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Website: www.aarf.asia Email : editor@aarf.asia , editoraarf@gmail.com

EFFECT OF AFRICAN BLACK PEPPER SEED OIL ON COWPEA SEED WEEVIL CALLOSOBRUCHUS MACULATUS(F.) (*COLEOPTERA: BRUCHIDAE*).

Jibrin, A.D[1*], Fagam, A.S[2]., Sabo, U.M[2]., And Abdallah, Y.[3]

[*1]Graduate School, University of the Philippines LosBanos, Laguna 4031, Philippines

[2]Department of Crop Production, Faculty of Agriculture and Agricultural Technology, Abubakar Tafawa Balewa University, P.M.B 0248 Bauchi,Nigeria.

[3]Crop Protection Unit, Department of Agriculture and Technical Services,Bauchi State Agricultural Development Program (BSADP) P.M.B.0050, Bauchi state, Nigeria.

ABSTRACT

The effect of West African black pepper (Piper guineense) seed oil on development of Callosobruchus maculatus (F.) on cowpea seed (Borno brown) inEntomology laboratory (40-30^{o}C temperature and 59-63% relative humidity) of Faculty of Agriculture,University of Maiduguri,Nigeria were examined under three studies (Post-oviposition, Pre-oviposition and lethal dose concentration (Lc_{50}) in January, 2016. Seventy two (72) glass jars of 100ml size was used , experiment was set up in Completely randomized design (CRD) , during each of the studies 10kg of cowpea seed (Borno brown) were weighted into 24 100ml glass jars covered with a muslin cloth and infested with 1-2 day old C. maculatus, thereafter, 50, 100, 150, 200, and 250mg of piper guineense seed oil were delivered in 0.2ml acetone onto four replicates each of 10g cowpea seeds in the glass jars, Control treatment was 0.02ml acetone. Data on number of eggs laid, number of adult first filial generation, percentage seed damage, weight loss and number of Callosobruchus maculatus mortality were collected. Results showed five dosages of Piper guineense seed oil were effective, causing adult mortality, reducing and stopping

oviposition, adult emergence, percentage weight loss and damage to cowpea seeds and was significantly difference (P≤0.05) from control. Result is indicative of the potential for the use of piper guineense seed oil in protecting stored cowpea seed against Callosobruchus maculatus by the resource poor farmers who form the bulk of cowpea seed producers in most cowpea producing areas.

Key words: Black Pepper, Callosobruchus maculatus, Cowpea seeds, Infestation.

Introduction

Cowpea vigna unguiculata (L.) (Walp) is a dicotyledonous annual crop. There is a high diversity of the cultivated species in west Africa where both dry and wet season cultivation is supported by the satisfactory crop growth conditions in the region (Opeke, 2006).Cowpea has beenidentified as amajor important crop in the livelihood of millions of relatively poor people in less developed countries of the tropics (Isubikalu et al., 2010).Rural families various derive food, animal feed, and cash together with spillover benefits to their farmlands through in- situ decay of root residues, uses of animal manures, and ground cover from its spreading and low growth habit (Ezeand Ogbonna 2011). Cowpea grain is widely traded out of the major production areas and it provides a cheap and nutritious food for relatively poor urban communities (Quin, 1997).

Nigeria accounts for up to 70% of the total world cowpea production (Soyinka*et al*., 1997). However, insect pest attack is an important limiting factor to optimum cowpea production in the country (Felicia *et al*., 2013). The cowpea seed is subject to serious infestation from insect pests during storage and unprotected cowpea could be completely infested following 3-5 months storage (Adedire and Lajide, 2001).

The Bruichid ,Collasobruchus maculatus Fabricious (Coleoptera: Bruchidae) , is economically the most important borer of stored cowpea in Nigeria (Maina *et al*., 2012) causing heavy post-harvest losses to the crop (Bamaiyi *et al.,* 2006) with infestation reaching up to 100% in unprotected store cowpeas (Dauda *et al.,* 2012).The larvae of the bruchid do all the damage. They spend their entire immature lives in individual legume seeds where they cause weight loss, decrease in germination potential and also diminish the market value of the crop. A Larva can consume up to 13% of the cowpea seed (Lale and Maina 2005).

As a major factor responsible for serious loss of cowpea in store, the search for the control of C.maculatus is continuing. The non-availability as well as the high cost of Synthetic pesticides, coupled with the potential hazards posed by these pesticides to the environment, humans and livestock, have necessitatedthe search for various local plant product for use in the control of this insect pest of cowpea in storage. Currently, Nigeria farmershave stated utilizing local plant productsto control insect pests in stored produce (Onu andAliyu 1995). Powdered fruits of pepper is one of such plant product.

In recognition of the above the continued selection and usage of syntheticchemicals in protecting stored grains is becoming an old fashion practice hence increasingly unpopular, there by necessitating the genuine search for natural, edible, cheap and safe plant materials that would not contaminate food products in acting as grain protectants. Other problems associated with the continuous use of synthetic insecticides, such as resistance and residuals, will stimulate the use of any effective, easy to use, inexpensive, Biodegradable and safe alternatives which are already a part of our diet. It was against the above stated reasonsthat this research was initiated and conducted in the laboratory for a period of one month to determine the insecticidal effect of black pepper (piper guineense Schum and Thonn) on the biology of cowpea bruchid and time of application in order to control cowpea bruchid C. maculatus.

Materials and Methods

Materials: 3kg of cowpea seeds identified as Borno brown was obtained from the local market in Bauchi, Bauchi state, Nigeria ($10^0$22' N' $09^0$04' E).The seeds was sorted out to remove damaged ones and later disinfected. West African black pepper (piper guineense) was also obtained from same market. The seeds were cleaned by removing dirt, those clean seeds were then pulverizedusing a warming milling machine. Test insects (adults C. Maculatus) that were used for the experiment were obtained from an already culture of cowpea bruchids raised in the Agronomy laboratory of the Department of crop Production,Abubakar Tafawa Balewa University, Bauchi. Insect cultures was raised under laboratory weather conditions.Seventy two (72) glass jars of 100ml each were used for the experiments.The experiments were conducted under laboratory conditions of 40-30^0c and 59-63% relatively humidity.

Oil extraction: 1020g pulverized seed of west African black pepper (p. guineense) was extracted for 6 hour with 3 liters n-Hexane, at 68-69^0c using a soxhlet extractor. Concentration of oil was

done by heating the flask in a ventilated oven for 45 minute at $103+2^0$c. The oils was stored in glass jars and weighted out for different assay that were carried out. Atotalof 750ml oil was obtained from 1020g pulverized seed.

Experiment Design: The experiment was set up in a completely randomized design (CRD) and consist of three studies which were replicated four times each (post-oviposition treatment, pre-Oviposition treatment and determination of LC50 at different dosage of oil).

Post-Oviposition: in this experiment 10g of cowpea seeds (Borno brown)were weighted into 24 100ml glass jars. Each glass jars was infestedwith 1-2 day old five female and two male, adult of Callosobruchus Maculatus and allowed to mate and ovipositeggs for four days before they were removed. Thereafter, 50, 100, 150, 200 and 250mg of West African black pepper oil were delivered in 0.2ml acetone onto four replicates each of 10g cowpea seeds in the glass jars. Control treatment was set along and treated with only 0.2ml acetone.At each time of oil application, treatments were stirred thoroughly with glass rod in order to have an even distribution of oil over the grain surface. The glass jars were left open for about 10 minutes in order to allow the carrier (acetone) to completely evaporate before lid coverswere replaced. The number of eggs laid in each treatment and replicate was counted and recorded.Treatment were examine from progeny emergence for a period of one month and total number of emergence in each treatment was counted and recorded. Cowpea seeds was subsequently assessed for percentage damage and weight loss after a period of one month.

Pre-Oviposition treatment: In this experiment 10g of cowpea seeds were weighted out into 24 100ml glass jars. 50, 100, 150, 200 and 250mg of west African black pepper was used to treat seeds before infestation with test insects. Infestation was carried out using the same number of insects and age in each treatment as describe earlier. All eggs laid in each replicate were counted and recorded. Treatments were examinedfrom progeny emergencefor a period of one month, number of insects emerging over a period of one month were counted and recorded. Percentage weight loss and damage was assessed after a period of one month.

Toxicity test (LC50): In this toxicity test, 10g of cowpea seeds were weight out into 24 100ml glass jars. Also, 50, 100, 150, 200 and 250mg of West African black pepper was used to treat seeds before infestation with test insects. Thirty (30) adult Callosobruchus maculatus of mixed sex were introduce into each glass jar containing the 10g of cowpea seeds. After 24 hours the

contents was observed thoroughly, the number of death in each replicate was counted and recorded as mortality and number of eggs deposited were also counted and recorded in each replicate. The numbers of insects'emergence over a period of one month were counted and recorded. StatisticalAnalysis**,** Percentage egg mortality, Oviposition deterrents and adults emergence were calculated using a one way analysis of variance. All percentage data were subjected to arc sine values before analysis. Mean separation was done using least's significant difference (LSD) (P=0.05). Percentage seed damage and weight loss using Ivbijaro and Agbaje (1986) procedure.

Result

The Oviposition deterrent of P.guineenseoil on Callosobruchus maculatusis presented in Table 1. The result shows that cowpea seeds treated with Piper guineenseoil highly prevents Oviposition as there was a high significant difference between treatment at lower dosage of 50 and 100 compare to control. Also, there is no statistically significant difference between treatments with higher doses. The result indicates that Piper guineense oil deterredCallosobruchusmaculatusfrom Oviposition in treated cowpea seeds compared to untreatedcontrol. The pre-oviposition treatment result shown in Table 2 indicate that there was no statistically significant difference between treatments at lower doses, in comparison to the treatments with higher doses. Piper guineense oil completely prevented the emergence of adult cowpea bruchid in treatment involving 150, 200 and 250mg dosages.

Table 1: Effects of Piper Guineense seed oil on egg laying of C.maculatus.

Dosage of seed oil	No. of eggs laid ± SE
(mg/10g seed)	
0.00	14.50 ± 4.1
50.00	4.00 ± 1.2
100.00	5.75 ± 4.6
150.00	0.75 ± 0.5
200.00	0.25 ± 0.5
250.00	0.00 ± 0.00
LSD (P=0.05)	**4.22**

Result of Post-oviposition treatmentas shown in Table 2 shows that Piper guineense seed oil prevented adult emergence in treatment with higher dosages compare to control. However, ivbijaro (1990) has reported that a crude extract of black pepper was highly toxic to rice weevil(Sitophilus oryzae L.) and this was attributed to the presence of piperine in black pepper. The study has shown that treating cowpea seeds with 100, 150, and 250mg of P.guineense seed will completely prevent adult C. Maculatusemergence on cowpea seeds even if eggs has already been deposited on the seeds implying less damage to the cowpea seeds.

Table 2. Effect of piper Guineense seed oil on the emergence of C.maculatus.

Dosage of seed oil	No. of adult emergence ± SE	No. of adult emergence ±SE
(mg/10g seed)	Pre-Oviposition	Post-Oviposition
0.00	64.87 ± 26.5	21.20 ± 5.2
50.00	10.00 ± 20.0	0.30 ± 0.6
100.00	8.35 ± 16.7	0.00 ± 0.00
150.00	0.00 ± 0.00	0.00 ± 0.00
200.00	0.00 ± 0.00	0.00 ± 0.00
250.00	0.00 ± 0.00	0.00 ± 0.00
LSD (P=0.05)	**20.86**	**3.24**

The resultsin Table 3 Indicate that there was no significant weight loss under pre-oviposition experiment treated with different dosages of Piper guineense seed oil. Thus, the ability of the seed oil to prevent significant infestation is remarkable due to its pungent active component (piperine) that prevented most of the eggs from hatching. The same trend was noticed under the post-oviposition treatment. However, the weight loss that was recorded in untreated control was significantly different from the treated ones. This result shows that P.guineense seed oil is very effective in protecting cowpea seed from damage by Callosobruchus maculatus.

Table 3. Weight loss in cowpea seeds treated with piper Guineense seed oil due toCallosobruchus maculatus infestation.

Dosage of seed oil	Weight loss ± SE	Weight loss ± SE
(mg/10g seed)	Pre-Oviposition	Post-Oviposition
0.00	15.25 ± 21.3	46.00 ± 14.30
50.00	0.05 ± 0.1	1.25 ± 1.5
100.00	0.75 ± 1.5	0.00 ± 0.00
150.00	0.00 ± 0.00	0.00 ± 0.00
200.00	0.25 ± 0.5	0.00 ± 0.00
250.00	0.00 ± 0.00	0.00 ± 0.00
LSD (P=0.05)	**13.19**	**8.74**

The result of the toxicity test (LC50) showed that there was a total and complete mortality of adult C. maculatusafter 24 hours of the application of P.guineenseseed oil on cowpea seeds. However, the result of the untreated control was significantlydifferent from the treated ones (Table 4). The result has proof that Piper guineenseseed oil is very effective in causing adult mortality of Callosobruchus maculatus.

Table 4. Effect of piper guineense seed oil on survival (mortality) of C.maculatus Adults (LC_{50} test).

Dosage of seed oil	No. of adult mortality ± SE
(mg/10g seed)	
0.00	16.35 ± 23.5
50.00	100 ± 0.00
100.00	100 ± 0.00
150.00	100 ± 0.00
200.00	100 ± 0.00
250.00	100 ± 0.00
LSD (P=0.05)	**10.00**

Discussion

Oviposition: The significant difference effects obtained could be due to the high and strong irritating or repulsive odour cause by piperine which is the main component of p. guineense oil. Yahaya (2002) and Maina (2006) reported that the oil and powder of p. guineense significantly reduced the egg laying capacity of c. maculatus weevils and that the ovicidal effects of or its 'guineense 1'components which causes suffocation of adult bruchids, thereby, preventing physical contact or hindering Oviposition success.

Pre-oviposition. The non-significant difference and highly significant difference recorded in this study among treatment at lower dosage control and 50, 100, 150, 200 and 250mg suggest that application of Piper guineense seed oil, especially at 150, 200 and 250mg/10g cowpea seeds, highly reduced the number of bruchid larval that survive till the emergence of adults C.maculatus. Maina et al., 2012 , Dauda et al., 2012, and Seck et al., 1991 were of the view that this could be due to either higher morality of immature larval or emerging adults contacting the Piper guineense oil while gnawing their way out of the cowpea seed.

Post-oviposition. Result indicate that Piper guineense seed oil significantly reduced weight loss of cowpea seed due to C.maculatus infestation. Olaifa and Exhun (1988)observed that weight loss is a measure of degree of damage in stored grains especially in the tropics. The reduced (no emergence) of adults Callosobruchus maculatus noticed as a result of the application ofvarious dosage of piper guineense seed oil might have contributed to reduced and no adult emergence of Callosobruchus maculatus which proof its ability as a cowpea seed protectant and deterrant against Callosobruchus maculatus. Ivbijaro (1990) showed that the volatile oil of piper guineense completely prevented post-oviposition at 0.02% and cause 100% mortality of bruchid females at 2 and 3ml kilogramme of cowpea within 24hours.

Toxicity test (LC_{50}). The 100% mortality of bruchid within 24 hours of the application of Piper guineense seed oil at all dosage could be attributed to piperine and chavicine which are the main constituent of piper guineense seed oil causing stomachor contact toxicity resulting in quick death (mortality) of the Callosobruchus maculatus coming into contact with it. Yahaya and Magaji (1997) recorded a highly significant effects of piper guineense seed oil whichcauses a reduced egg laying capacity of adults C.maculatus and their survival on cowpea grains. The

leaves of piper nigrum has been reported to increase the mortality of adults C.maculatus on cowpea grains (Rajapakse, 1998).

Conclusion and Recommendation

Different dosages of the seed oil of Piper Guineense was studied and their efficacyon the development,biology and mortality of Callosobruchus maculatus was studied. The result of this study revealed that, Piper guineense seed oil has a high potential in killing and deterring cowpea bruchid from Oviposition. Thus, leading to low weight loss and damage. Hence, the use of P.guineenseseed oil in treating cowpea seed can be a source of means of preventing C. maculatus from causing menace during storage and a source of hope to poor resource formers who cannot afford synthetic insecticides.

Conflict of interest: The authors have declared that no conflict of interests exist.

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