammatoïdea* sp.

Ten pairs of *Trichogrammatoidea* sp., were each introduced into a box measuring 5.4 cm in height and 2.2 cm in diameter. The male of the trichogramma is generally distinguished from the female by the fact that the males' antennae bear bristles (Manjunath, 2016).

Each box contains a batch of 30 *N. blitealis eggs* previously irradiated in a dark room with the 4W UV tube lamp (UVP, USA, 254 nm) (Figure 1), for 30 minutes to slow down the embryogenesis process.

The batch of 30 eggs are glued, using a non-toxic glue (Figure 2A), on Bristol paper measuring 4 cm in height and 2 cm in diameter and carrying a few droplets of honey which serves as food to insects (Figure 2B).

Every day, a pair of *Trichogrammatoidea sp.* is transferred to a new box containing a new batch of 30 eggs. The male that dies is replaced by a new one and the death of the female puts an end to the experiment. The same experiment was carried out with *Corcyra* eggs *cephalonica* under the same conditions for comparison. The boxes containing the parasitized eggs are placed in incubation until the emergence of new *Trichogrammatoidea* prog-

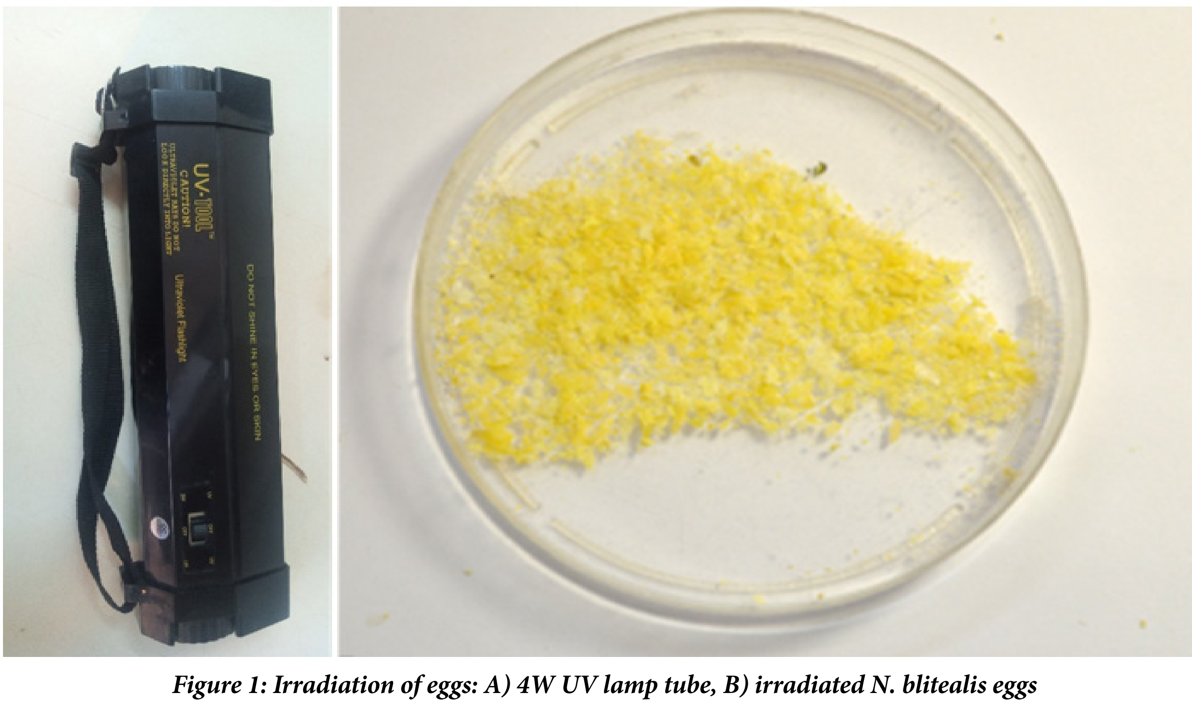

Figure 1: Irradiation of eggs: A) 4W UV lamp tube, B) irradiated N. blitealis eggs

eny. These experiments made it possible to determine, depending on the host species, the rate of parasitism, the number of eggs laid per female and the number and sex of adults emerged per female.

Testing the parasitic capacity of *Habrobracon hebetor*

This test was carried out by introducing newly emerged *H. hebetor* adults into a mating cage for 24 hours and a cotton ball soaked in 10% honey solution.

Subsequently, ten females were introduced, each into a Petri dish containing ten 4th instar larvae of *N. blitealis*. Infested petri dishes were incubated until *H. hebetor* progeny emerged (Figure 3). At emergence, male and female adults are collected and counted. The same experiment was carried out with the 4th instar larvae of *Corcyra cephalonica* under the same conditions for comparison.

These experiments made it possible to determine, depending on the host species, parasitism, the longevity of females and the emergence of adults according to sex.

Evolution of predator colonies on Moringa during the year

Field experimental

The on station experiments took place from August 2020 to July 2021 at the International Institute for Crops Research in Semi-Arid Tropical Zones (ICRISAT) in Sadoré, in the Sahel bioclimatic zone. The station is located in the southwest part of Niger (13°15 north latitude and 2°18 east longitude) in the Tillabéri region (Niger), Kollo Department, Youri commune. The experiment was carried out in a completely randomized design comprising two treatments and three repetitions (=block) in an area of 12 m x 14 m. The treatments correspond to two varieties of *M. oleifera,* namely the Local variety and the improved PKM1 variety. Each plot is made up of 9 plants of a variety planted on 3 lines of 3 plants at spacings of 1m x 2 m. Each block includes 1 plot of each of the 2 varieties, i.e. 2 plots per block.

Experimental conduct

The dynamics of predator colonies on the two varieties of *M. oleifera* during the year was monitored. These are ants, spiders and praying mantises collected weekly on plants of each of the two varieties tested. All the plants in each of the 6 plots (54 plants in total) are observed every week, i.e. a frequency of four weeks per month during the 12 months of the experiment. Monthly accumulations of average numbers of predators per plant were calculated according to the time of year expressed in months.

Data analysis

Data were analyzed using SPSS software (IBM SPSS Statistics Version 25). Descriptive statistics (means and standard errors) were calculated for the different variables. The data were tested for statistical significance of the means using ANOVA (analysis of variance) compared by Student Newman Keuls tests at the 5% level. And when the ANOVAs are significant, the means are subjected to a Post hoc test using the Ficher test in order to determine the smallest significant differences (LSD) under SPSS.

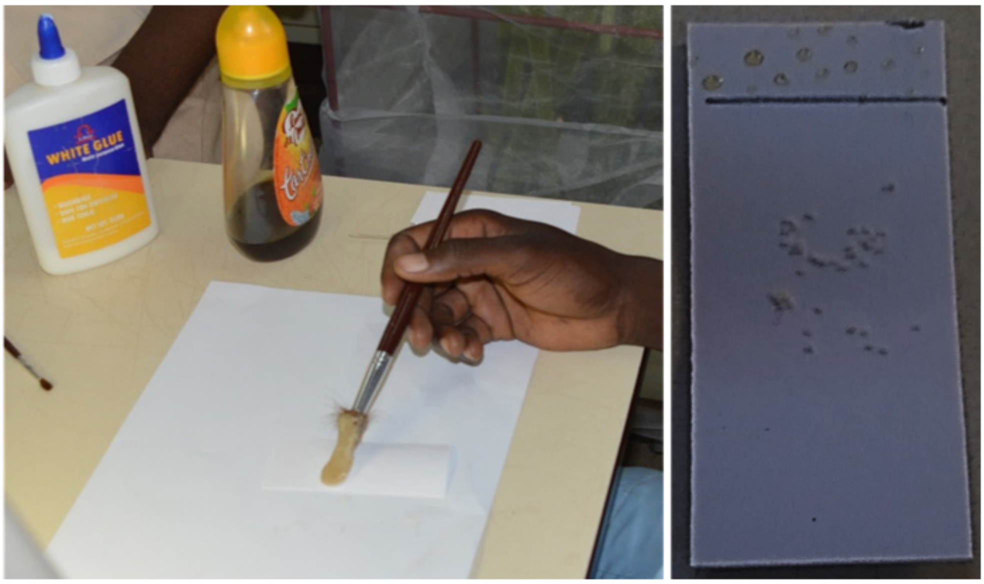

Figure 2: Preparing an egg plot: A) Spreading glue on the Bristol paper, B) Bristol paper carrying the eggs and honey droplets

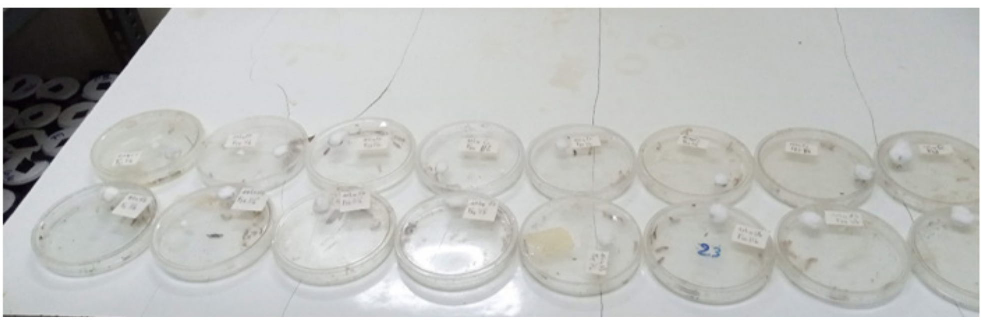

Figure 3: Parasitized larvae incubated for emergence

RESULTS

Parasitism and emergence of *Trichogrammatoidea* sp. on eggs of *N. blitealis* and *C. cephalonica*

Rate of daily egg parasitism

Figure 4 illustrates average parasitism rates based on host species and day of infestation. The rate of parasitism on eggs of the species *C. cephalonica* oscillates on average from 49 to 70% between the first and fourth day of infestation from where it begins to decrease and gradually up to 32% on the tenth day of infestation. On *N. blitealis* eggs, the rate of parasitism reached its maximum on the second day of infestation with approximately 41%. This rate decreases to between 17 and 39% between the 3rd and 7th day after infestation and then continues to drop to 1% on the 10th day after infestation. Host species significantly influenced egg parasitism with higher rates recorded on eggs of the species *C. cephalonica* compared to *N. blitealis* regardless of the rank of the day of infestation. The number of *N. blitealis* eggs parasitized per female *Trichogrammatoidea* sp., are on average 6.1 eggs per day of infestation, an average rate of 20.3%. While up to 16.4 eggs of the *C. cephalonica species* were parasitized with an average rate per day of infestation of 54.7%. Female longevity is significantly longer on *C. cephalonica* eggs with an average duration of 12.4 ± 0.9 days compared to the duration observed on the eggs of the species *N. blitealis* which is 9.2 ± 0.7 days.

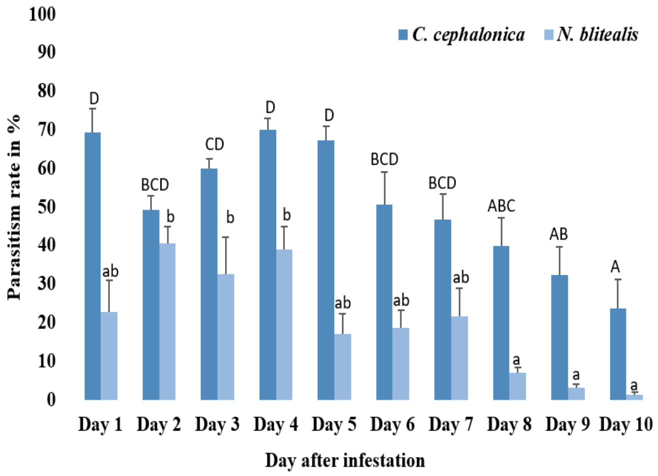

Figure 4: Rat of daily egg parasitism according to host species

Daily emergence rate of adults

C. cephalonica eggs without any significant difference between the days of infestation and from 20 to 73% for the species *N. blitealis* (Figure 5). The rank of the day does not influence the emergence rate on *N. blitealis* eggs from the 1st to the 9th day of infestation. The rate recorded on the batch of infested eggs on the 10th day is significantly lower compared to the first 9 days of infestation (Figure 5). The same is true for the average number of individuals emerged. Indeed, an average of 13.6 individuals per day of infestation emerged on *C. cephalonica* eggs, i.e. a rate of 82.3%, with a female/male sex ratio of 1.3/0.7. This number of adults emerged from the eggs of *C. cephalonica* is

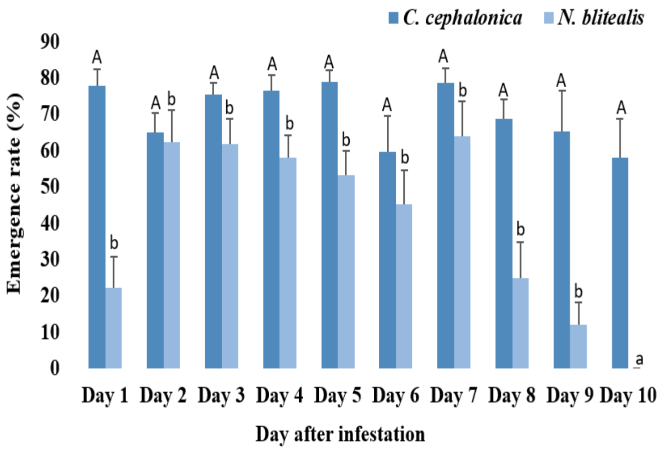

Figure 5: Daily egg emergence rate depending on host species and day after infestation

significantly higher than the 4.1 individuals emerged per day of infestation on the eggs of *N. blitealis*, i.e. a rate of 62.2%, with a sex ratio Female/Male 1.8/0.5.

Parasitism and emergence of *Habrobracon hebetor* on L4 larvae of *N. blitealis* and *C. cephalonica*

Figure 6 illustrates the number of male and female adults and the totals of individuals emerged from the batch of ten infested larvae per female depending on the host species. Larval parasitism was 100% on the two species tested from the first 24 hours after infestation. We observe that a total of 32.8 individuals on average emerged from a batch of ten larvae of *C. cephalonica*, from a female of *H. bracon*, with a sex ratio of 53.6% female. While only nine individuals on average emerged from a batch of ten *N. blitealis larvae* with a sex ratio of 69.0% female. The host species significantly influenced both the number of emerged male adults ($F_{(1, 18)} = 16.8$; $P = 0.001$), and the number of emerged female adults ($F_{(1, 18)} = 16.7$; $P = 0.001$) and the total number of individuals emerged ($F_{(1, 18)} = 35.6$; $P < 0.001$).

It was observed that the female candidate of *H. hebetor* lived for about nine days on average on the larvae of *C. cephalonica* compared to 7.3 days on *N. blitealis*. The duration of pre-imaginal development of male adults was 13.5 days for the larvae of two species but for female adults pre-imaginal development lasted 13.1 days on average at the level of species *N. blitealis* against 12.8 on the larvae of the species *C. cephalonica*.

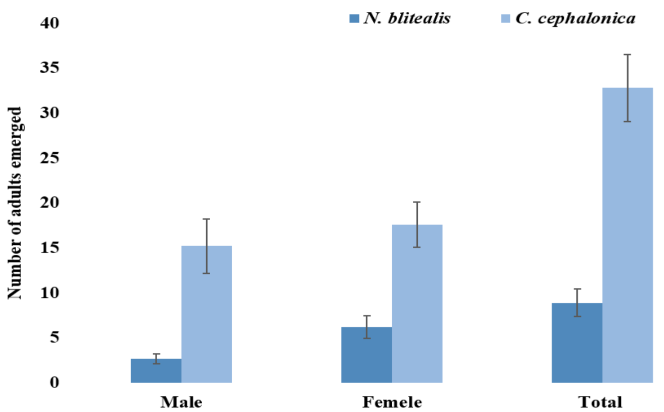

Figure 6: Average number of adult males and females of H. hebetor emerged depending on the host species

Evolution of colonies of generalist predators on the two varieties of *Moringa oleifera* during the year

The study of the dynamics of colonies of insect predators of larvae and eggs, namely, ants, spiders and mantises, shows that their populations are present on the plants of two varieties of *M. oleifera* almost throughout the of the year, with abundances varying greatly from one season to another.

Evolution of ant colonies

The ant population increases gradually from August to reach its peaks in October with an average of 69 individuals on V-Locale and 210 on V-PKM1 per plant, then decreases sharply from November to December. Variety does not significantly influence the number of ants whatever the time of year (Figure 7).

Evolution of spider colonies

The average monthly number of spiders varies from 0 to 10 individuals per plant regardless of the variety. We observe progressive growth from the month of August to reach the peak in October with an average number of 28 individuals per plant on V-Locale and 46 on V-PKM1 (Figure 8).

Evolution of mantis colonies

The mantis is almost absent during the period from March to July. It appears intensively and reaches its peak in the same month of August with an average of 6 individuals on V-Locale and 10 on V-PKM1 per plant, decreasing gradually from October to February (Figure 9).

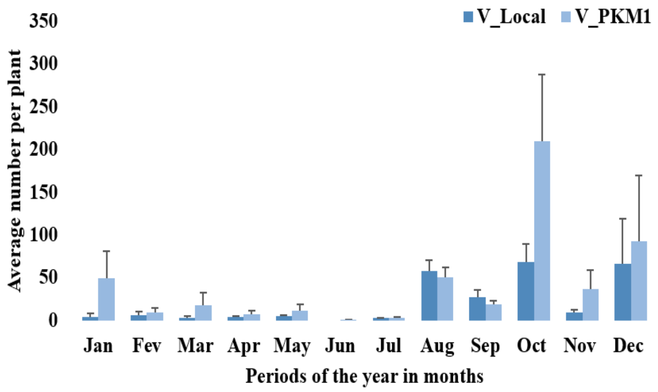

Figure 7: Dynamics of ant populations per plant during the year on two varieties of *M. oleifera*

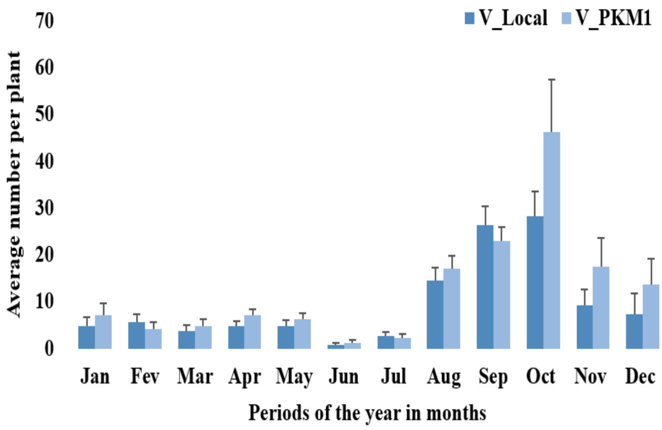

Figure 8: Dynamics of the abundance of spider populations per plant during the year on two varieties of *M. oleifera*

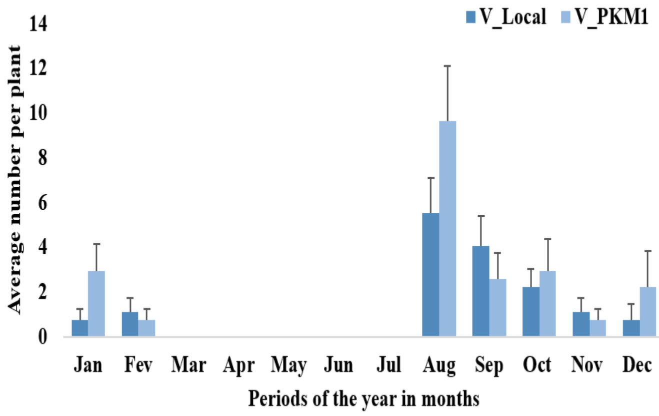

Figure 9: Dynamics of the abundance of mantis populations during the year on two varieties of *M. oleifera*

DISCUSSION

The present laboratory study made it possible to obtain low rates of parasitism of *N. blitealis eggs* by *Trichogrammatoidea* sp., low adult emergence rates and the cumulative number of parasitized eggs per female during her life, compared to the different rates obtained with the eggs of *C. cephalonica*. However, it has been reported that in Indonesia, *T. armigera* parasitizes a whole range of insect pests of the order Lepidoptera including species of the family Crambidae such as *Crocidolomia pavonana* Fabricius (= *C. binotalis*) (Lepidoptera: Crambidae) and *Scirpophaga incertulas* Walker (Lepidoptera: Crambidae) (Buchori *et al.*, 2008). But the results of this study are similar to those obtained by Karimoune *et al.* (2018) according to whom, the eggs of species of the Crambidae family, namely *C. ignefusalis* and *N. blitealis,* were weakly parasitized in the laboratory by a parasitoid of the same genus as that which was tested, *Trichogrammatoidea armigera*. Knowing that an endogenous parasitoid can only be used in the biological control of a pest when it can cause a satisfactory rate of parasitism (Karimoune *et al.* 2018; Van Lenteren *et al.*, 2018). These results show that *Trichogrammatoidea* sp. cannot be designated as a good candidate that can serve as a successful biological control agent against *N. blitealis*. The poor performance of *Trichogrammatoidea* sp. on *N. blitealis* eggs could be due to variation in their egg nutritional quality, which varies from one host species to another as has been observed with other parasitoid trichogramma species (Saidou *et al.*, 2020; Karimoune *et al.*, 2018; Kishani *et al.*, 2016).

As for the test of the parasitic capacity of *Habrobracon hebetor* (Say), the results of the study obtained in the laboratory showed that the number of emerged adults was also very low on the larvae of *N. blitealis* compared to the number obtained on the larvae of *C. cephalonica.* However, *H. hebetor* (Say) (Hymenoptera: Braconidae) is an ectolarval parasitoid that attacks a wide range of insect pests of crops and stored goods of the order Lepidoptera (Amadou *et al.*, 2017; Ba *et al.*, 2014; Milonas, 2005). The results of this study also reveal that *H. hebetor* does not develop optimally on *N. blitealis* larvae.

The low emergence rates of *H. hebetor* and *Trichogrammatoidea* sp. can be explained by the fact that Hymenoptera generally parasitize and lay eggs on the majority of Lepidoptera species, especially in situations of «no choice» as occasional hosts. While emergences depend on several parameters including the quality and biochemical content of the host species (Karimoune *et al.*, 2018).

In short, *H. hebetor* and *Trichogrammatoidea* sp. do not appear to be good candidates for biological control agents against *N. blitealis.*

The observations also interested the colonies of certain species of predatory arthropods, generalist in nature, which shelter the Moringa plants. Ants, mantises and spiders were almost present in the plots throughout the year with more intense invasions during the periods from September to October. These periods correspond to times when *N. blitealis* infestations are more significant, in particular, the cessation of rains creates favorable conditions for its development until before the onset of the cold seasons, December to February in the conditions of Niger (Halilou *et al.*, 2021; Halilou, 2023).

The predatory habit is widespread in the insect and arachnid classes. Juvenile predators use their prey for growth, while adults use them for maintenance and reproduction (Van Driesche and Bellows, 2001).

Previously studies carried out by Berg and Cock (1993) and Nagusu (2005) clearly showed the regulatory role of ants (*Myrmicaria* sp) and mantises on populations of *N. blitealis*. Predatory ants (Formicidae) can be a huge source of non-specific predation and play an important role in suppressing pests in forests and crops (Weseloh, 1990). Conservation of native ant species can be an important source of natural pest control and deliberate manipulation of ants to control citrus pests was practiced in China over 1900 years ago (Coulson *et al.*, 1982). However, ants should not be introduced outside their native range, because the generalist nature of their predation and the numbers that some species can reach per hectare are such that native invertebrates may be threatened by predation or competition (Howarth, 1985). In China and Yemen, ant colonies have been moved between sites to control pests of tree crops of citrus (*Citrus* spp) and dates (*Phoenix dacylfera*) (Coulson *et al.*, 1982). The Mantidae group constitutes a family of large, mainly tropical predators with rapacious forelegs. Some species have been introduced to new areas, for example the Chinese mantis, *Tenodera aridifolia sinensis*, and the European mantis, *Mantis religious*, both introduced to the United States.

Saha *et al.* (2014) reported that spiders frequently harbor in large numbers on buds thereby contributing to the regulation of the growth of *N. blitealis* populations. The role of spiders in biological control is different from that of hymenopteran parasitoids which have been widely used when introduced to new locations to control specific immigrant pests (Van Driesche and Bellows, 2001). Most spiders lack host specificity, but exhibit habitat specificity. As such, they are poorly suited for introduction to new regions to control specific pests, but instead may be better utilized using agricultural practices that conserve native spiders for the suppression of pest groups in crops (Van Driesche and Bellows, 2001). Characteristics of spider biology that have an important influence on their action as biological control agents include the relatively high number of spiders per unit of land surface and the responsiveness of their movements within and of crops to local conditions of heat and humidity. In China, spiders have also been manipulated for pest control purposes (Sparks *et al.*, 1982).

Practices of using predators in pest control are thousands of years old and were developed by farmers through direct observation of these predators, which are large enough to be visible and whose life cycles are easy to understand.

CONCLUSION

The inconclusive results of laboratory tests, the parasitic capacities of *H. hebetor* and *Trichogrammatoidea* sp., as part of this study did not allow us to repeat the station tests. Because the performance of these two parasitoid biological control agents is not up to par on populations of *N. blitealis*. It is therefore crucial to continue to prospect for parasitoids dependent on *N. blitealis* in the field.

However, the presence and importance of the three recorded colonies of these predators, namely ants, mantises and spiders, were promising and call into question the application of agricultural practices likely to protect them in order to contribute to ecologically balancing the damage of *N. blitealis*. Thus the use of biopesticides and other reasonable control methods that can allow predators to establish themselves should be encouraged and integrated into programs to combat *N. blitealis* in particular and other pests of Moringa and other crops in general.

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