STUDIES ON ACTIVE CHANGES IN DEFENSE RELATED ENZYMES IN PEPPER RESPONSES TO *HELICOTYLENCHUS MULTICINCTUM* AND *MELOIDOGYNE INCOGNITA* INFECTIONS

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SUMMARY

An experiment was conducted at the screen house of Niger State College of Agriculture, Mokwa to evaluated nine accessions and a variety of pepper *Capsicum* species for changes in activities of phenolic compounds accumulation in relation to resistance or susceptibility to combined parasitism of *Helicotylenchus multicinctum and Meloidogyne* incognita. The trial was laid out using completely randomized design with three replications. Each of the accessions and the variety was inoculated with approximately 2000 mixed population, 1000 each of *H. multicinctum and M. incognita* on the root and adhering soil. Root samples were collected a day before inoculation and at day 7, 14 and 21 post inoculation. Standard methods were employed to quantified phenolic content and tannins. Changes of pepper phenolic contents on total antioxidant capacity of peroxidase, polyphenol oxidase and tannins on their response to *H. multicinctum and M. incognita* infection were recorded. In comparison, extracts from the roots of five pepper accessions viz NGB00581, NGB00587, NGB00629, NGB00684 and NGB00574 had high concentrations of peroxidase, polyphenol oxidase and tannins as compared to the remaining varieties tested, indicating the strong level of their responses to combined *H. multicinctum and M. incognita* attack. Findings from this study suggest the potential of pepper phenolics in reducing nematode infection in pepper production. The result of the present study shows that five of the ten pepper accession/variety possess high quantities of natural antioxidants and can be further investigated for possible use in the management of pepper nematode infections.

Keywords: Accession, Antioxidant, Infection, Phenolic, Nematodes

PLANT-PARASITIC nematodes are important pest of crop. Abundant in nature, they are found in association with virtually all important agricultural crops and constitute a significant constraint to global food production (Gregory *et al*., 2017). Over the years, the relationship between plants

and nematodes has led to the evolution of the plant-parasitic nematodes studies. As widely spread pathogens affecting vascular plants, huge losses in crop yields have been accredited to the incidence of plant parasitic nematodes (PPN). The complex relationship between plants and parasitic nematode has resulted in an "evolutionary arms race". Parasitic nematodes have developed strategies to suppress host plant immune responses for the growth of feeding sites. Therefore, plants have evolved specialized molecules to identify pathogens signaling the establishment of immune responses. Researches in alternative nematode control methods are gaining attention, as a result of decline in use of chemical pesticides. The use of nematode-resistant cultivars in crop breeding programs is an efficient nematode management strategy (Gregory *et al*., 2017)

Pepper plant is composed of significant quantity of phenolic compounds that includes; phenol, tannin and antioxidants (Ogunlade *et al.,* 2012). Varieties of secondary metabolites, a biologically active substance, produced by plants take part in plants defense against insect pests and diseases (Pagare *et al*., 2015). Phenolic compounds, alkaloids and terpenoids form the major categories of secondary metabolites. Of these three, phenolic compounds are often used to refer to a group of structurally different plant secondary metabolites (Mintel *et al*., 2017). They have in common an aromatic ring containing single or multiple hydroxyl substituents. Mainly, the phenolic compounds are polyphenols and are classified into five groups: - phenolic acids and simple phenols, flavonoids, phenyl propanoids, quinone and tannins (Mintel *et al*., 2017). The metabolism and activation of phenolics in plants, in responding to attack or injury by plant pathogens, like nematodes, fungi, bacteria and viruses, have been investigated and documented (Orhi and Pannu, 2010; Kihika *et al*., 2017; Oliveira *et al.,* 2019). Natural resistance is considered by many when accessible, to be the best alternative for the management of plant- parasitic nematodes, for the reason that it is not only compatible with other management methods but it is cost-effective and eco-friendly (Molinari, 2011). Ravindra *et al.* (2014) reported differential host response of some pepper varieties to *M. incognita*. The objective of the present study was therefore, to evaluate the

activity of polyphenol oxidase, peroxidase, Tannin and the phenolic content in pepper roots before and after inoculation with mixed populations of *H. multicinctum*and *M. incognita*

MATERIALS AND METHODS

Description of the Study Location

The experiment was conducted at the screen house of the Niger State College of Agriculture, Mokwa. Geographically, Mokwa is located on Latitude 9.3044^{o} N and Longitude 5.06 6^{o} E of the Equator, situated in the Southern Guinea Savanna agro - ecological zone of Nigeria. The trial was conducted during the 2018 cropping season. Mokwa has a mean annual rainfall of 1200 mm, which normally begins in April and ends in October. The temperature ranges between 35 and 37.5 ^{o}C, with relative humidity between 40 and 80% (Anon., 2018).

Sources of planting materials

Nine accessions and a variety of the red pepper seeds (*Capsicum* species) which belong to the *Capsicum frutescens*: sweet pepper (Atarodo) bird pepper (Ata wewe) and *Capsicum frutescens* (cayenne pepper) (Ata sombo) and *Capsicum annum* were used. Nine of the accessions: NGB00574, NGB00581, NGB00586, NGB00587, NGB00624, NGB00629, NGB00631, NGB00684 and NGB00702 were obtained from the National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan and the local variety was purchased from Adalinci Agrochemical store, Mokwa, Niger State, Nigeria.

Raising of pepper seedlings in the nursery and transplanting

Nurseries for each of the accession/variety were raised separately in plastic pots of 14 cm ×15 cm filled with 3 kg heat-sterilized soil as described by Paiko *et al*. (2019). Two seedlings each of the pepper accession/variety were transplanted at four weeks old into 3 kg plastic pots containing heat sterilized soil, shaded and watered as required.

Preparation of nematode inocula

Helicotylenchus multicinctum was isolated from pepper plants during a survey of plant parasitic nematodes in Niger state, Nigeria and cultured on plantain plants in IITA, Ibadan as described by Speijer and De Waele, 1997), and was used for the nematode treatment. Nematodes were collected from the plantain infected roots in distilled water using White head tray method and applied on the roots and adhering soils. For the *M. incognita,* infected galled roots were collected from pepper during a survey of plant parasitic nematodes in Niger state, Nigeria and cultured on tomato. The galled tomato roots were uprooted, washed, chopped and the egg mass extracted. Second-stage juveniles (J2) were recovered from hatched eggs by incubation in sterile distilled water at 28 ± 1°C. The suspensions were made up with distilled water until 1000 juveniles/adults nematodes were counted for each.

Inoculation

A week after the establishment of the seedlings, each pot was inoculated with approximately 2000 mixed population, 1000 each of *H. multicinctum* and *M. incognita* nematode. The 20 ml aqueous nematode suspensions were poured into three holes, 2 - 4 cm deep around the roots of the plants. The plant in the control pots with no nematodes received 20 ml distilled water.The trial was laid in Completely Randomized Design (CRD) and replicated 3 times in a screen house with a photoperiod of 12 h and an ambient temperature of 24±4◦C, and irrigation done as required.

Treatments and samples collection

A week, after the establishment of the seedlings, treatment was applied to each pot with inoculation of 2000 mixed nematode population, 1000 each of *H. multicinctum* and *M. incognita*. The 20 ml aqueous nematode suspension (Juveniles/Adults) of the treatment was poured into three holes, 2 - 4 cm deep around the roots of the plant. Similarly, the soil in the control pots (a day before inoculation) received 20 ml of nematode-free water. The holes were created using pencil. Tagged root pieces from each accessions and the variety were removed, chopped, snap-frozen in liquid N_2

and stored at −80◦C a day before inoculation or sample at 0 days. The plants were maintained in the screen house for another 7 days. At day 7, day 14 and day 21, one each of the remaining root pieces per plant was collected, chopped, snap-frozen in liquid N_2 and stored at −80◦C.

Enzyme extraction and assay

Enzyme extraction and assay were done following the Aguilar *et al*. (2000) method. About 1 ml pre-cold 0.5 M Sodium acetate buffer of pH 5 was added to each 1 g root sample in 2 ml Eppendorf tube, after which 5 mg of polyvinyl pyrolidone was added. The mixture was centrifuged at 14000 rpm for 20 min at 4˚C. The supernatant was collected into new tubes and used for polyphenol oxidase (PPO), peroxidase (PO) and tannin enzymes assay.

Peroxidase (PO) Assay

The assay of Peroxidase was done by adding 0.67 ml of enzyme extract to 1 ml of reaction substrate containing 80 ml of 0.1M Sodium phosphate buffer of pH 6, 1 ml of 1mM H_2O_2 and20 ml guaiacol and incubated at 25˚C till used. Spectrophotometer was used to record changes in absorbance at 470 nm at intervals of 3s per minute. Blank was prepared from reaction substrate without enzymes extract. The activity was expressed as changes in absorbance at 410 nm expressed as changes in the absorbance unit g^{-1} tissue according to the formula described by Kokkinakis and Brook (1979) as below:

$$\text{Unit g} - \text{1 tissue} = \frac{\text{optical density} \times \text{dilution factor}}{\text{g of tissue used in the assay}} \times 100$$

Polyphenol oxidase (PPO) activity

The activity of polyphenol oxidase was determined by observation in colour change intensity of pyrrol oxidation products. The reaction mixture consisted of a 100 µl of the enzyme extract of each sample added to 1.5 ml of 0.2 M sodium acetate buffer at pH 5 and temperature of 4˚C, modified by replacing pyrogallol with catechol at 200 µl of 0.02 M. The activity was expressed as changes

in absorbance at 410 nm expressed as changes in the absorbance unit g^{-1} tissue according to the formula described by Kokkinakis and Brook (1979) as below:

$$\text{Unit g} - 1 \text{ tissue} = \frac{\text{optical density} \times \text{dilution factor}}{\text{g of tissue used in the assay}} \times 100$$

Tannins Activity

The concentration of tannin was determined by adding 2 ml of the aqueous pepper extract to 2 ml of distilled water, and 2 drops of diluted ferric chloride solution was thereafter added. A blue-green or dark green coloration showed the presence of tannins

RESULTS

Qualitative phenol screening of pepper plants infected with H. multicinctum and M. incognita

The qualitative phenolic screening of the pepper root extracts studied (Table 1) revealed that Peroxidase (PO) at a day before inoculation, tested positive (showed presence of the enzymes) in only four **(**NGB00574, NGB00624, NGB0062 and NGB00684) of the nine accessions and a varieties evaluated, while at day 7, all tested negative (absence of the enzymes). However, at day 14 and 21, all tested positive (showed presence of the enzymes**.** Similarly, Polyphenol Oxidase (PPO) in the nine accessions and a varieties analyzed tested negative (absence of the enzymes) at both a day before, 7 and 14, except for NGB00631 that tested positive at the 14^{th} day**;** they were all tested positive (showed presence of the enzymes) at day 21 in five of the nine accessions and a variety **(**NGB00586, NGB0**0**624, NGB00629, NGB00631 an LV (Table 1)

However, qualitative phenolic screening showed that (Table 1) Tannins at a day before inoculation all tested negative (absence of the enzymes) with the exception of NGB00587**,** NGB00631 and NGB00684 and LV that were positive. At day 7, 14 and 21, the pepper root extracts tested positive (showed presence of the enzymes) for all the accessions and a variety (day 21).

Table 1: Phytochemical test of root extracts of nine accessions and a variety of Pepper inoculated with *H. multicinctum and M. incognita*

Pepper varieties	A day before inoculation	Peroxidase (PO)			0 Day	Polyphenol Oxidase (PPO)			0 Day	Tannins		
		Day 7	Day 14	Day 21		Day 7	Day 14	Day 21		Day 7	Day 14	Day 21
NGB00574	+	-	+	+	-	-	-	-	-	+	+	+
NGB00581	-	-	+	+	-	-	-	-	-	+	+	+
NGB00586	-	-	+	+	-	-	-	+	-	+	+	+
NGB00587	-	-	+	+	-	-	-	-	+	+	+	+
NGB00624	+	-	+	+	-	-	-	+	-	+	+	+
NGB00629	+	-	+	+	-	-	-	+	-	+	+	+
NGB00631	-	-	+	+	-	-	+	-	+	+	+	+
NGB00684	+	-	+	+	-	-	-	+	+	+	+	+
NGB00702	-	-	+	+	-	-	-	-	-	+	+	+
LV	-	-	+	+	-	-	-	+	+	+	+	+

Effect of mixed population of *H. multicinctum and M. incognita on* the activities of antioxidant enzymes (Tannins) in infected Pepper roots

The activities of Tannins in the infected roots of *Capsicum species* by *H. multicinctum* and *M. incognita combined* were evaluated, as they take part in defense responses of plants to nematodes infection. The result of the present study revealed that at a day before inoculation) the activities of Tannins in the pepper roots were almost the same (Figure 1) except for NGB00631 that exhibited lower enzymatic activities. At day-7, however, accession NGB00702 had higher enzyme activities followed closely by LV, NGB00574, and NGB00624 compared to others that were almost the same. At day-14; higher enzyme activities were recorded in LV and NGB00586 as compared to others that were almost the same. At day-21, however, Tannin activity declined in all the accessions/ variety except in LV, NGB00702, NGB00624 and NGB00574 that had remarkable increase.

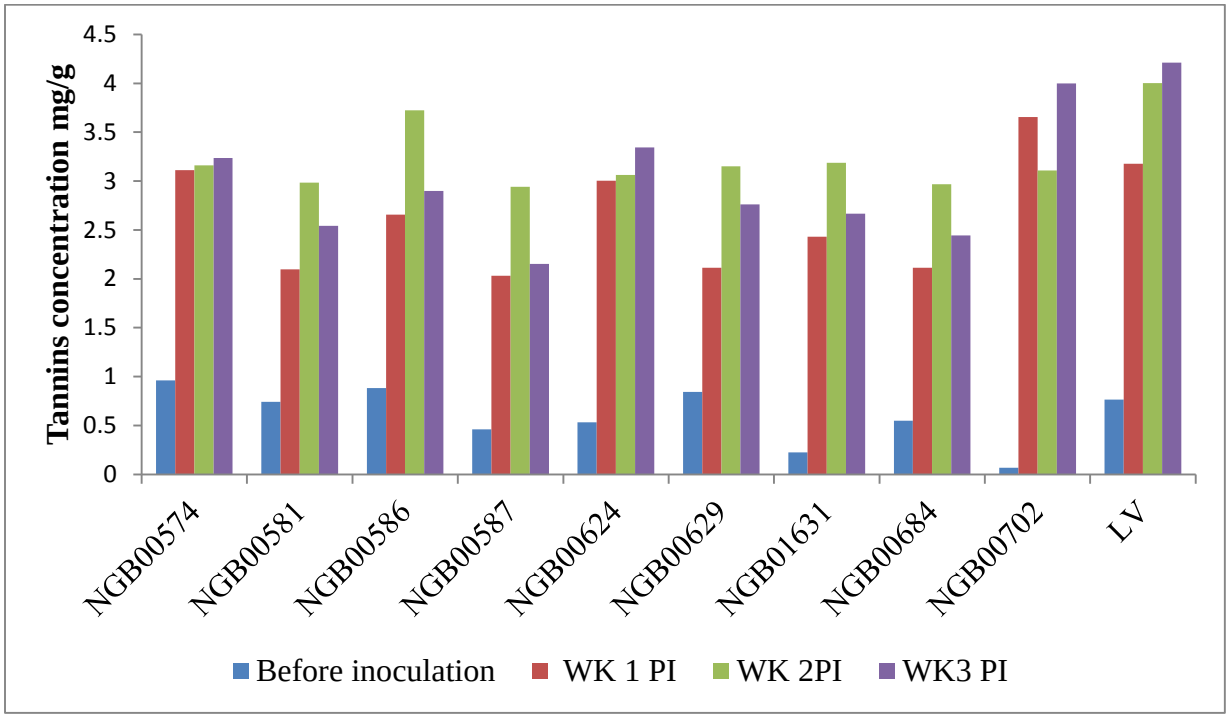

Figure 1: Tannins concentration from Pepper roots challenged with 2000 mixed population, 1000 each of *H. multicinctum and M. incognita*

Effect of mixed population of *H. multicinctum and M. incognita* on the activities of antioxidant enzymes (Polyphenol oxidase) in infected Pepper roots

The analysis of the activities of polyphenol oxidase in the infected roots of *Capsicum species* by mixed population of *H. multicinctum and M. incognita revealed* their presence and participation in pepper responses to infection. The results from this investigation showed increase in the activities of polyphenol oxidase of the pepper Accessions and a variety at a day before inoculation were almost similar, except for NGB00684 that showed higher enzymatic activities. However, at day 7, 14 and 21 post inoculation, NGB00684 increased with increase in the number of days followed by NGB00624, while NGB00587 recorded the least enzymatic activities.

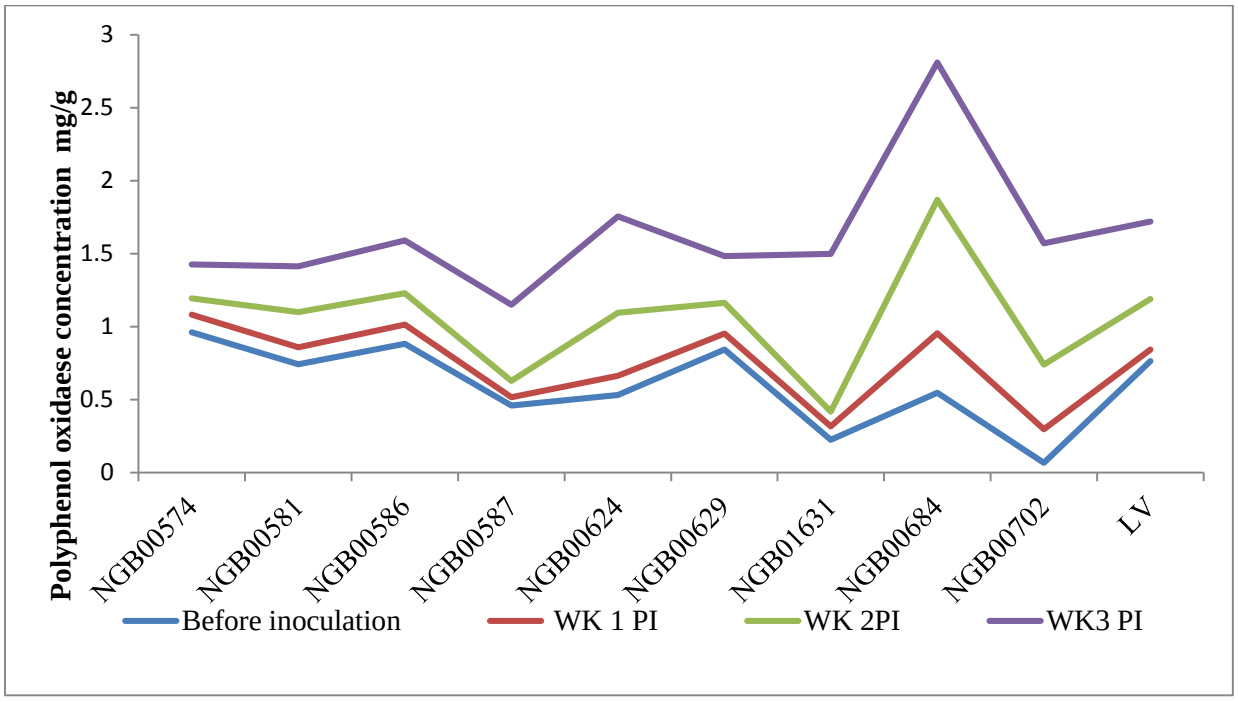

Figure 2: Polyphenol oxidase concentration from pepper roots challenged with 2000 mixed population, 1000 each of *H. multicinctum and M. incognita*

Effect of mixed population of *H. multicinctum and Meloidogyne* on the activities of antioxidant enzymes (Peroxidase) in infected Pepper roots

The concentration of peroxidase in the infected roots of Capsicum species by *H. multicinctum and M. incognita combined* were analysed. The results revealed that at a day before inoculation, the enzymatic concentration of the pepper accessions/variety were similar all through. However, at day 7, 14 and 21, NGB00587, NGB00629 and NGB00684 had higher enzymatic activities in defense responses of the plants against the nematode pathogens while the activities in other varieties were almost similar.

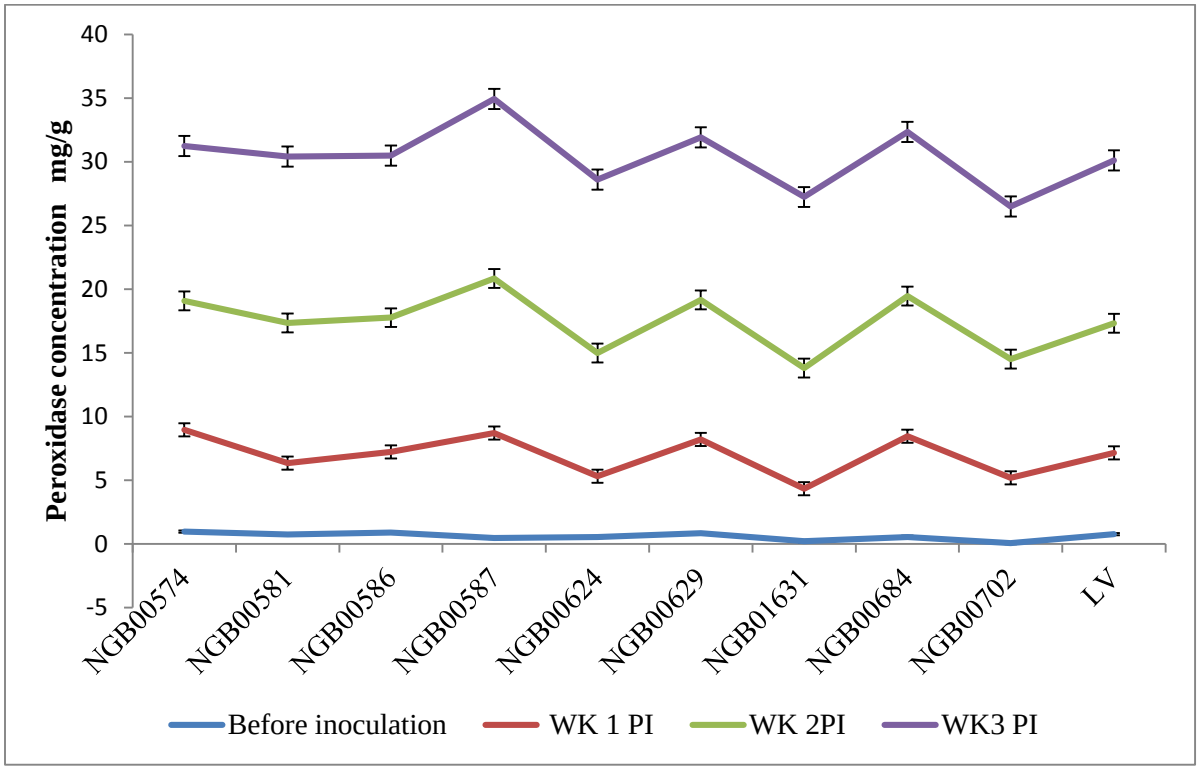

Figure 3: Peroxidase concentration from Pepper roots challenged with 2000 mixed population, 1000 each of *H. multicinctum and M. incognita*

DISCUSSION

The involvement of widely distributed and natural occurring substances in plant resistance against pathogen attack is a well-established phenomenon (Materu *et al*., 1995). The result of the present-day investigation evidently demonstrated that *H. multicinctum* and *M. incognita nematodes* infection of the pepper roots induced fast accumulation of phenolic compounds in the resistance peppers compared to the susceptible ones that responded slowly to the compounds accumulation. The accumulation of phenolics at the pathogen infection sites is a common attribute of plant defense response to pathogenesis, which results in rapid cell death, retarding the development and penetration of the pathogen (Vaganan *et al*., 2014). Most of the phenolics compounds are found in

plants in conjugated ester form with a sugar molecule attachment in one or two hydroxyls of phenolics. Looking from nematodes infection point, the wall-bound conjugated ester phenolic acids are very important as the esterification of phenols to the cell wall material. As a common biochemical process of resistance and accumulation of polymerized phenols, it is also known to occur as a rapid response to pathogen infection (Vaganan *et al*., 2014). In view of the findings of this study and reports from previous investigation, it could therefore hold that high activities and accumulation of phenolics in some of the accessions/variety evaluated constitute part of their resistance mechanism to *H. multicinctum* and *Meloidogyne* nematodes attack. Earlier reports by Aguilar *et al*. (2000) observed difference in PO activity in banana against Fusarium wilt. In another study, Bajaj and Bhatti (1984) found tomato cultivar infected with *M incognita* to show higher PO and PPO activity in resistance cultivar than in the susceptible). In nematode-plant interactions, PO is considered to be involved in resistance response as with increase in activity after infection to greater levels in resistance cultivars. The results seem to show that the resistant pepper accessions/variety responded well to the nematode infection through strong synthesis and deposition of phenolics. Induced phenolics and lignin accumulation in infected plants roots is established as one of the numerous plant defense responses against pathogens including plant parasitic nematodes (Collingborn *et al*., 2000). The remarkable increase of phenolic metabolites in wall bound fraction from roots of the resistance pepper plants upon the nematode's infection explains the role played by the metabolites in resistance to nematodes infection.

The existence of remarkable quantity of phenolics compounds in the roots of some of the evaluated pepper accessions and a variety in this study showed that, these five: NGB00581, NGB00587, NGB00629, NGB00684 and NGB00574 upon *H. multicinctum* and *M. incognita* infection, exhibited a metabolic initiatives and chemical defenses to pepper to provide resistance by biochemical means to infection by nematodes. Similarly, lower activities of phenolics recorded in the remaining accessions/variety, signifies their level of susceptibility to nematodes infection.

CONCLUSION

The present study has demonstrated that, of the investigated nine pepper accession/variety, five accessions - NGB00581, NGB00587, NGB00629, NGB00684 and NGB00574-possessed high content of phenolic compounds and they can be exploited for their use as bio nematicides in the management of mixed infections of *H. multicinctum* and *M. incognita;* and other nematodes by pepper producers. Response of these accessions and a variety may have been influenced by their genetic make-up and thus exhibited appreciable level of immunity and tolerance to the nematodes attack.

Monitoring and management of these nematodes therefore is fundamental to sustainable pepper production. Seeds of these five accessions should be subjected to further investigation by breeders using appropriate biotechnological tools. The result will enhance the development of cultivars with multiple resistances to economically important plan parasitic nematodes, which can be made available to poor resource farmers to enhance pepper productivity.

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MORPHOMETRIC OVERVIEW AND SEXUAL DIMORPHISM OF THE SUB-FAMILY BRUCHINAE (COLEOPTERA: CHRYSOMELIDAE) USING SAMPLES COLLECTED FROM SAMARU ZARIA, NIGERIA.

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SUMMARY

***Callosobruchus maculatus, C. subinnotatus, Bruchidius. artrolineatus and Caryedon serratus* are the most common members of the subfamily Bruchinae found in Zaria. They were obtained from godowns in Samaru markets or cultured from host commodity infested with either eggs or larvae/pupae of the insects. Measurement of the major diagnostic features of the subfamily Bruchinae was illustrated and a brief description of specific observable variables and sexual dimorphism of these insects was also carried out in J.C. Deeming Insect Museum, Institute for Agricultural Research, Ahmadu Bello University Zaria. A total of twenty seven characters were used comprising of eleven measurable variables, nine explanatory variables and seven ratios. A handheld digitalized MiScope microscope (40-140x magnification) was used to capture photographs and made measurements of major measurable diagnostic traits of the subfamily Bruchinae. These insects are relatively small, variable in colouration and often alike leading to confusion in their identification. This paper elucidates the major morphological characteristics of the subfamily Bruchinae to ease their early detection, accurate identification and characterization being sine qua non for the adoption of effective management strategies. Accurate identification is also a prerequisite to World Trade Organizations (WTO) negotiations regarding the presence or absence of pests in any commodity for international transactions.**

Keywords: Godowns, Bruchinae, microscope, diagnostic and traits

The seed-beetle family (subfamily Bruchinae) has received much attention in recent times due to increasing demand for more food to meet the needs of the expanding world population. Focus has been on the need for better control of stored- product insects especially those attacking comestible leguminous seeds in ladders of developing countries and to reduce storage losses that are ruinous

not only from initial damage by bruchid larvae but also from ensuing invasions of other organisms (Howe, 1973). Losses encountered by bruchids range between 80-100% and have triggered researches in many countries like Colombia, England, France, United States, Japan and Nigeria into the biology, physiology, morphology, plant-host resistance and pheromones in relation to the principal cosmopolitan species (Kingsolver, 2004).

Members of this subfamily Bruchinae are characterized by a compact body invested with short hairs and short elytra covering all but the last abdominal segment called pygidium (Lale, 2002).

Over 1200 species have been reported from different parts of the world but species of economic importance recorded in legume seeds stores in Nigeria include *Callosobruchus maculatus* (F.), *C. chinensis* (L.), *C. subinnotaus* (Pic) and *Caryedon serratus* (Olivier).

The recognition and identification of Bruchinae is often tedious and difficult because many species are small in size (average length 1.0-6.0 mm) and sympatric in nature (closely related and look alike). A large gap exists in our ability to accurately identify and name these pests by mere observation of their diagnostic features being essential for the adoption of effective management strategies. In the light of the aforementioned, this paper intends to highlight the major specific measurable diagnostic features, their mode of measurement as well as some specific observable features that will aid in the identification of these pests species in Nigeria.

MATERIALS AND METHODS

The biological materials used to depict the major diagnostic characteristics of the sub-family Bruchinae were; *Callosobruchus maculatus, C. subinnotatus, Bruchidius. artrolineatus and Caryedon serratus* collected from godowns in Samaru Zaria markets or cultured from host commodity infested with either eggs or larvae/pupae of the insect. They were identified and sexed

in J.C. Deeming Insect Museum, Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria.

Raising of insect culture

Cultures of adult insects used were raised in 1- litre capacity, clear plastic containers (9 cm diameter, 16 cm high) with 8 cm diameter screw- type lids. Each container contained about 200 g seeds of a susceptible bruchinae host-legume seeds; cowpea (*Vigna unguiculata*); Bambara groundnut (*V. subterranea* (L.) Verdcourt; shelled and unshelled groundnut as well as tamarind fruits enough for producing bruchids in 3- 4 weeks (Bandara and Saxena, 1995). The lid of each container had a central circular perforation (3 cm diameter) covered with fine muslin cloth for aeration. These were kept under laboratory conditions at 28±2 ºC and 70±5% Relative humidity (RH) and observed daily until adult emergence. The lid and side walls of the container with active species (*C. maculatus, C. subinnotatus, B. artrolineatus*) were tapped repeatedly so that the adults gathering around the lid or the side walls dropped back unto the seeds and were chilled by refrigeration before collection.

The newly emerged species of *C. maculatus, C. subinnotatus* and *B. artrolineatus* were sieved out from the cowpea seeds while *C. serratus* were picked with forceps into vials (7.5 cm high and 2.5 cm diameter) containing 100% ethanol until use. The preserved samples were identified using keys on adult morphological characters by Southgate (1957) and Haines (1991), as well as, comparison with preserved specimens in the reference collections of the J.C. Deeming Insect Museum of Crop Protection Department, Faculty of Agriculture, Ahmadu Bello University Zaria. Preserved samples were washed using distilled water, transferred onto clean cardboard papers, whole/parts removed were allowed to dry and a handheld digitalized MiScope microscope (40-140x magnification) was used to illustrate photographs and of measurements of diagnostic features.

A total of twenty seven characters were illustrated comprising of eleven measurable variables, nine explanatory variables and seven ratios. *C. maculatus* and *C. subinnotatus* samples were used to depict mode of measurement of the measurable variables.

RESULTS

C. maculatus, B. artrolineatus, C. subinnotatus and *C. serratus* were the most common members of the sub-family Bruchinae found in comestible legumes in Samaru, Zaria. *C. maculatus* and *B. artrolineatus* were found on *Vigna unguiculata*, *C. subinnotus* on *Vigna subterranean* and *C. serratus* on tamarind fruits culture as shelled and unshelled groundnut seed cultures had no emergence.

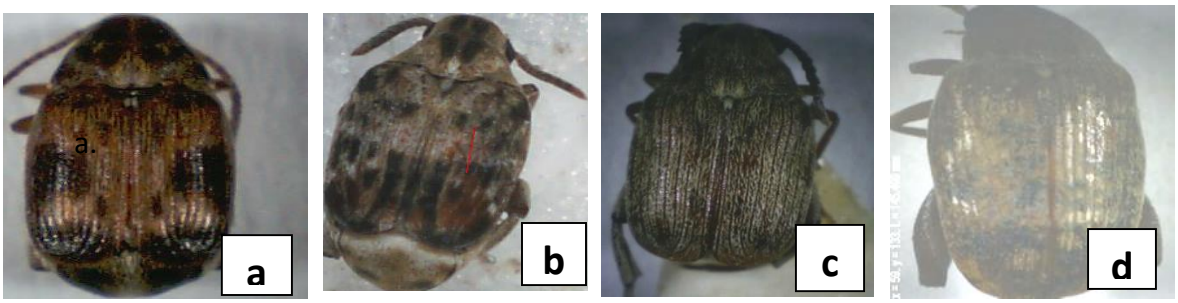


Plate 1 a, b, c and d: Dorsal Habitus of *C. maculatus, B. artrolineatus, C. subinnotatus* and *Caryedon serratus* respectively.

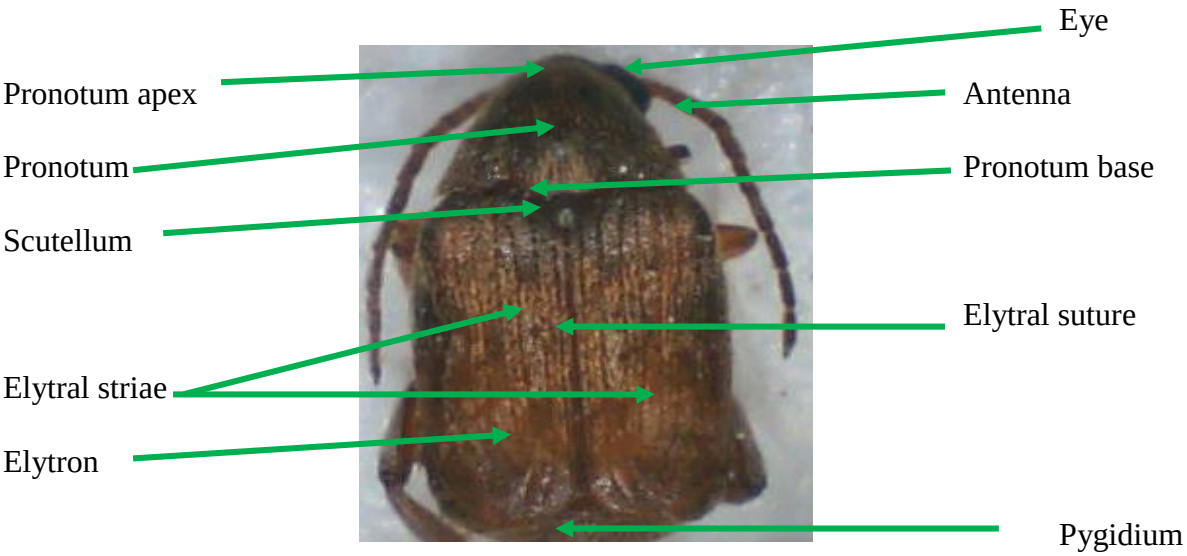


Plate: Dorsal view of a typical Bruchinae

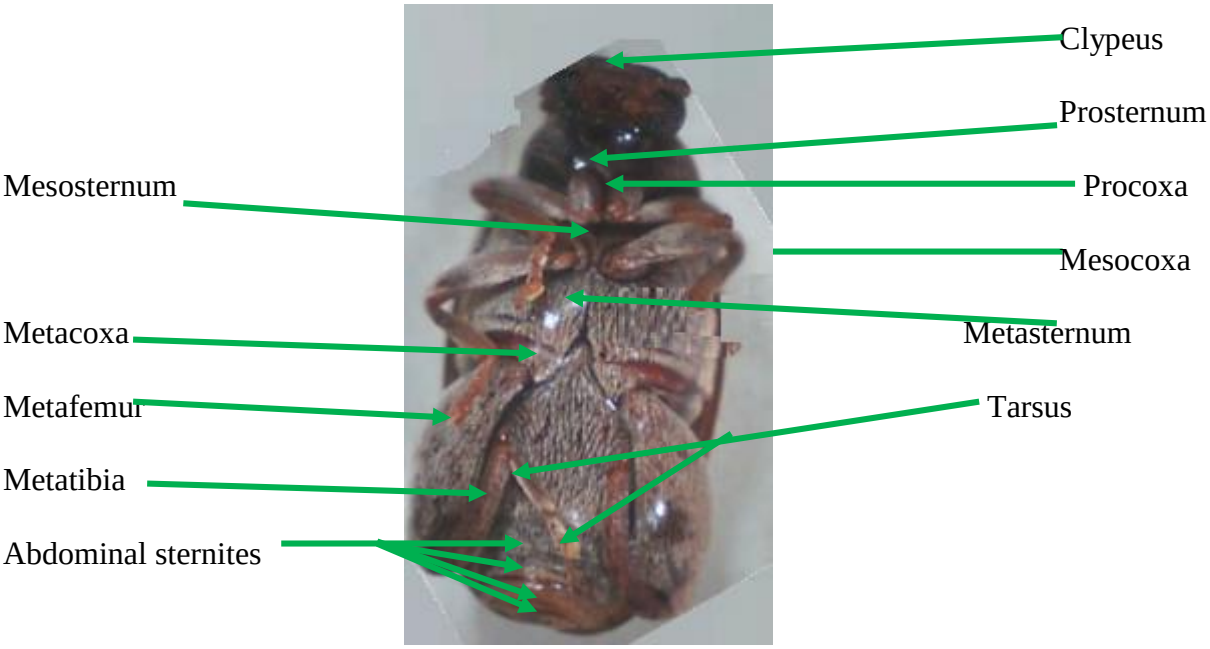


Plate III: Ventral view of a typical Bruchinae

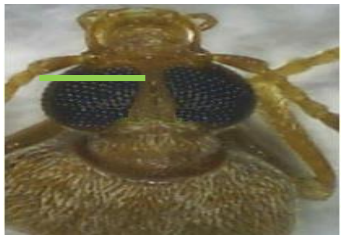
MEASURABLE VARIABLES

Features hitherto used by Southgate *et al.* (1957), Haines (1989), Bandara and Saxena (1995) as well as Kingsolver (2004) were adopted.

i. **narrowest width between eyes (nwe);** measured on a horizontal plane across the narrowest distance between eyes

ii. **greatest width across eyes (gwe);** measured on a horizontal plane across the greatest distance between eyes

iii. **Eye width (ew)** : measured as greatest width across eye from inner edges to side edges



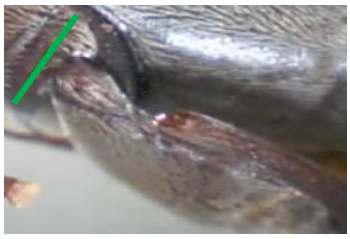
iv. **body length (bl);** measured centrally from anterior margin of head to tip of abdomen (bl).

v. **body width (bw)**; measured as greatest width across elytra

vi. **antennal length(al);** measured when antenna is fully extended with head in hypognathous position and covered the distance from the socket at base of antennal scape to apex of last antennal segment

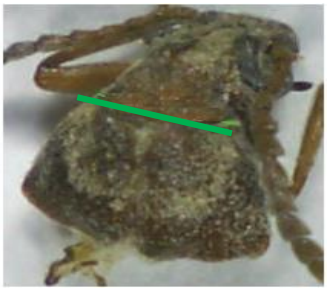
vii. **hind femoral width (hfw);** measured across greatest width of hind femur

viii. **Width of coxa (wc);** measured across the greatest distance of metacoxa



ix. **length of pronotum (lp)**; measured centrally on the dorsum from anterior margin to the posterior margin of the pronotum

x. **width of pronotum apex (wpa)**; measured across the narrowest width of pronotum from the anterior margin



xi. **width of pronotum base (wpb)**; measured across the greatest width of pronotum at the basal region

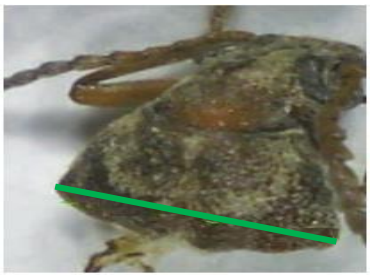


Plate IV i – xi: Mode of measurement of measurable variables

RATIOS USED IN BRUCHINAE MORPHOMETRICS

The most commonly used body parts ratio used in the morphometric studies of the members of the subfamily Bruchinae are listed and defined below:

i. Occular index (o:i); obtained by dividing the greatest width across eyes by the narrowest width between eyes.

ii. Eye width to greatest distance between eyes (ew:gbe); determine by diving eye width with greatest distance across eyes.

iii. Body length to body width (bl:bw) : calculated by diving body length with body width of the insect

iv. Elytral length to elytral width (el:ew); compared by dividing the length of elytra with width of elytra.

v. Elytral length to length of thorax (el:lt) ; obtained by dividing the length of elytra with the length of thorax.

vi. Hind femoral width to with of coxa (hfw:wc); can be obtained by dividing the hind femoral width of meta thoracic leg by the width of meta coxa.

vii. Width of pronotum base to width of pronotum anterior (wpb:wpa): can be calculated by dividing the width of pronotum base by width of pronotum apex.

EXPLANATORY VARIABLES

Emphasis here was largely on visible body parts devoid of dissection or dismemberment and these include; colour characteristic on elytra, number of elytra striae intervals, pronotal shape, antennal structure or shape, type of hind femoral tubercle, shape of tibia, tarsal segments, shape of pygidium and an ecological factor which is mode of pupation.

BRIEF DESCRIPTION AND SEXUAL DIMORPHISM OF SPECIES

C. maculatus: Elytral colour of adult male *C maculatus* collected in Zaria and environs were generally brownish in colour and without markings on the elytra unlike the females which had strong markings on the elytra consisting of two large lateral dark patches mid-way along the elytra and smaller patches at the anterior and posterior ends, leaving a pale –brown cross-shaped area covering the rest of the body. In addition, males usually have shorter abdomen and dorsal side of terminal segments curved sharply downwards in contrast to the females which have omparatively longer abdomen and the dorsal side of terminal segments only slightly bent downwards

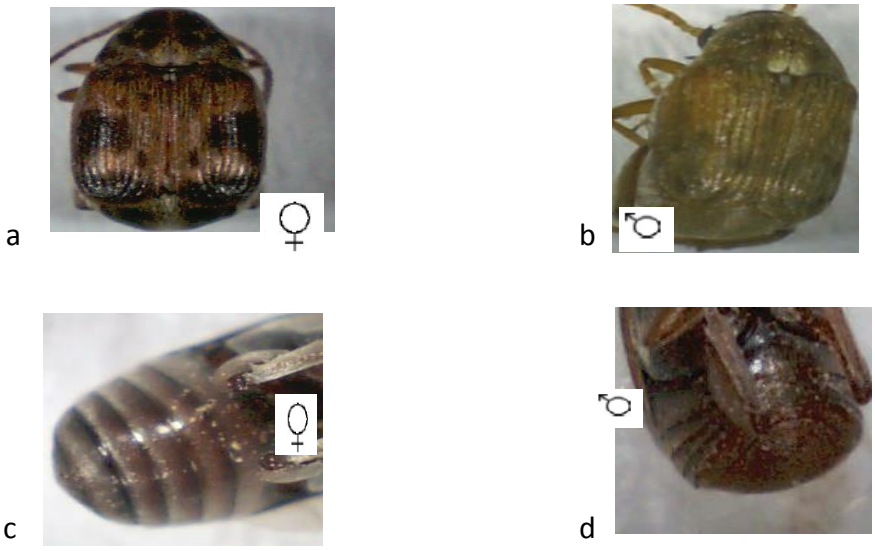


Plate V: Diagnostic view of female (a. and c.) and male (b. and d.) *C. maculatus.*

Striae are of irregular sizes, slightly punctured and consist of ten intervals on each elytra while the pronotum is dark- red to black in colour usually with a pattern of paler hairs forming two white oval spots closed together at the middle of the base. Scutellum triangular and covered with white scale-like pubescence. Antennal structure in both sexes of *C. maculatus* (Fabricius) are slightly serrate withoutmarked dimorphism between sexes. They are segmented and inserted at the mouth of eye emargination (Southgate, 1958)

Bruchidius artrolineatus: Also called the African cowpea bruchid, is *a species that* predominates in the fields and cannot breed in stores. It is sympatric in distribution and superficially similar to *C. maculatus* in shape but differ in the absence of one tooth on the ventral side of the hind femur.

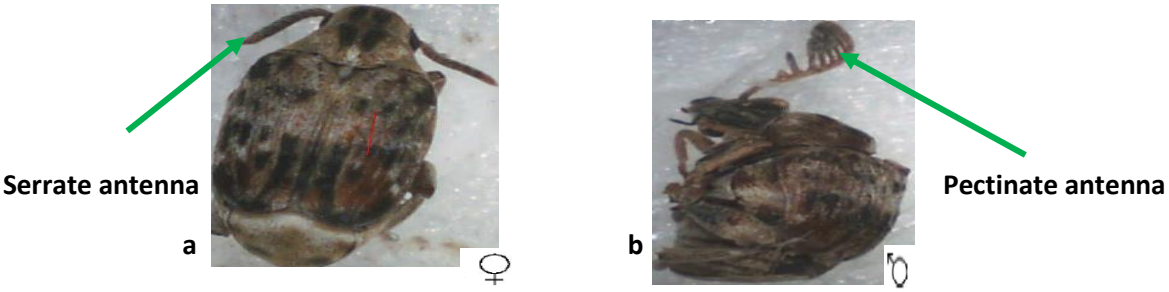


Plate VI: Female (a) and male (b) of *Bruchidius artrolineatus*

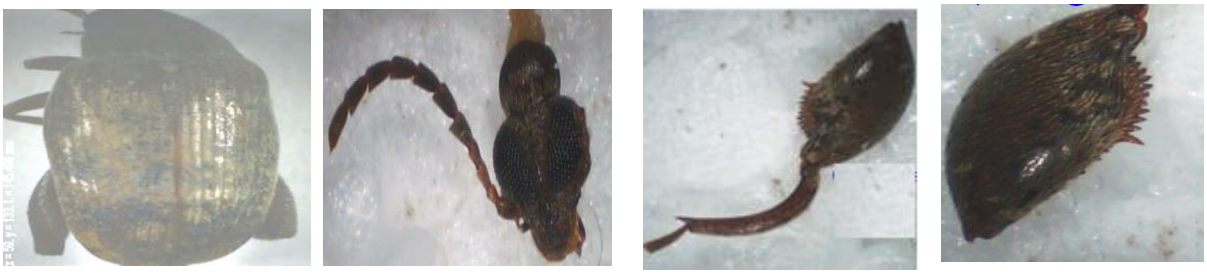
Furthermore, male *B. artrolineatus* possess an unusual profound pectinate antennal shape which is subserrated in the female.

Callosobruchus subinnotatus: Body colour is uniformly dark brown to black with pale setae. It closely resembles *C. maculatus* though larger in size. Dimorphism between sexes is not well pronounced however females in contrast to males have dark fuscous to black derm with whitish setae forming a pattern on the elytra and antenna less serrated in females compared to the males.

Plate VII: Dorsal habitus of *Callosobruchus subinnotatus*

Males possess head with large bulbous eyes that are prominent, coarsely faceted and deeply emarginated. Pronotum conical and evenly convex with lateral margins slightly sinuate. Scutellum pubescent with white scale-like setae. Pygidium nearly vertical with sides arcuate and clothed with golden setae. Legs reddish testaceous, hind femur bicarinate on ventral margin with inner tooth acutely triangular and slightly longer than the outer one with straight tibia just as in *C. maculatus*.

***Caryedon serratus*:** A large robust Bruchinae with reddish brown colour and clothed with grey brown setae. The elytron is strongly convex with sub-parallel striae intervals. Antenna elongate and coarsely serrate and reaching metacoxa in the males while in females, it reaches the middle of episternum. Pronotum trapezoidal with lateral margins rounded anteriorly while scutellum is rectangular and flat. The pygidium narrow, apically rounded and densely setosed. Each hind femur swollen dorsoventrally flattened and bears a conspicuous ventral comb (one large spine and 8-12 smaller ones) with a curved tibia unlike other Bruchinae.



a b c d

Plate VIII a, b, c and d: Dorsal habitus, coarsely serrate antenna, curved tibia and dosoventrally flattened hind femur of *C. serratus* respectively.

DISCUSSION

The knowledge of the morphometric variability that exists among members of the subfamily Bruchinae would ease species identification, sex differentiation and may lead to ecotype or biotype

discovery. Sexual dimorphism is generally common among insects with females being bigger than the males (Teder and Tammaru, 2005).

The significance of linear measurable traits, body parts ratio and explanatory variables in the morphometric studies of the subfamily Bruchinae cannot be over emphasized. Parameters like body length (bl), body width (bw), elytral length (el) and antennal length (al) could have specific ranges peculiar to some species which could aid in their identification. For instance, Magaji *et al*. (2013) reported that the bl, bw and al of *B. artrolineatus* collected from different ecological zones of Northwestern Nigeria, ranged between (1.6 – 2.9 mm), (1.1 – 1.7 mm and (1.5 – 1.6 mm) respectively. Similarly, Lale (2002) reported *C. maculatus* body length to range between 2.5 – 3.5 mm, antennae twice as long as thorax and that *C. subinnotaus* was at least twice as large as *C. maculatus* with body length ranging between 4.5-5.5 mm and the elytral 1.2 times as long as broad. Howe (1973) reported the body length of *Caryedo serratus* collected from different regions of the United States and Canada to range between 3.5 to 6.8 mm and the width 1.8- 3.0 mm and that, the elytral length was 1.5 times as long as wide. Ocular index and antennal structures are dimorphic in some species and aid in sex differentiation. This is in agreement with the report of Magaji *et al*. (2013) who showed the ocular index of *Caryedon serratus* to be 6.75:1 in males and 6.2:1 in females while antennal structures were reported to be serrate to coarsely serrate in both male and female *C. maculatus, C. subinnotatus* and *caryedon serratus* but pectinate in the males of *B. artrolineatus*.

Pronotal ratios, presence or absence of metathoracic hind femoral pectin as well as hind femoral to coxal ratios, are essential in the morphometrics of the subfamily Bruchinae. Width of pronotum at base was three times the anterior width in male and female *C. serratus* but approximately twice the anterior width in *B. atrolineatus, C. maculatus* and *C. subinnotatus* (Magaji *et al*., 2013).

Some explanatory variables such as elytral colour, striae intervals, pronotal shape, hind femoral tubercle, shape of tibia, tarsal segments, and shape of pygidium and mode of pupation are of taxonomic importance and could be used to identify a species or discriminate among individuals of different populations while some have constant values and are not discriminatory in nature but rather are characters specific to the species. Delobel and Tran (1995) supported this idea when he established the number of teeth as a non-discriminating parameter in *C. maculatus*.

Colour characteristics could be used to identify *C. subinnotatus* and, *Caryedon Serratus* but not *C. maculatus* because the later are variable in colours leading to confusion in their identification with others members of the genus (Southgate *et al*., 1957).

Unlike other members of the subfamily Bruchinae which have straight metathoracic tibia, the hind femoral leg of *Caryedon serratus* is dorso-ventrally swollen bearing a row of denticles, possesses straight tibia and the only Bruchinae that pupates externally.

CONCLUSION

Illustrations on the major morphological characteristics of some members of the subfamily Bruchinae found in Zaria will help in the detection and accurate identification of these species. Effective management strategies including biological control are only possible if the accurate identities of both the pests and their parasites or parasitoids are known to enable successful introduction of biocontrol agents. Mistakes in species concepts or identifications as well as the use of inappropriate biocontrol agents often accounts for most failures in biocontrol measures and could sometimes cause great impact on non-target species.

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