EFFECTS OF DURATION ON THE EFFICACY OF AQUEOUS EXTRACT OF NEEM SEED *Azadirachta indica* IN THE CONTROL OF *Callosobruchus maculatus* IN STORED COWPEA

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ABSTRACT

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Aqueous extracts of Azadirachta indica seed were evaluated as a grain protectant against cowpea beetle Callosobruchus maculatus (F) in the Laboratory at 1.0, 1.5 and 2.0 (%v/w) concentrations. The experiment was done using a completely randomised design. The aqueous extract and the control served as the treatments. Each treatment was replicated three times. The parameters assessed include adult mortality, larval and pupal emergence and adult emergence. Mortality was assessed at 1, 14 and 28 days after infestation with adult C. maculatus at a sex ratio of 3:3 (3 males: 3 females). A. indica at 2.0 (%v/w)/16.7g seeds resulted in a significant reduction in adult mortality, larval, pupal and F1 progeny emergence 1 day after treatment. The aqueous extract did not affect the physical appearance of the cowpea seeds as no mouldiness was observed though there was a bitter taste noticed during the consumption of the seeds.

Keywords: Bruchid beetle, botanicals, control, cowpea, duration.

INTRODUCTION

Cowpea *Vigna unguiculata* L. (Walp) is one of the most important crop grown in the tropical and subtropical regions of the world (Ntoukam et al., 2000). It is also a multipurpose crop grown as human food and fodder (Patterson et al., 2000). According to FAO (1996), Nigeria produces the highest quantity of cowpea with an annual production of 2.1 million tonnes, closely followed by Niger, 65,000 tonnes and Mali, 110,000 tonnes. In Nigeria, about 80% of cowpea production is from the northern part of the country due to low rainfall in this region (FAO, 1996).The world cowpea production in 2001 was estimated at 3.3 million tonnes grown on 122.5 million hectares (FAO, 2004). West and Central Africa is the leading cowpea producing regions in the world. These regions produce about 64% of the total estimated 3.3 million tonnes of cowpea seeds produced annually (FAO/PESA, 2007).

Cowpea plays a critical role in the lives of millions of people in Africa and other parts of the developing world where it is a major source of dietary protein (IITA, 2007). It is commonly grown as a secondary crop in association with other staples such as maize, sorghum, millet and cassava and constitutes the cheapest source of dietary protein and energy (IITA, 2007). It is used as both a vegetable and grain containing between 23-25% protein and 50-67% starch (FAO, 1996; Okigbo, 1978). Cowpea is utilised as fodder for livestock feed and it is a valuable component of farming systems in many areas because it has the ability to restore soil fertility for succeeding cereal crops grown in rotation with it (Adedire, 2009). It is also a valuable commodity that produces income for farmers and traders (Adedire, 2009). Even though cowpea is popular and nutritionally important to many people, its cultivation is under threat from insect pests, both in the field and in storage with *Callosobruchus maculatus* being the most damaging insect pest of cowpea in storage (Monti *et al*., 1997). It attacks the cowpea before harvest causing qualitative and quantitative losses to seeds in storage (Shade *et al*, 1996). *C. maculatus* multiplies rapidly in storage, giving rise to a new generation every month (Ouedrago *et al*., 1996). Infestation on stored cowpea grains may reach 50% within 3-4 months of storage (Pascual-villalobus and Ballesta-Acosta, 2003).

Different methods have been used over the years to protect cowpea in storage, with synthetic pesticides been the most favourable. Although various synthetic insecticides have been developed over the years for the control of *C. maculatus*, the cost of purchase, residual effect, health hazard to grain handlers and the widespread development of resistance in insect pests are still issues of great concern (Udo, 2011). It is in the light of the above problems that the need to develop alternative control strategies that will be affordable and eco-friendly becomes expedient.

MATERIALS AND METHODS

Cowpea seeds variety IAR 48 obtained from the National Seed council were used for the experiment. A culture of *C. maculatus* was set up in the laboratory of the Department of Crop Protection University of Ilorin at ambient temperature. Aqueous extract of neem seed obtained from freshly harvested neem seeds were used as the bio pesticide treatment in the experiment. The Low-Tech method of extraction as described by Ganguli (2000) was used in the extraction of the bio pesticide.

16.7g of cowpea seeds were placed in a plastic container measuring 10 cm x 8cm. The contact method of application was used to apply the treatments to the seeds. The bio pesticide was rubbed on to the seeds uniformly to ensure that they were all properly coated. Four different concentrations namely 0 ml, 1.0ml, 1.5ml and 2ml of aqueous neem extract each were added to the cowpea seeds in a completely randomised design. There were three replicates per treatment. All the cowpea seeds were treated on the same day with the bio pesticides. Six newly emerged adults of *C. maculatus* in a ratio 1:1 (3 males: 3 females) were introduced into each plastic container at 0 day after treatment (immediately after application of the bio pesticide), 14 days after treatment with the bio pesticide and 28 days after treatment with the bio pesticide.

The following data were collected from the experiment namely adult mortality, larval emergence, pupal emergence and F1 progeny emergence. The data had zero values and 0.5 was added to all entries and transformed using the square root transformation. Data were analysed using Genstat and the means were separated using the Least Significant Difference (LSD).

RESULTS

At 0 day after treatment (DAT) the bio pesticide at all levels of concentration caused an increase in adult mortality of *C. maculatus* but it was not significantly different at P=0.05 (Table 1a). The highest mortality of *C. maculatus* was at 2.0ml/16.7g of seed and this was significantly different from the control. At 14 DAT, there was mortality of *C. maculatus* at all levels of concentration of the bio pesticide with 2.0ml/16.7g of seed showing the highest mortality. There was no significant difference at P=0.05 between the different treatments concentration and the control (Table 1b). The mortality rate at 28 DAT follows a similar trend to what occurred at 14 days after infestation (Table 1c).

The bio pesticide at all levels of concentration was able to reduce both the larval and pupal emergence when compared to the control (Table 2a). Again, 2.0ml per 16.7g of seed had the highest reduction in larval emergence while 1.0ml/16.7g of seed had the lowest pupal emergence. There was a significant difference between all the treatments and the control at P=0.05 for larval emergence, but there was no significant difference between all the treatments and the control for pupal emergence at P=0.05 (Table 2a). At 14 DAT and 28 DAT respectively, the bio pesticide was able to lower the larval and pupal emergence (Table 2b and Table 2c), but there was no significant difference between the treatments and the control at P=0.05

The F1 progeny was highly reduced at 2.0ml per 16.7g of seed when treated at 0 DAT (Table 3a). There was a significant difference between the bio pesticide at 2.0 ml per 16.7g of seed and the control at P=0.05. At 14 DAT, the F1 progeny emergence was highly reduced when compared to the control and it was significantly different at P=0.05 (Table 3b), while at 28 DAT, there was very little reduction in the F1 progeny emergence between the different levels of treatments and the control (Table 3c). There was no significant difference at P=0.05 between the treatments and the control at 28 DAT.

DISCUSSION

The result of the experiment shows that aqueous neem seed extract significantly reduced the incidence, growth and development of *C. maculatus* on the treated cowpea seeds. The effect on *C. maculatus* was related to the concentration and the time of treating the seeds. Treating cowpea seeds immediately after harvest with 2.0ml/16.7g of seed of the aqueous neem seed extract was the most effective providing adequate protection for the seeds. The study also showed that aqueous neem seed extract cannot be used for long storage of cowpea as the potency is reduced with increase in time. This corroborates the work of Khalid and Shad (2000) who stated that the toxic effect of neem is normally of an ephemeral nature disappearing within 14 – 21 days. The organoleptic test showed that the cooked seeds had a bitter taste as opposed to the palatable taste it should have and the bitterness increased with increasing concentrations of the aqueous extract. Due to the bitter taste observed during cooking, it is recommended that the aqueous extract be used as a seed dressing for seeds to be planted and not for seeds for consumption.

Table 1a: Mean mortality of adult *C. maculatus* 1 day after treatment (1 DAT)

Concentration	Days after Treatment													
ml/16.7g	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
1.0	0.87	0.70	1.05	0.70	1.05	0.99	1.34	0.70	1.26	1.34	0.87	0.70	0.70	0.70
1.5	1.05	1.22	1.47	0.87	1.22	0.87	0.87	1.05	0.87	0.87	0.70	0.70	0.70	0.70
2.0	1.17	1.22	1.56	1.17	0.70	1.22	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Control	0.70	0.70	0.70	0.70	1.22	0.70	0.70	1.58	1.22	1.58	1.22	1.22	0.70	0.70
LSD	0.66	0.54	0.67	0.53	0.30	0.74	0.39	0.30	0.66	0.30	0.30	0.70	0.70	0.70

Table 1b: Mean mortality of adult *C. maculatus* 14 days after treatment (14 DAT)

Concentration	Days after Treatment												
ml/16.7g	D14	D15	D16	D17	D18	D19	D20	D21	D22	D23	D24	D25	D26	D27
1.0	0.70	0.87	0.70	1.22	1.05	0.87	1.17	0.70	1.46	0.99	0.87	0.70	0.70	0.70
1.5	0.70	0.87	0.70	0.87	1.34	0.87	1.17	0.87	1.17	0.70	0.70	0.70	0.70	0.70
2.0	0.70	0.99	0.99	1.05	0.99	1.29	1.22	1.05	0.70	0.70	0.70	0.70	0.70	0.70
Control	0.70	0.70	0.87	0.70	0.70	1.58	0.70	0.70	1.22	1.22	1.22	0.70	1.22	0.70
LSD	0.70	0.69	0.63	0.39	0.60	0.60	0.00	0.49	0.50	0.50	0.30	0.70	0.70	0.70

Table 1c: Mean mortality of adult *C. maculatus* 28 days after treatment (28 DAT)

Concentration	Days after Treatment												
ml/16.7g	D28	D29	D30	D31	D32	D33	D34	D35	D36	D37	D38	D39	D40	D41
1.0	0.70	0.70	0.70	1.34	1.05	1.58	0.87	0.70	0.87	1.17	0.87	0.70	0.70	0.70
1.5	0.70	0.87	0.70	1.05	0.99	1.22	0.87	0.70	0.70	0.70	0.70	0.70	0.70	0.70
2.0	0.70	0.87	0.70	1.65	1.17	0.87	1.35	0.87	0.70	0.70	0.70	0.70	0.70	0.70
Control	0.70	0.70	0.70	0.70	1.58	0.70	0.70	1.22	0.70	0.70	0.70	1.22	0.70	0.70
LSD	0.70	0.46	0.70	0.51	0.80	0.30	0.84	0.87	0.46	0.44	0.12	0.52	0.70	0.70

Table 2a: Mean values of larval and pupal emergence 1 day after treatment (1 DAT).

Concentration ml/16.7g of Seeds	Larval Emergence	Pupal Emergence
1.0	1.34	1.17
1.5	0.87	1.34
2.0	0.70	1.22
Control	2.12	1.84
LSD	0.60	0.51

Table 2b: Mean values of larval and pupal emergence 14 days after treatment (14 DAT).

Concentration ml/16.7g of Seeds	Larval Emergence	Pupal Emergence
1.0	1.65	1.90
1.5	1.16	1.90
2.0	1.05	1.52
Control	2.12	2.54
LSD	1.18	2.24

Table 2c: Mean values of larval and pupal emergence 28 days after treatment (28 DAT).

Concentration ml/16.7g of Seeds	Larval Emergence	Pupal Emergence
1.0	2.11	1.99
1.5	2.26	1.77
2.0	1.95	1.95
Control	2.54	2.34
LSD	2.14	2.32

Table 3a: Mean value of F1 progeny emergence of *C. maculatus* 1 Day after treatment (1 DAT)

Concentration	Days after Treatment									
ml/16.7g	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11
1.0	1.32	2.84	2.63	2.88	3.36	2.56	0.99	1.93	1.05	0.87	0.70
1.5	1.05	1.09	2.02	1.54	1.29	1.38	0.99	1.93	1.05	0.87	0.70
2.0	0.70	0.70	0.70	0.87	0.70	0.70	0.87	0.87	0.70	0.70	0.70
Control	3.80	4.30	3.53	3.53	3.42	3.67	4.30	3.80	3.08	3.67	2.34
LSD	0.40	0.79	0.97	1.13	0.82	0.73	0.73	0.38	0.35	0.35	0.70

Table 3b: Mean value of F1 progeny emergence of *C. maculatus* 14 Days after treatment (14 DAT)

Concentration	Days after Treatment												
ml/ 16.7g	D12	D13	D14	D15	D16	D17	D18	D19	D20	D21	D22	D23	D24
1.0	3.97	3.32	3.41	3.36	2.70	2.52	1.17	1.94	1.52	1.52	1.44	1.34	0.70
1.5	2.04	2.40	2.82	3.07	2.63	2.60	2.06	2.00	1.22	1.22	1.81	0.87	0.70
2.0	2.03	2.66	2.88	2.83	2.18	1.38	2.01	1.58	0.87	0.87	0.87	0.87	0.70
Control	4.30	4.06	2.93	4.06	4.18	2.73	3.08	3.08	1.58	1.22	0.07	0.70	0.70
LSD	1.26	1.36	0.95	0.95	0.98	1.25	0.94	0.94	0.64	0.64	0.67	0.67	0.70

Table 3c: Mean value of F1 progeny emergence of *C. maculatus* 28 Day after treatment (28 DAT)

Concentration	Days after Treatment												
ml/16.7g	D28	D29	D30	D31	D32	D33	D34	D35	D36	D37	D38	D39	D40	D41
1.0	4.15	4.44	4.00	4.20	4.49	3.86	4.91	3.82	3.61	3.42	3.26	3.32	3.16	2.67
1.5	3.72	3.79	4.40	3.74	3.78	3.69	3.36	3.93	3.75	3.17	3.47	3.00	2.99	2.62
2.0	3.57	4.00	4.26	4.12	3.61	3.81	3.33	3.53	3.71	3.08	3.38	2.63	2.92	2.83
Control	4.74	4.52	4.30	4.52	3.93	4.41	4.18	3.08	4.06	3.67	3.24	4.18	3.80	2.73
LSD	0.94	0.90	0.79	1.07	0.81	0.93	0.63	0.84	0.52	0.90	0.89	0.48	0.59	1.28

REFERENCES

Adedire, C. O. 2009. Safety and policy issues in formulation and use of medicinal plants; In formulation of medicinal plants for crop and animal production in Nigeria: Realities and Challenges, proceedings of the 5th conference (Akure-Humboldt Kellog) pp 9-12.

FAO/PESA Nicaragua, 2007. Project documentation of the special program for food security. P5

FAO, 2004. Economic and Social Department. http://www.gmocompass.org/eng/crop/18.genetically-modified-cowpea-eu.html

FAO, 1996. Food and Agricultural Organisation year book production, FAO Rome, Italy.

IITA, 2007. www.iita.org/research/summaries/sumproj 8.html

Khalid, S. and Shad, R. A. 2002. Potential advantage of recent allelochemical discoveries and agro ecosystems. *Progressive. Farming*. 11:30-35.

Monti, L. M., Murdock, L. L. and Thottapplly, G. 1997. Opportunities for biotechnology in cowpea.

Ntoukam, G., Murdock, L.L., Shade, R. E. and Kitchand Endondo, L.W. 2003. Managing insect pests of cowpea in storage. Bean/Cowpea, Midcourse 2000 Research Meeting, April 9-14,Senegal. P3-4.

Okigbo, B. N. 1978. Cropping systems and related researches in Africa. Association of Advancement of Agricultural Science in Africa (AAASA). *Occasional Publication Series.* OT-1. 81pp.

Ouedrago, A. P., Sou, S. and Sanon, A. 1996. Influence of temperature and humidity on population of *C. maculatus* (Coleoptera:Brudhidae) and its parasitoid *Dinarmus basalis* (Peteromalidae) in two climatic zones of Burkina Faso. *Bulletin of Entomological Research*. 86: 695-702.

Pascual-Villalobos, M. J. and Ballesta-Acosta, M. C. 2003. Chemical variation in an *Ocimum bacilicum* germplasm collection and activity of the essential oils on *Callosobruchus maculatus*. *Biochemical Systematics and Ecology*. 31: 673-679.

Patterson, S. P., Mcwatters, K. H., Hung, C., Chinnan, M. S. and Phillips, R. D. 2000. Consumer acceptability of Akara (fried Cowpea paste) made from three different varieties of cowpea. Research program on sensory. www.griffin.peachnet.edu/fst/Pages/Consumer.html

Shade, R. E., Kitch, L. W., Mentzer, P. and Murdock, L. L. 1996.Selection of a cowpea weevil (Coleoptera: Bruchidae) biotype virulent to cowpea weevil resistant Landrace TVu 2027. *Journal of Economic Entomology.* 89: 1325-1331.

Udo, I. O. 2011. Potentials of *Zanthoxylum xanthoxyloides* (Lam.) for the control of stored product insect pests. *Journal of Stored Products Postharvest Research*. 2(3): 40-44.