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acta SATECH 11 (1): 69 – 77 (2019)

Journal of Life &Physical Sciences

Evaluating larval pathogens influencing larval population of *Cirina forda* westwood (Lepidoptera: Saturniidae) on the field in two local government areas of Niger State, Nigeria.

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Abstract

The *C. forda* larvae when processed are cheap alternative protein source for less privileged farmers. The processed larvae are good source of animal feeds, cost effective, with nutritive and commercially viable. Unfortunately, there are entomopathogenic factors depleting their population. Fifteen Individually handpicked larvae from each replicate of indisposed, sluggish and dying or recently dead individual larvae from each site of 10m by 10m quadrat throws were sampled. *C. forda* larval population handpicked were transferred under sterile condition for pathogenic examination of parasitic agents. The thoracic and abdominal regions of each larva were lacerated using sterile forceps to examine under a dissecting microscope for parasitic microbes. The teased out visceral contents were stained and examined microscopically after placing on a flame to remove air bubbles. The isolates from the larvae were identified. The results revealed Gurara LGA had, two virus- *Nuclear Polyhedrosis Virus* (NPV) and *Granulosis Virus (*GV) with (75.5% and 24.5%) respectively and high intensity of occurrence. In Katcha LGA recording four , 44.2% of *Nuclear Polyhedrosis Virus* (NPV) impacted more followed by 29.3% of (*Nomeraea rileyi (*Nr) and 15.1% of *Beauveria bassiana* (Bb) with the least of 11.4% of *Granulosis Virus (*GV) across all the sites.

Keywords: Pathogens, Larval, Population, Field.

Introduction

Ande (2003) posited that both the mature larvae and penultimate larvae showed mineral composition that will meet man's daily needs and compared the mineral composition of the larvae with other food items. That *C. forda* larvae proved to be a better source of mineral salts with respect to Ca, Cu, Fe, Mg, Mn, P, K, Se, Na, S, and Zn. Omotosho (2006) and Ande (2002 reported similar ranges of Sodium and Potassium contents of about 45.00 mg/100g and 65.00 mg/100g, respectively. Paiko *et al*, (2014) documented that a Calcium concentration of about 31.21 ± 0.09 mg/100 g in *C. forda* caterpillar and suggested that this amount of calcium could improve the calcium intake of the body and therefore play a role in blood clotting process. Paiko *et al*, (2014) documented that *C. forda* has a desirable high crude protein content of about 64.28% on dry weight basis. *C. forda* is reported to be rich in essential amino acids largely due to reasonable concentrations of amino acids such as threonine, leucine, valine, phenylalanine, isoleucine, methionine, threonine, and lysine (Ande, 2002). Unfortunately, this important larvae's population is threatened by many factors among them are entomopathogens and their individual effect on the larvae had not been worked upon.

The aim of the study is therefore to survey the larval entomopathogens influencing the larval population of *C. forda* in the wild.

Significance of the study is to find ways to improve the larval population so that the nutritional requirement of poor resource farmers can be improved.

Materials and methods

The *Cirina forda* larvae, through handpicking of 15 individual each from each replicate (15 by 3) of indisposed, sluggish and dying or recently dead individual larvae from each of the three sites from 10m by 10m quadrat throws as *C. forda* larval population was difficult to estimate because they were quite mobile particularly at later instars. The thoracic and abdominal regions of each larva were lacerated using sterile forceps and the content examined under a dissecting microscope for parasitic agents. The teased out visceral contents were also carefully stained and examined microscopically under high power magnification (500x) after placing on a flame to remove air bubbles (Odebiyi *et al*, 2003b), for pathogenic viral agents. Surveillance for organisms infesting the larvae of *C. forda* entailed scrutiny of the body and vicinity of the *C. forda* with the aid of hand lenses and binoculars (MARCO-model 750/8 m-988000 m), dissecting and phase contrast microscopes and direct visual assessment as the case may require. Sample from each larval suspension was diluted to one ml saline solution and inoculated inside of unrefrigerated cabinet maintained at 22^0 C and 70% RH for 25 days. The isolates from the larvae were identified according to (Glare and Inwood, 1998).

Results and discussion

In Gurara, *Nuclear Polyhedrosis Virus* (NPV) and *Granulosis Virus (*GV) were pathogens identified with very (75.5% and 24.5%) high intensity of occurrence in the Local Government Area (Table 1). The role of each pathogen in terms of intensity of occurrence was recorded as followed. NPV had the highest infection in the local government area. The highest value was recorded in Sarkin Fulani site (39), followed by Kudan with 38, Fulani settlement with 36, Suleiman Pnapi recorded35, Galadima resident site with 33, Domi had 32 and Tanko Gbeyidna site with 30. The site with the highest value of GV infestation was Tanko Gbeyidna with 15 larval infestation rate followed by Domi site with 13, Galadima site 12, Suleiman Pnapi site with 10, and Sarkin Fulani site with 6.

Nuclear Polyhedrosis Virus (NPV) :- This virus strain is found protected in a polygonal shaped capsid. This capsid enables the virus to invade the cells more easily and helps in rapid reproduction and multiplication of the virus. When the protected capsid is liberated inside the host, the virus strains are ruptured into the tissues and start reproduction and pathological changes begin to appear with symptoms showing on susceptible larvae when ingested the contaminated foliage as the larvae are vegetarians. The host will eventually die by turning black as it is decaying (Plate 1)

Granulosis virus (GV). This is another important pathogen with over 28.8% infection across the local government areas. The infection by GV differs from NPV because GV- infected larvae immediately turned milky not black and did not feed. But in both situations the body tissues of the dead larvae are liquid ruptures and then release the infectious virus. The dead larva from the virus infection occurs between 1 and 4 days under laboratory room temperature condition (Plate 2)

Reports from Table 2 showed that in Katcha Local Government Area, four pathogens were recorded instead of two from Gurara such that (44.2%) of *Nuclear Polyhedrosis Virus* (NPV) impacted more followed by (29.3%) (*Nomeraea rileyi (*Nr) (Plate 3) and (15.1%) *Beauveria bassiana* (Bb) (Plate 4), and the least was (11.4%) *Granulosis Virus (*GV) across all the sites probably because all sites in Katcha local government area had unique average altitude above sea level, this under investigation. In the Local Government Area, Kansannagi site had the highest value with 21; Kataeregi site also along with Kansannagi and Kpatamisu had highest value followed by Badeggi site with 20, Eshanti worth 20, and Ganabigi with 20 larval infestation then Ebba and Kambari with19 and Goyinekeni site least value of 18.

*Nomeraea rileyi (*Nr) recorded the highest entomopathogenic fungus infesting the larvae more at Badeggi (15), Eshanti (15), and Ganabigi (15) sites followed by Kataeregi (13), Kansannagi (13), and Kpatamisu (13). Ebba took turn with 12 along with Kambari (12) and Goyinekeni (11). *Beauveria bassiana* (Bb) followed as the third most infesting pathogen but more in Goyinekeni (9) then Ebba and Kambari with 8, Kataeregi, Kansannagi and Kpatamisu with 7 each and least Badeggi, Eshanti, Ganabigi with 5 each. *Granulosis Virus* (GV) was the least in the Local Government Area with highest value in Goyinekeni (7) and Ebba and Kambari (6) each. The dead larvae featuring this disease were frequent in Katcha LGA with infection rate of 29.35%. The infected larvae were more often inactive and became stiff just before their death.

Beauveria bassiana is an entomopathogenic fungus. *Beauveria bassiana* infection was 15.09% in Katcha LGA only. It is often found in an upright position; more often attached to a stem in the upper part of the host trees.

The biological agents include predators and pathogens (Muhammad and Ande, 2014) but intensity of each pathogen was revealed in this work. Gurara and Katcha LGAs had pathogens identified with very high intensity in all the sites with *Nuclear Polyhedrosis Virus and Granulosis Virus* but in addition Katcha LGA reported additional two *Nomuraea rileyi and Beauveria bassiana* fungal infection recorded as observed by (Muhammad and Ande, 2014). Certain factors still under investigation made the development of fungi (*Nomuraea rileyi and Beauveria bassiana)* favorable in Katcha Local Government Area. All these entomopathogens recorded contributed to the reduction in population values of *C. forda* larvae significantly, especially at the mature larva stage of fourth and fifth instars.

Conclusion. *C.forda* larvae the most destructive stage of the insect life cycle and the edible stage had more factors that could exterminate its population on the field.

Acknowledgement; Effort of the local government staff is hereby acknowledged.

Plate 1. *Nuclear Polyhedrosis Virus* infested larva hanging head down turning in death.

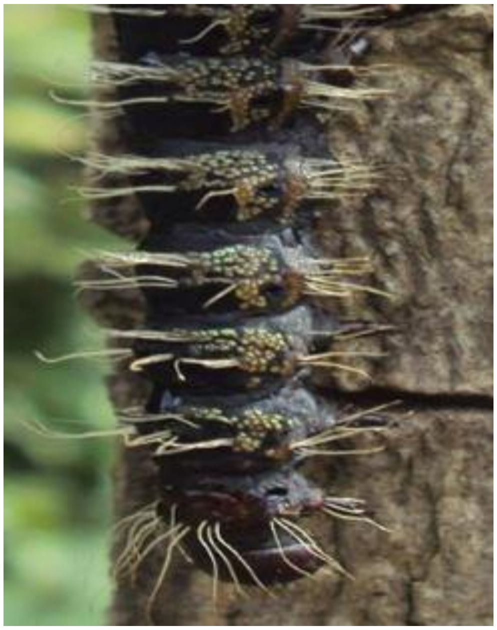

Plate 2. *Granulosis Virus* infested larva turned milky and ceased feeding

Plate 3 is the infested larva on the field with *Nomeraea rileyi (*Nr)

Plate 4 is the infested larva on the field with *Beauveria bassiana* (Bb)

Table 1: Identities and intensities of various viral pathogens associated with *C. ford*a larvae in the various sites of Gurara LGA of Niger State

LGA	Sites	Organisms Identified	Taxonomy	Intensity of occurrence
(Gurara)	Kudan	1.*Nuclear Polyhedrosis Virus*	Baculoviridae:Eubaculovirinae	1. NPV-38
		11. *Granulosis Virus*	Baculoviridae: Eubaculovirinae	11. GV- 7
	Domi	1.*Nuclear Polyhedrosis Virus*	Baculoviridae:Eubaculovirinae	1. NPV-32
		11. *Granulosis Virus*	Baculoviridae: Eubaculovirinae	11. GV-13
	Fulani settlements	1.*Nuclear Polyhedrosis Virus*	Baculoviridae:Eubaculovirinae	1. NPV- 36
		11. *Granulosis Virus*	Baculoviridae: Eubaculovirinae	11. GV- 9
	Galadima resident	1.*Nuclear Polyhedrosis Virus*	Baculoviridae:Eubaculovirinae	1. NPV- 33
		11. *Granulosis Virus*	Baculoviridae: Eubaculovirinae	11. GV- 12
	Tanko Gbeyidna	1.*Nuclear Polyhedrosis Virus*	Baculoviridae:Eubaculovirinae	1. NPV-30
		11. *Granulosis Virus*	Baculoviridae: Eubaculovirinae	11. GV- 15
	Sarkin Fulani	1.*Nuclear Polyhedrosis Virus*	Baculoviridae:Eubaculovirinae	1. NPV- 39
		11. *Granulosis Virus*	Baculoviridae: Eubaculovirinae	11. GV-6

Table 2: identities and intensities of various viral pathogens associated with *C. ford*a larvae in the various sites of Katcha LGA of Niger State

LGA	Sites	Organisms Identified	Taxonomy	Intensity of occurence

(Katcha)	Badeggi	1.*Nuclear Polyhedrosis Virus* 11. *Granulosis Virus* 111.*Nomuraea rileyi* IV.*Beauveria bassiana*	Baculoviridae:Eubaculovirinae Baculoviridae: Eubaculovirinae Monililes: Moniliaceae Hypocreales:Cordycipitaceae	1. NPV- 20 11. GV-5 111.Nr-15 1V.Bb-5
	Kataeregi	1.*Nuclear Polyhedrosis Virus* 11. *Granulosis Virus* 111.*Nomuraea rileyi* *IV.Beauveria bassiana*	Baculoviridae:Eubaculovirinae Baculoviridae: Eubaculovirinae Monililes: Moniliaceae Hypocreales:Cordycipitaceae	1. NPV-21 11. GV-4 111.Nr-13 1V.Bb-7

Ebba	1.*Nuclear Polyhedrosis Virus* 11. *Granulosis Virus* 111.*Nomuraea rileyi* *IV.Beauveria bassiana*	Baculoviridae:Eubaculovirinae Baculoviridae: Eubaculovirinae Monililes: Moniliaceae Hypocreales:Cordycipitaceae	1. NPV- 19 11. GV- 6 111.Nr-12 1V.Bb-8
Eshanti	1.*Nuclear Polyhedrosis Virus* 11. *Granulosis Virus* 111.*Nomuraea rileyi* *IV.Beauveria bassiana*	Baculoviridae:Eubaculovirinae Baculoviridae: Eubaculovirinae Monililes: Moniliaceae Hypocreales:Cordycipitaceae	1. NPV- 20 11. GV- 5 111.Nr-15 1V.Bb-5
Kansannagi	1.*Nuclear Polyhedrosis Virus* 11. *Granulosis Virus* 111.*Nomuraea rileyi* *IV.Beauveria bassiana*	Baculoviridae:Eubaculovirinae Baculoviridae: Eubaculovirinae Monililes: Moniliaceae Hypocreales:Cordycipitaceae	1. NPV-21 11. GV- 4 111.Nr-113 1V.Bb-7
Ganabigi	1.*Nuclear Polyhedrosis Virus* 11. *Granulosis Virus* 111.*Nomuraea rileyi* *IV.Beauveria bassiana*	Baculoviridae:Eubaculovirinae Baculoviridae: Eubaculovirinae Monililes: Moniliaceae Hypocreales:Cordycipitaceae	1. NPV- 20 11. GV-5 111.Nr-15 1V.Bb-5
Goyinekeni	1.*Nuclear Polyhedrosis Virus* 11. *Granulosis Virus* 111.*Nomuraea rileyi* *IV.Beauveria bassiana*	Baculoviridae:Eubaculovirinae Baculoviridae: Eubaculovirinae Monililes: Moniliaceae Hypocreales:Cordycipitaceae	1. NPV- 18 11. GV-7 111.Nr-11 1V.Bb-9
Kambari	1.*Nuclear Polyhedrosis Virus* 11. *Granulosis Virus* 111.*Nomuraea rileyi* *IV.Beauveria bassiana*	Baculoviridae:Eubaculovirinae Baculoviridae: Eubaculovirinae Monililes: Moniliaceae Hypocreales:Cordycipitaceae	1. NPV- 19 11. GV-6 111.Nr-12 1V.Bb-8
Kpatamisu	1.*Nuclear Polyhedrosis Virus* 11. *Granulosis Virus* 111.*Nomuraea rileyi* *IV.Beauveria bassiana*	Baculoviridae:Eubaculovirinae Baculoviridae: Eubaculovirinae Monililes: Moniliaceae Hypocreales:Cordycipitaceae	1. NPV- 21 11. GV-4 111.Nr-13 1V.Bb-7

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