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EFFECT OF *Callosobruchus subinnotatus* (PIC.) INFESTATION ON NUTRIENT COMPOSITIONS OF *Cassia tora* (L.) SEEDS IN KATSINA STATE, NIGERIA.

*[1] Muhammad, A., Oshoke[2], J. O. and Bashir[3], K. A.
[1]Department of Crop Production and Protection, Federal University Dutsin-ma
[2]Department of Fisheries and Aquaculture, Federal University Dutsin-ma
[3]Department of Biological Sciences, Faculty of Science, Federal University Dutsin-ma, Katsina State, Nigeria
*Correspondence: amuhammad1@fudutsinma.edu.ng; Tel.: +234 (0)8024776111

ABSTRACT

An investigation was carried out to identify the insect pest attacking seeds of *C. tora* in Dutsi-ma, Katsina State, Nigeria and to evaluate the influence of this attack on the proximate composition of the seeds. The insect was identified as *Callosobruchus subinnotatus* Pic. [Coleoptera: Bruchidae] at the Insect Museum, Institute for Agricultural Research, Ahmadu Bello University, Zaria. The result showed that moisture and lipid contents were higher in un-infested seed whereas ash, protein, fibre and carbohydrate contents were higher in infested seeds. The infestation due to this insect could lead to loss of lipid which is required in oil industry or its utilization as ingredients in fish meal.

Keywords: *Callosobruchus subinnotatus*, infestation, nutrient composition, *Cassia tora*

1. INTRODUCTION

Cassia tora (L.) syn. *Cassia obtusifolia* (L.) is an annual or perennial herb growing wild as a weed. It is commonly known as *Foetid cassia*, sickle senna or sickle pod and "t*afasa*" in HausaThe plant grows wild and is widely distributed in the Sudan and Sahel savanna ecological zones of Nigeria (Aliyu, 2006). *C. tora* was found in states of Uttar Pradesh and Madhya Pradesh in India Shreejiagro (2014). Dasuki *et al*. (2014) reported that, it is a common weed of open undisturbed areas of Hawaii, Arid lowlands and disturbed areas such as drainage channels and overgrazed pastures. It belongs to the family Fabaceaea, sub family Caesalpiniaceae. It grows erect and branch profusely reaching up to 90cm in height (Akobundu and Agyakwa, 1987). The leaves are obovate and alternate, consisting of three pairs of leaf-lets. The leaf-lets are opposite and pinnately arranged. The flowers are bright yellow in colour. The rhombohedral brown seeds are enclosed in highly beaked straight or curved brown pods of 12-18cm long (Plate 1) with 18-28 seeds enclosed. It is a dehiscent pod-bearing plant and therefore disperses its seeds by explosive mechanism. All parts of this plant is useful, the leaves (Plate 2), seeds and roots (Shreejiagro, 2014; Aliyu, 2006) *C. tora* is an invasive weed which if not adequately controlled on a farm can invade and colonize farmlands due to high seeding ability. The leaves are widely eaten as a delicacy mixed with groundnut cake "kuli-kuli" amongst the Hausa community as an alternative source of food. Due to its vigorous growth habit, it's usually the first plant that germinates immediately after first rainfall preceding dry season and as such serves as pasture to grazing animals. *C. tora* seeds are valued as raw materials in cosmetic industry as well as in the formulation of livestock feeds (Altrafine, 2013; Dasuki *et al*., 2014). The leaves are highly valued food commodity across international borders. The insufficient sources of water supply such as of rainfall in semi-arid regions threatens food production. The dried leaves of *C. tora* are traded across international borders, between Nigeria and Niger Republic (Aminu, personal communication). The weed was widely reported to be used in ethno medicine for treatment of medical ill-conditions. Chandan *et al*. (2011) and Aliyu (2006) reported utilization of *C. tora* leaves as mild laxative, eye diseases, liver stimulant, heart tonic, dysentery and antihelmentics as well as relieving constipation. Altrafine (2013) reported that the dried and fresh leaves are used in northern Nigeria in the treatment of ulcers, ring worm and other parasitic skin diseases. The seeds of the plant are used greatly by the Ancient Chinese as herb (Sheejiagro, 2014). Altrafine (2013) reported that the leaves of *C. tora* contained natural chemicals such as chrysophanic acid-9-anthrone, an important fungicide often used as natural pesticides in the organic farms in India. *C. subinnotatus* is an insect that was widely known to attack grain legumes. Maina and Lale (2004) stated that *C. subinnotatus* is one of the field-to-store bruchid pest that infest grain legumes in tropical Africa and Asia. It is an economic and primary insect pest restricted to Bambara groundnut, *Vigna subterranaen* (L.) Verdcourt (Ofuya, 2001; Maina and Lale, 2004; Ibrahim and Isah, 2014 and Maina *et al*., 2011). The life cycle of this pest is similar to that exhibited by cowpea seed bruchid, *Callosobruchus maculatus* (F.) (Ofuya, 2001). Infestation starts from the field and continues in storage. The bruchid attack on Bambara nuts causes substantial losses both in quality and quantity to ripening seeds after the pods were harvested and left to dry on the farm (Ibrahim and Isah, 2014; Maina *et al*., 2011). The larva lives and feed within the seeds turning it in to a mass of brown powder. This research aims at the effect of *Callosobruchus subinnotatus* on the nutritive content of *C. tora*.

2. MATERIALS AND METHODS

This investigation was carried out in the Biology laboratory, Federal University Dutsin-ma, Latitude 11° 07' 49" to 13° 22' 57" N and between Longitude 6° 52' 03" to 9.9° 02' 40" E in the Sudan savanna ecological zone. Dried seeds were collected after rainy season of 2015 at the outskirts of Dutsin-ma town. The seeds which were naturally infested (from the field) were collected and stored in polythene bag and kept in the laboratory. The larva was cultured in a plastic cup measuring 8.80cm (outside lid diameter) cut at the centre (5.80cm inner diameter) and covered with a tissue paper. This is to ensure free air circulation and also to stop the adult insect from escaping. The cup was kept in the laboratory under ambient temperature 20-25°C ± 2° until completion of metamorphosis. The emerged adult insects were identified at the Insect Museum, Department of Crop Protection, Faculty of Agriculture/Institute for Agricultural Research, Ahmadu Bello University. Proximate analysis after separating infested and uninfested seeds was carried out at the Produce Development Programme, Institute for Agricultural Research, Ahmadu Bello University, Zaria, Nigeria.

2.1 Nature of damage of Callosobruchus subinnotatus on Cassia tora

Sharp, circular punctured holes were observed on the surface of *C. tora* pods. This indicated that eggs were laid and upon hatching, the first instar larva bore through the pods and in to the developing weed seeds (Plate 8) and destroys the seed cotyledons turning it in to mass of reddish powder. The larva of this insect does the damage. Eggs were observed to be laid singly on the dried seeds. Exit holes were seen as evidence of larval activity on the seeds (Plate 9). Pupation occurred in a hard pupal case outside the seeds (Plate 10). Proximate analysis (AOAC, 1990)

2.2 **Determination** of Moisture content:

Aluminum dishes were washed and dried to constant weight in an oven at 100°C. They were removed and cooled in a dessicator and weighed (W1). Two grams of the ground (powder) sample was placed in the weighted moisture dish (W2). The dish containing the sample was kept in an oven for about 3 hours, the sample was removed and cooled in the desiccator and weighed (W3).

% moisture = W2-W3/W2-W1 x 100

2.3 **Determination** of ash content

Crucibles were cleansed and dried in the oven at 60^0C for 45 mins and cooled in the dessicator and weighed (W1). Two grams of the cassia powder was placed in the crucibles and weighed (W2). They were transferred into the Searchtech Muffle Furnace (Model SXL-1008) at 550°C then removed and cooled in a dessicator and weighed again (W3).

% Ash = W3-W1/W2-W1 x 100

2.4 Determination of fibre content

Two grams of Cassia seed powder was place in a beaker containing 1.2 Mol H_2SO_4 per 100 ml solution and boiled for 30 minutes. The residue was filtered and washed with hot water. The residue was transferred to a beaker containing 1.2 g of NaOH per 100 ml of solution and boiled for 30 minutes. The residue was washed with hot water and dried in an oven and weighed (C2). The weighed sample was incinerated in a furnace at 550°C, removed and allowed to cool and weighed (C3).

$$\% \text{ fibre} = \frac{C2 - C3}{W} \times 100$$

Where: C2 = weight of residue after washing and oven drying, C3 = weight of residue after incineration in a furnace, W = weight of sample.

2.5 Determination of lipid content

Two hundred and fifty millilitres (250) ml boiling flask was dried in an oven. It was transferred in to a dessicator and allowed to cool. Two grams of Cassia seed powder was placed in the thimbles (filter paper). The flask was filled with petroleum ether on N-hexane. Soxhlet apparatus was assembled and allowed refluxing for 8 hours. The apparatus was removed and transferred to an oven to dry. Later, it was transferred into a dessicator and allowed to cool and reweighed.

$$\% = \frac{\text{Weight of extracted fat}}{\text{Weight of sample}} \times 100$$

2.6 Determination of protein

2.6.1 Digestion

Two grams of Cassia seed powder was weighed and placed into Kjeldahl tubes. One tablet of catalyst (copper) and 25 ml conc. H2SO4 were added. The mixture was heated in a fume cupboard until green colour was assumed. It was cooled and washed down any black particles showing at the mouth and neck of the flask with distilled water. After cooling, the digest with washing into 250 ml with distilled water.

2.6.2 Distillation

Before usage, steam was allowed through the Markham distillation apparatus for about 15 minutes. Under the condenser, 100 ml conical flask containing 10 ml of boric indicator was placed. Ten (10) ml of the digest was pipetted into the body of the apparatus via the small funnel aperture. It was washed down with distilled water followed by 10 ml of 40 % NaOH solution. Steam was allowed through for about 5-7 minutes to collect Ammonium sulphate. The receiving flask was removed, the tip of the condenser was washed down into the flask.

2.6.3 Titration

The solution was titrated in the receiving flask using 0.001N HCl and the nitrogen content was calculated and hence the protein content of the sample.

% protein = nitrogen (%) x conversion factor (6.25)

$$\text{Nitrogen } (\%) = \frac{(A\text{-}B) \times 0.01N \text{ HCl} \times 14.007}{W} \times 100$$

Where: A = Titre value of test sample, B = Titre value of blank, W = weight of sample in milligram, 14.007 = atomic mass of nitrogen in ammonia, 0.01 N normality of HCl used as titrant.

2.7 Determination of carbohydrate content:

This was obtained by difference having estimated all other fractions by proximate analysis using Pearson (1976) procedure. Carbohydrate was obtained by simple calculation:

100 – (% moisture + % Ash + % protein + % fibre + % lipid)

Proximate analysis on infested and un-infested seeds was replicated three times with un-infested seeds serving as control. Data obtained was subjected to descriptive statistics using percentages.

3. RESULTS

The results of the effect of *C. subinnotatus* on the major nutrient compositions of *C. tora* are presented in Table. 1. The result showed that moisture and lipid contents of un-infested seeds were observed to be higher than those of infested seeds. Generally, it was observed to be higher in the un-infested seeds. On lipid contents, this investigation found out that the un-infested seeds were higher in lipids than the infested even though the protein content was lower. There was a decrease in the moisture content of the infected seeds. Protein content of *C. tora* was observed to be higher in infested seeds than that found in un-infested. Carbohydrate increased with increasing infection of the seeds by *C. subinnotatus*. Also, fibre was found to be higher in infested seeds as compared to the un-infested.

4. DISCUSSION

The decrease in moisture content of infested seeds could be attributed to metabolic and or respiratory processes of the insects which utilized the moisture for growth and other activities. This result is at variance with the report of Mbah and Silas (2007) which showed increase in moisture content with increase in infestation level of *Callosobruchus maculatus* on '*kananado*" cowpea variety. It however agrees with the report of Collier (1964) who stated that time of the year affect moisture content of produce. The result of the present study was at variance with the findings of Mbah and Silas (2007) that observed increase in lipid due to increase in protein content. The high amount of oil in form of lipids made it suitable as raw material in cosmetic and detergent industries. Ash content was found to increase in infested seeds compared with un-infested seeds. There was a decrease in the moisture content of the infected seeds. This could be attributed to the feeding activity of *C. subinnotatus* on *C. tora* seeds which led to reduction in the overall tissue mass and hence water content of the lost tissues. This is similar to the findings by Mbah and Silas (2007). Protein content of *C. tora* was observed to be higher in infested seeds than that found in un-infested. This is similar to the report by Sowunmi (1977) who robserved increase in crude protein in moderately infested seeds when compared with un-infested cowpea seeds. The increase in protein may be due to remains of the eggs, egg cases, carcasses of larvae and pupae, pupal exuviae, insect carcass and other excretory products that were left behind after removal of larvae, pupae and adult insects prior to analysis. Altrafine (2013) however reported high protein content of healthy seeds of *C. tora* which predisposes it to attack by *C. subinnotatus*. Carbohydrate increased with increasing infection of the seeds by *C. subinnotatus*. The result is in disagreement with the report of Kwatamdia (1983) that carbohydrate decrease with severity of infestation. The monosaccharide fractions in the un-infested seeds have all been converted in to polysaccharides in infested seeds. This finding was also in disagreement with the report of Mbah and Silas (2007) that carbohydrates decreased with severity of infestation of cowpea by *Callosobruchus maculatus*. Fibre content was observed in this study to be higher in infested seeds (14.30%) compared to un-infested seeds (11.61%).

5. CONCLUSION

The result of this investigation clearly indicated the loss of moisture and lipid as a result of Carbohydrate increased with increasing infection of the seeds by *C. subinnotatus* infection of the *C. tora* seeds and the increments in the other nutrients. More work should be done to unravel the mechanisms that led to increases in the level of the nutrients reported.

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Table 1: Nutrient composition of *C. tora* seeds as affected by *Callosobruchus subinnotatus* (Pic.)

Nutrients	Uninfested seeds	Infested seeds
Moisture	10.04	5.07
Ash	3.34	5.88
Protein	3.57	14.40
Fibre	11.61	4.21
Carbohydrate	62.94	70.44

Plate 1: *Cassia tora* pods

Plate 2: Edible part of *C. tora* (leaves)

Plate 3: Cooked *C. tora* leaves being

Plate 4: Healthy *C. tora* seeds traded in Katsina town

Plate 5: *C. subinnotatus* larvae

Plate 6: Seeds of *C. tora* infested with *C. subinnotatus* eggs

Plate 7: *C. subinnotatus* Adults

Plate 8: Punctured hole by *C. subinnotatus* on *C. tora* pod

Plate 9: An exit hole left by *C. subinnotatus* on a *C. tora* seed

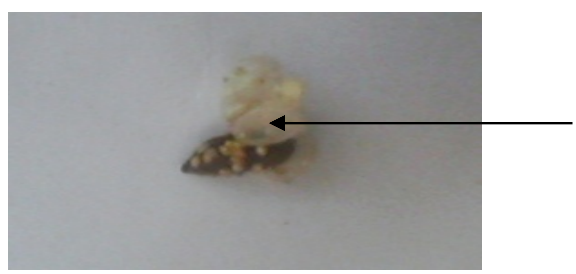

Plate 10: *C. subinnotatus* pupal case