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Natural Enemy: An Underutilized Cornerstone of Integrated Pest Management of *Hymenia recurvalis* on Amaranthus species

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Abstract

This research has been focused toward identifying non-chemical methods of pest control, which are safe, cheap, easy to access, and eco-friendly. Therefore, this study was to investigate the potential of using natural enemies for the management of *Hymenia recurvalis* in Ibadan, Nigeria. The study assessed the function of natural enemies (predatory insects, pathogens, parasitoids) in the management of *Hymenia recurvalis* on three Amaranthus species. Samples were collected biannually over a two-year duration. The experiment was designed in a randomized complete block design, with three treatments and four replicates. The three treatments were neem extract, control group (water only) and karate. Laboratory analysis enabled the identification and quantification of natural enemies. Statistical comparisons were conducted to examine seasonal variations and differences among Amaranthus species. Data collected were analyzed using descriptive statistics and ANOVA at $P<0.05$. Twelve predators and three larva and pupa parasitoids were encountered on *Amaranthus* species throughout the seasons. Of great importance are parasitoids which caused significant nonproductive larval mortalities in the host plants. The implications of these findings for mass rearing of the parasitoid as well as for conservation and augmentative biological control of amaranth lepidopteran leaf-webbers in Southwest Nigeria are examined and discussed

Keywords: *Hymenia recurvalis*, natural enemies, biological control, amaranthus, integrated pest management

Introduction

Amaranth production suffers a severe setback due to insect infestation (Seni, 2018). Over-reliance on organochlorides and organophosphates or their derivatives as a control approach for pests is facing criticism due to the growing influence on the environment and health of human beings and their animals (Lengai *et al.*, 2020). This is attributed to the persistence and bioaccumulation of toxic residuals in soils (Hierlmeier *et al.*, 2022). However, environmentally sustainable pest management measures involving the use of biological control agents, insecticides derived from natural sources, cultural control of pests, and judicious use or complete abstention from persistent pesticides are the way ahead in the management of insect pests (Kagali, 2014). An efficient integrated pest management strategy includes accurate identification of insect pests and a full knowledge of their biology as a critical pillar to their control (Fahad *et al.*, 2021). This requires monitoring the pest population as well as tracking the stage of insect development and only deploying control methods when the pest numbers reach economic damage thresholds (Morrison III *et al.*, 2021). However, natural enemies including predators, pathogens, and parasitoids are ignored biological agents in the Integrated Management of lepidopteran insect pests (Furlong, 2015). They are known to inhibit the growth of the insect pest population (Fischbein and Corley, 2022). But, the excessive use of toxic and broad-spectrum pesticides in controlling these insect pests on the host plant has weakened the role of natural enemies as a crucial component of the IPM block-chain of lepidopteran pests (Anderson *et al.*, 2019). Meanwhile, biological management has been regarded as an environmentally acceptable method of pest control (Kidanu and Hagos, 2020). But the single curtailable unfavorable effect of imported natural enemies is their prospective impact on non-target species (Hepler *et al.*, 2020). This loophole necessitates the establishment of acceptable international phytosanitary protocols (IPPs) for the introduction of exotic natural enemies (STA, 2017) to ensure the safety of biological control agents (BCAs) in the local ecosystem since international trade of agricultural products is indispensable (Abd-Elgawad, 2020). In the past, efforts have been made to deploy a generic natural enemy in controlling lepidopteran pests. For instance, microbial control agents, entomopathogenic fungi (EPF) play a crucial role in controlling insect pests in humid tropics (Rajula *et al*., 2020). Several studies have proved the efficacy of entomopathogenic fungi against various pests on plants (Sani *et al*., 2020). The ovicidal and pupicidal activities of this fungus have been reported in various lepidopteran pests (Raguvaran *et al.*, 2022). *Paecilomyces farinosus* (Holmsk) have also been proven to infect and kill larvae of leaf webbers like Psara basalis Walker and *S. recurvalis* on amaranth (Othim and Tarmogin, 2019). Microbial pesticides based on the soil-borne bacterium *Bacillus thuringiensis* (Berliner) (Bt) are among the most commonly administered kinds of biopesticides (Belousova *et al*., 2021). In combination with other biological control agents or on their own, *Bacillus thuringiensis* formulations have been found to be effective against a spectrum of lepidopteran parasites (Singh *et al*., 2019). Due to their high toxicity to some pests, compatibility with integrated pest management (IPM) strategies, narrow host specificity, high amenability to genetic engineering, and safety to non-target species, these formulations represent a workable substitute to conventional pesticides (Ndolo *et al*., 2019). Additionally, it has been disclosed that formulations of the bacterium *Bacillus* *thuringiensis* are useful in minimizing leaf webbers including *S. recurvalis* and *Herpertogramma bipunctalis* (F) (Lepidoptera; Crambidae) (Omburo, 2016). Natural enemies, which include both parasitoids and predators, are vital in regulating parasite populations (Othim and Tarmogin, 2019; Omburo, 2016). Several parasitoids have been connected to webworms, including egg parasitoids like Trichogramma species (Hymenoptera: Trichogrammatidae) and larval parasitoids like Apanteles species, Cardiochiles species, Phanerotoma species, *Cotesia marginiventris* Cresson (Hymenoptera: Braconidae), Campoletis species, *Venturia infesta* species (Hymenoptera: Ichneumonida). However, there is still a scarcity of comprehension on the natural adversaries associated to some parasites (Lotfalizadeh and Mohammadi-Khoramabadi, 2021). In order to establish particular natural adversaries of *H. recurvalis* on Amaranthus species, a critical component of an integrated pest management strategy in the Amaranthus ecosystem, the study was carried out. Therefore, the study was conducted to establish specialized natural enemies of *H. recurvalis* on Amaranthus species, an undervalued cornerstone of an integrated pest management strategy in the Amaranthus ecosystem.

Materials and Methods

Survey and identification of natural enemies of *H. recurvalis*

A survey of predators related to the Amaranthus plant was conducted following the initial observations of the predating activities of numerous ant species on *Hymenia recurvalis* larvae and eggs in the field. In the laboratory, the effectiveness of the identified ant species as predators was subjected to testing. A sampling of ant species was done on a monthly basis from February 2009 on Amaranthus grown at a spacing of 5 cm by 30 cm in absolute monoculture. Each *Amaranthus* plant was thoroughly inspected for ant species around the stems, branches, flowers, leaves, and soils around the bases of plants. Camel hairbrushes were utilized to facilitate the collection of small and fragile insects. Ants were swept into 2 lb-sized Kilner jars containing cotton wool soaked with 95% ethyl acetate which was then covered with filter paper. A few ants were placed in containers without ethyl acetate for future examination in the laboratory. The ants' species seen were later identified at the Insect Reference Collection Centre in the Department of Crop Protection and Environment Biology at the University of Ibadan employing taxonomic keys, a hand lens as well as a light microscope for checking fine features which are referred to as the small and intricate characteristics of insects that are difficult to see with the naked eye, such as their compound eyes, the number and shape of segments on the antennae, the arrangement of veins on the wings, arrangement of hairs, the shape and size of mouth parts and the texture of the exoskeleton. These fine features are often crucial in identifying different species of insects, and therefore, a hand lens as well as a light microscope may be required to examine them in detail. Other characteristics of the ant-plant interactions and the uniqueness of the connections between the *Amaranthus* plant and its ant occupants, as well as the occurrence of homopterans (scale insects and aphids), were examined in the field.

Predatory efficiency of ants on *Hymenia recurvalis* in the laboratory

An experiment was set up to examine the predatory efficiency of ant species as natural enemies of *Hymenia recurvalis* eggs and larvae. Field-collected ant species were separately put into transparent plastic rearing containers. These containers were put with tissue paper at the base to minimize dampness. A leaf with one field-collected larva was inserted inside each container. The leaves were replaced often over the duration of the observation. Five ants of the same species and the same age were later introduced separately into each of these containers and replicated five times. The containers were covered with plastic lids that featured a portion of roughly 14 cm by 12 cm (length x width) made of muslin cloth to allow for aeration. The setup was positioned randomly on an elevated platform inside the Entomology Laboratory, Department of Crop Protection and Environmental Biology, University of Ibadan, for observation. A control experiment with the leaves and larvae was equally set up without any of the ant species. The temperature and relative humidity were maintained at ambient levels of 27 ± 30C and 75 ± 3%, respectively. The set-up was monitored and reviewed at two hourly intervals for a length of 5 days. Data acquired on mortality of juvenile stages of H. recurvalis at 24hrs after the introduction of ant species were examined using ANOVA and descriptive statistics.

Host Plants

Amaranthus cruentus, a local vegetable species of amaranth, was developed and employed under laboratory conditions in experimental cages at the Entomology Laboratory, Department of Crop Protection and Environmental Biology, University of Ibadan. The selection of this cultivar was based on its widespread cultivation and consumption in Nigeria, rapid growth rate, and its huge leaves. The seedlings were grown in trays in the screen-house from seeds acquired from a certified distribution source, the National Horticultural Research Institute (NIHORT), Ibadan, Nigeria.

***Hymenia recurvalis* Colonies**

Colonies of *Hymenia recurvali*s were reared and maintained in the laboratory at CPEB on *Amaranthus cruentus* for eight generations preparatory to their trial use. In June and July 2009, at the Farm Practical Year Training Plot in Ibadan, a comprehensive field assessment on amaranth lepidopteran pests led to the initial acquisition of the adults and larvae of *H. recurvalis*. The moths were placed in transparent ventilated rearing cages with a sleeve and a netting material at the back. They were fed on cotton wool which was saturated with a 10% solution of honey as a source of sustenance and introduced into the potted amaranth plants for oviposition at 25 ± 2°C, 50–70% RH, and 12:12 L:D (Photoperiod) (Photoperiod) (Photoperiod). The plants were collected every 24 h and stored in separate holding wooden cages with ventilation on the sides and at the top until the eggs hatched. Newly hatched larvae were allowed to feed on the live plants for 3 days and then placed into vented plastic lunch boxes lined with paper towels to absorb excess moisture. Fresh amaranth leaves were supplied to the larvae as a food source until pupation. The pupae were nurtured under equivalent rearing settings until adult emergence.

Colonies of *Apanteles hymaniae*

A colony of the braconid wasps larval endoparasitoid, *A. hymaniae* was established at the CPEB laboratory from pupal samples collected during a survey conducted in the University of Ibadan Practical Farm Training Plot in March 2009. Adults were placed in a ventilated Perspex cage with a sleeve on one side and fed honey from strips of paper. The rearing was conducted at 25 ± 2°C, 50–70% RH, and 12:12 L: D photoperiod. Potted plants containing 3-d-old larvae of *H. recurvalis* were then introduced into the cage for the parasitoids to oviposit. The exposed larvae were removed on a daily basis and placed in ventilated plastic lunch boxes lined with a paper towel. Fresh amaranth leaves were added into the lunch boxes as required until pupation. The parasitoid pupae were harvested and transferred in clean Petri dishes (9 cm diameter), into a Perspex cage under similar conditions for adult emergence. These parasitoids were mass-reared separately on each host pest until the fifth generation to ensure adaptability before their use for experiments.

Data Analysis

The data obtained from plant height, leaf area, stem girth, number of insects, number of defoliated leaves per plant, and yield of Amaranthus. The experiment was designed in a randomized complete block design, with three treatments and four replicates. The three treatments were neem extract, control group (water only) and karate. Data collected were analyzed by ANOVA and descriptive statistics and significant means were separated using Student Newman Keuls (SNK) ($P < 0.05$).

Results

The occurrence and diversity of ant species associated with *Hymenia recurvalis* and their associated coccoids varied with the number of leaves on susceptible *Amaranthus* sp. in Ibadan Southwest, Nigeria as shown in Table 2. The highest ant species (37.21) was *Pheidole megacephaly* associating with the mealybug, *Planococcus citri* followed by *Neivamyrmex apacithorax* (26.19) which was not significantly ($p>0.05$) different from *Dorymyrmex insanus* (23.83) population while *Odontomachus clarus* (13.41) was the least. In general, there were three parasitoids *A. hymaniae* and *A. marginiventris* (Cresson), an unidentified Ichneumonid, probably *Xanthopimpla emaculata*, and an unidentified Braconid while the other rubber fly, *Efferia pogonias* (Plate 1A), Ants of various species (e.g. Plate 1C) as earlier mentioned, Assasin bug, *Rhynocoris bicolor*, Beetles of various species (e.g. Plate 1B), Dragonfly, *Ophiogomphus susbehcha*, and Grasshopper of various species (e.g. Plate 2A) are predators of *Hymenia recurvalis*.

Table 2: Occurrence and diversity of ant species associated with *Hymenia recurvalis* and their associated coccoids on *Amaranthus* sp. in Ibadan Southwest, Nigeria (n=10)

Natural Enemy	Subfamily	No./Plant	No. of leaves	Coccoids
Pheidole megacephaly	Myrmicinae	37.21a	12.37a	*Planococcus citri/ Toxoptera auranti*
Crematogaster larreae	Myrmicinae	18.16c	5.13c	*Planococcus citri*
Dorymyrmex insanus	Dolichoderinae	23.83b	6.15c	*Planococcus citri*
Neivamyrmex apacithorax	Ecitoninae	26.19b	7.21b	*Planococcus citri*
Camponotus laevigatus	Formicinae	20.26c	6.37c	*Planococcus citri*
Odontomachus clarus	Ponerinae	13.41d	4.87c	*Planococcus citri*

Means followed by the same letter in the same column are not significantly different ($P>0.05$) using Student Newman Keuls (SNK) ($P < 0.05$)

A

B

C

Plate 1: Natural enemies: A= Grey predatory rubber fly *Efferia pogonias* with *Hymenia recurvalis* moth as prey; B= African black beetle adult, *Heteronychus arator* feeding on 1^{st} Instar of *Hymenia recurvalis*; C= Ant *Crematogaster larreae* predating on the 5^{th} Instar of *Hymenia recurvalis*.

A

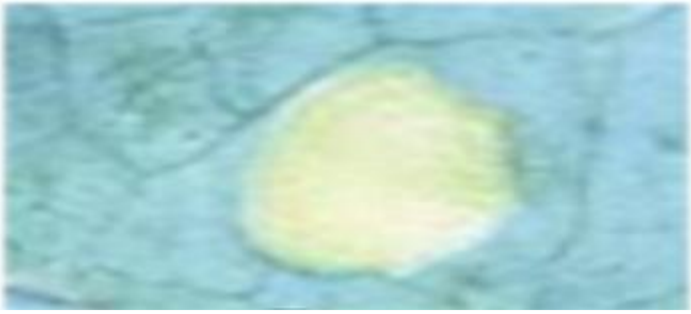
B

C

Plate 2: Other Natural Enemies A=Short-horn grasshopper on *Amaranthus blitum* during the prevalence of immature stages of *H. recurvalis*. B= Salk-eyed fly, *Diopsis longicornis* feeding on eggs of insects other than *H. recurvalis*. C= Parasitoid wasp (*Apanteles hymeae*) bred from parasitized pupae.

A

B



C

Plate 3: A= Ants tendering aphids (*Myzus persicae*) for their honeydew on Amaranthus leaf**,** B=Aphids (*Myzus persicae*) coexisting with mealybug on *Amaranthus cruentus*, and C= Newly laid eggs (in batch) of *Hymenia recurvalis* at the underside of *Amaranthus* leaf as viewed under microscope.

Predatory efficiencies of natural enemies on *Hymenia recurvalis*

The predatory efficiency of six collected ant species in the laboratory varied with the number of *Hymenia recurvalis* (Table 3). *Pheidole megacephaly* had the highest predatory efficiency on *H. recurvalis* with mean mortalities of 100.0%, 100.0%, 100.0%, 85.2%, 68.1%, and 62.3% recorded 1, 4, 6, 10, 13, and 18 days after eclosion (DAE) at 24hours after treatment, respectively (Table 3). The Big-headed ants, *Pheidole megacephaly* killed and ate up all the first, second, and third instar larvae and no remains were left. *Neivamyrmex apacithorax* caused 63.1% kill of first instar 24 at the end of 24 hours, while *D. insanus* had 51.2% predation on the first instar within the same period. All the ant species exhibited varying degrees of predation on different stages of *Hymenia recurvalis* larvae in the Cage. Larvae in the control set-up were all intact at the end of the 24 hours (Table 3).

Table 3: Percentage Mortality of immature stages of Beetworm Moth, *Hymenia recurvalis* by ant species in laboratory cage at 24 hrs after treatment.

Ant species	Days After Emergence					
	1	4	6	10	13	.18
Pheidole megacephaly	100.0a	100.0a	100.0a	85.2b	68.1c	62.3d
Crematogaster larreae	47.1a	33.1b	30.4b	19.1c	11.0d	7.9d
Dorymyrmex insanus	51.2a	42.0b	45.6b	25.0c	19.7d	10.3e
Neivamyrmex apacithorax	63.1a	61.9a	64.0a	30.1b	21.1c	15.3d
Camponotus laevigatus	48.0a	36.0b	32.9b	21.3c	13.1d	9.3e
Odontomachus clarus	45.0a	27.1b	23.9b	15.0c	10.1d	3.3e
Control	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a

Means followed by the same letter along the same row are not significantly different (P>0.05) using Student Newman Keuls (SNK) ($P < 0.05$)

Percentage mortality of *H. recurvalis* by parasitoid *Apanteles hymeniae* varied significantly across immature stages of *H. recurvalis* infested in the laboratory as presented in Table 4 matrix. An adult of *Apanteles hymeniae* (Cresson) was bred out of infested immature stages of *H. recurvalis* in the laboratory (Plate 2C).

Table 4: Mortality Percentage of Hymenia recurvalis Immature Stages Induced by Apanteles marginiventris Parasitoid (n=10)

Life Stage	Total	1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar	6th Instar	Pupae	Adult	% Mort.
1st Instar	-	54.3	-	-	-	-	-	-	-	256.5
2nd Instar	-	56.6	54.3	-	-	-	-	-	-	190.4
3rd Instar	-	62.5	56.6	54.3	-	-	-	-	-	120.1
4th Instar	-	62.3	62.5	56.6	54.3	-	-	-	-	80.9
5th Instar	-	60.5	62.3	62.5	56.6	54.3	-	-	-	60.7
6th Instar	-	58.7	60.5	62.3	62.5	56.6	54.3	-	-	50.3
Pupae	-	68.9	58.7	60.5	62.3	62.5	56.6	54.3	-	35.6

Note: The values represent the percentage of mortality for each life stage induced by Apanteles marginiventris parasitoid.

Discussion

Insects inhabit host plants for varying purposes such as feeding, protection, shelter, and reproduction. However, some insect pests utilize host plants for all three activities. A specific example is the beetworm moth (BWM), *Hymenia recurvalis* which lays its eggs on *Amaranthus* leaves, later hatched into larvae that feed voraciously and completed their life cycles under the webbed leaves. Meanwhile, other categories of insects called natural enemies that prey on beetworm moths are either for direct feeding or as a prerequisite intermediate host to complete their life cycle. One of the ways by which the activities of *H. recurvalis* could probably be checkmated is by planting Amaranthus varieties with erect leaves to distort nocturnal feeding habit by *Hymenia recurvalis* (Ujagir and Byrne, 2009). In this study, ants of varying species were found in a tri-trophic relationship in the *Amaranthus* ecosystem. Aphids engage in a detrimental relationship by sucking sap from *Amaranthus* shoots and making it become stunted while the injured shoot is predisposed to viral infection. On the other hand, ants engaged in a symbiotic relationship with aphids. Ants harvest honeydews from aphids while aphids enjoy protection from ants. This relationship can be broken to deter *H. recurvalis* adults from laying eggs on the host plant. In this regard, an artificial diet that is preferable to ants could be formulated and placed in strategic positions on the *Amaranthus* farm. Parasitoids develop on or in a single insect host and finally kill the host. Adults are typically free-living and may be predators. They may also feed on other resources, such as honeydew, plant nectar, or pollen. However, the irreplaceable roles of parasitoids in this study cannot be overemphasized. Two major braconid wasps were bred out from mummified larvae and pupae collected from the field. *Apanteles marginiventris* was bred out from mummified larvae while *A. hymeniae* was bred out of mummified pupae. This implies that mass rearing and release of these parasitoid wasps into the *Amaranthus* field are vital to maintaining an ecological balance in ecosystems because of their ability to respond in a density-dependent fashion to the population number of their hosts thereby regulating the population density of BWM below an economic threshold level. One of the major findings of this study was the predatory capacity of the rubber fly, *Efferia pogonias H. recurvalis* moth. Other important insect predators observed in the study site are assassin bugs, *Rhynocoris bicolor*, beetles of various species, dragonflies, *Ophiogomphus susbehcha*, and grasshoppers of various species. However, insects that were previously of little economic concern often become severe pests when liberated from the control of their natural enemies. On the other hand, when a non-toxic technique is identified to control a primary pest, the lower consumption of pesticides and higher survival of natural enemies often reduce the numbers and damage of secondary pest species. Moreover, Insects are also attacked by various species of nematodes that inflict disease or death. Under certain climatic settings, diseases may arise and spread naturally among an insect population.

Conclusion

One predator, *Efferia pogonias*, rubber fly (Moth predator), 4 species of ants (egg and larva predator), and one parasitoid, *Apanteles hymeniea* (pupal parasitoid), were identified in this study to have promising potential as a biological control method of *H. recurvalis*. However, from this study, it is evident that no single biological control agent can give outright control of insect pests. Therefore, the natural enemies encountered in this study can be developed further to form a major blockchain in the integrated management of *H. recurvalis* and other insect pests of Amaranthus.

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