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Efficacy of Integrating Varietal Resistance and Neem (*Azadirachta indica* A. Juss) Seed Oil for the Management of *Callosobruchus maculatus* Infesting Bambara Groundnuts in Storage

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ABSTRACT

The efficacies of three rates (0.5, 1.0 and 1.5 mg/20 g seed) of application of neem (*Azadirachta indica*) seed oil (NSO) in controlling *Callosobruchus maculatus* infesting stored bambara groundnut were assessed on four local cultivars (Ole, Bakingangala, Bulmono and Jor) with different susceptibilities to bruchid attack. Untreated batches of the cultivars were included to serve as control. Treated and untreated batches were infested with adults of *C. maculatus* under prevailing storage conditions. Combining application of NSO especially at the rate of 1.5 mg/20 g seed with varietal resistance significantly reduced oviposition and progeny development in *C. maculatus*. No adult progeny developed in seeds of Bakingangala that were protected with 1.5 mg of oil. Treatment of seeds with NSO at this rate reduced seed damage from 97, 72.3, 58.1 and 87.9% in unprotected Ole, Bakingangala, Bulmono and Jor to 3.0, 3.4, 0.0 and 18.3%, respectively. The interaction of the strategies also significantly reduced the severity of damage (number of adult emergence holes per seed) and overall susceptibility of the bambara groundnut cultivars. Susceptibility indices (SI) values for unprotected bambara groundnuts were 11.92, 10.52, 9.09 and 11.43 for Ole, Bakingangala, Bulmono and Jor, respectively. Comparable SI values for bambara groundnuts that were treated with NSO at the rate of 1.5 mg/20 g seed were 0.0, 1.12, 0.0 and 2.82 for Ole, Bakingangala, Bulmono and Jor, respectively.

INTRODUCTION

Bambara groundnut, *Vigna subterranea* (L.) Verdcourt, is susceptible to infestation by field-to-store pest bruchids. It is often infested simultaneously by two species of Bruchidae, *Callosobruchus subinnotatus* (Pic.) and *Callosobruchus maculatus* (F.) in West Africa (Haines, 1991; Mbata, 1992). Of these two bruchids, *C. maculatus* is the more destructive on account of its shorter life cycle and higher intrinsic rate of increase (Lale and Vidal, 2001). Whilst *C. maculatus* attacks a wide range of grain legumes, *C. subinnotatus* is restricted to bambara groundnut (Haines, 1991; Silim Nahdy, 1998). These bruchids commence infestation in the field once bambara groundnuts have been harvested and left to dry, reducing the quantity and quality of the grains during storage (Prevett, 1966; Dike, 1994).

In Nigeria, a major producer of bambara groundnut, several local cultivars and a number of improved varieties differing in maturity time, grain size and colour, are available (Rawal, 1973). There are limited reports in the literature on effective methods of controlling *C. maculatus* on bambara groundnut in storage. The integration of insecticidal products from locally available plants for use in storage, and the growing of varieties of grain legumes with some resistance to *C. maculatus* may lead to the sustainable management of the bruchid especially in subsistence agriculture (Lale and Mustapha, 2000).

Oil and powder obtainable from neem (*Azadirachta indica* A. Juss) seeds have been reported to provide sustained protection of stored grains (Ivbijaro, 1983; Makanjuola, 1989; Ogunwolu and Idowu, 1994; Lale and Ajayi, 1996). However, complete dependence on the application of products from neem seed for the control of bruchids on grain legumes may result in the emergence of resistant strains of the bruchid through selection (Lale and Mustapha, 2000). Varietal resistance in the crop species (complete or partial), though a relatively cheap and safe method of controlling *C. maculatus*, is also vulnerable to the emergence of biotypes of the bruchid that are tolerant of the resistant mechanism(s) of the host plant. There are examples of this in the literature (Dick and Credland, 1986a, b), which amply justify the need for an IPM approach to insect control (NRI, 1992).

The integration of varietal resistance and application of neem seed oil (NSO) for bruchid control is likely to be more successful than a sole dependence on either of them. In an integrated bruchid control programme, a resistant or partially resistant variety of grain legume may improve the effectiveness of insecticides. This study reports the findings of the integration of NSO and four cultivars of bambara groundnut with

different levels of resistance to infestation by bruchid species in the genus ***Callosobruchus*** (Ajayi and Lale, 2001a; Ahmed, 2002).

MATERIALS AND METHODS

Insect rearing and source of bambara groundnut cultivars

A batch of pristine cowpeas (cv. Borno brown) was obtained locally in Maiduguri (latitude 11°50'N and longitude 13°05'E) and used to rear a laboratory culture of *C. maculatus* under variable conditions (20-27°C and 18-54% r. h.). The experiment was also conducted under these conditions. Four cultivars (Ole, Bakingangala, Bulmono and Jor) of bambara groundnut obtained directly from farmers in Maiduguri and environ, were used for the study. These cultivars were previously reported to have varying susceptibilities to *C. maculatus* or *C. subinnotatus*: Jor was identified as susceptible; Ole and Bakingangala as moderately susceptible whereas Bulmono was identified as slightly susceptible (Ajayi and Lale, 2001a; Ahmed, 2002).

Extraction of neem seed oil

Mature, fallen neem seeds were gathered from a neem plantation in Maiduguri. These were washed, sun-dried for at least 5 h, decorticated and pulverized using a locally operated mill.

Five hundred grammes of the pulverized neem seed were weighed into a 5 litre plastic bowl and pre-boiled water was added, a little at a time, and kneaded into the powder. The paste was then subjected to a hydraulic pressure and the oil was expressed out manually into a 250 ml beaker.

Experimental procedure

Three dosages (0.5, 1.0 and 1.5 mg) of neem seed oil (NSO) were applied separately in 0.5 ml of analytical grade of acetone to 20 g of seeds (i.e. 25, 50 and 75 mg/kg seed) in each of the four cultivars of bambara groundnut in 100 ml glass jars. Treated seeds were stirred with a glass rod until seeds were uniformly coated and until the acetone had completely evaporated. Control seeds were treated with 0.2 ml of pure acetone and dried as described previously. The experiment was set up as a randomized complete block design with three replications.

Three pairs of 2-day old *C. maculatus* were then introduced into each jar with the aid of a pooter and the females were allowed to lay eggs for 4 days. On day 4, all insects were removed and the eggs laid on seeds in each jar were counted. Adult progenies that developed were counted and the number of damaged seeds (seeds bearing adult

emergence holes) in each jar was also counted. The number of damaged seeds wa expressed as a percentage of the total number of seeds in each replicate. Severity o seed damage (number of adult emergence holes per seed) was obtained by dividing th number of adult progenies by the number of damaged seeds. Susceptibility index (SI was obtained for each cultivar according to the method of Dobie (1974) and is given as:

$$SI = \text{Log}_e F_1 \times 100/D$$

where F_1 is the total number of emerging adults and D the median developmental perio (estimated as the time from middle of oviposition to the emergence of 50% of the F generation).

Data obtained were subjected to two-way ANOVA and means were compared usin the LSD statistic at the 5% level of probability.

RESULTS

Application of NSO at the rate of 1.5 mg/20 g seed significantly reduced egg layir by *C. maculatus* females on Ole and Jor. On untreated seeds, however, *C. maculat* laid significantly more eggs on Ole than on Bulmono (Table 1). Ole supporte significantly higher populations of *C. maculatus* adult progenies than any of the oth cultivars of bambara groundnut except Jor (Table 2). The application of NSO at t various rates obliterated observable differences in susceptibilities of the cultivars. Tab 2 also shows that application of NSO at the rates of 1.0 and 1.5 mg/20 g se significantly suppressed adult progeny development in Ole, Bakingangala or Jor seeds

Table 1. Mean number of eggs laid by *Callosobruchus maculatus* on different cultivars bambara groundnut that were protected with neem seed oil (NSO)

	Dosage of NSO (mg/20 g seed)				
Cultivar	0	0.5	1.0	1.5	Mean
Ole	120.0	86.0	67.0	43.3	79.1
Bakingangala	91.0	79.3	64.0	51.7	71.5
Bulmono	77.3	76.3	57.0	38.3	62.3
Jor	88.7	66.0	61.7	34.0	62.6
Mean	94.3	76.9	62.5	41.8	

SED = 10.2, LSD (> 0.05) (Cultivar); SED = 10.2, LSD (0.05) = 20.8 (NSO); SED = 20.4, I (0.05) = 41.6 (Interaction)

Table 2. Mean number of *Callosobruchus maculatus* adult progeny that developed in bambara groundnut cultivars that were protected with neem seed oil (NSO)

	Dosage of NSO (mg/20 g seed)				
Cultivar	0	0.5	1.0	1.5	Mean
Ole	54.0	35.0	4.0	0.7	21.4
Bakingangala	35.3	24.7	12.3	1.0	18.3
Bulmono	22.3	17.0	4.3	0.0	10.9
Jor	49.0	38.0	26.0	3.7	29.2
Mean	40.2	28.7	11.7	1.3	

SED = 4.5, LSD (0.05) = 9.2 (Cultivar); SED = 4.5, LSD (0.05) = 9.2 (NSO); SED = 9.0, LSD (0.05) = 18.4 (Interaction)

The two higher dosages of NSO also reduced percentage damage in Ole, Bakingangala or Bulmono seeds significantly when compared to the levels of damage in untreated seeds or seeds that were protected with 0.5 mg of NSO (Table 3). At the application rate of 1.0 mg/20 g seed, Jor was damaged to a significantly greater extent than Ole, Bakingangala or Bulmono. In untreated seeds, Jor was the most severely damaged of all the cultivars infested with *C. maculatus* (Table 4). In each of the cultivars, seeds protected with NSO at the rates of 1.0 and 1.5 mg/20 g seed were significantly less severely damaged than untreated seeds or seeds that were treated with 0.5 mg of NSO. Numbers of adult emergence holes per seed were 1.76, 1.82, 2.47 and 3.37 for untreated seeds of Bakingangala, Bulmono, Ole and Jor, respectively. Comparable figures for seeds treated with NSO at the rate of 1.5 mg/20 g seed were 0.33, 0.0, 0.67 and 0.76 for Bakingangala, Bulmono, Ole and Jor, respectively.

Table 3. Mean percentage of seeds of bambara groundnut cultivars protected with neem seed oil (NSO) that were damaged by *Callosobruchus maculates*

	Dosage of NSO (mg/20 g seed)				
Cultivar	0	0.5	1.0	1.5	Mean
Ole	97.0	87.7	15.3	3.0	45.7
Bakingangala	72.3	61.2	23.1	3.4	40.0
Bulmono	58.1	61.1	21.6	0.0	35.2
Jor	87.9	78.6	60.6	18.3	61.3
Mean	78.8	67.1	30.1	6.2	

SED = 8.6, LSD (0.05) = 17.4 (Cultivar); SED = 8.6, LSD (0.05) = 17.6 (NSO); SED = 17.3, LSD (0.05) = 35.3 (Interaction)

Table 4. Mean severity of damage (number of adult emergence holes per seed) caused by *Callosobruchus maculatus* to bambara groundnut cultivars protected with neem seed oil (NSO)

	Dosage of NSO (mg/20 g seed)				
Cultivar	0	0.5	1.0	1.5	Mean
Ole	2.47	2.08	1.17	0.67	1.59
Bakingangala	1.76	1.58	1.29	0.33	1.24
Bulmono	1.82	1.46	1.00	0.00	1.07
Jor	3.37	2.72	2.19	0.76	2.26
Mean	2.36	1.96	1.41	0.44	

SED = 0.16, LSD (0.05) = 0.33 (Cultivar); SED = 0.16, LSD (0.05) = 0.33 (NSO); SED = 0.32, LSD (0.05) = 0.65 (Interaction)

There were no significant differences in the resistance of untreated seeds of the four cultivars of bambara groundnut cultivars screened. However, application of NSO at the rate of 1.5 mg/20 g seed significantly reduced susceptibility of the cultivars to infestation by *C. maculatus* (Table 5).

Table 5. Mean susceptibility indices for bambara groundnut cultivars that were protected with neem seed oil (NSO) and infested with *Callosobruchus maculatus*

	Dosage of NSO (mg/20 g seed)				
Cultivar	0	0.5	1.0	1.5	Mean
Ole	11.92	10.16	3.09	0.00	6.29
Bakingangala	10.52	9.36	5.76	1.12	6.69
Bulmono	9.09	8.10	2.72	0.00	4.98
Jor	11.43	10.65	8.82	2.82	8.43
Mean	10.74	9.56	5.10	0.98	

SED = 0.90, LSD (0.05) = 1.84 (Cultivar); SED = 0.90, LSD (0.05) = 1.84 (NSO); SED 1.80, LSD (0.05) = 3.67 (Interaction)

DISCUSSION

The combination of varietal resistance with application of NSO has been shown in this study to be a possible integrated pest management strategy that could be used to protect bambara groundnuts against *C. maculatus* in storage. The protection of these cultivars of bambara groundnut with NSO especially at the rate of 1.5 mg/20g seed reduced both egg-laying and adult progeny development. Successful infestation of grain legumes is determined by the number of eggs laid initially by bruchids (Dick and Credland, 1986), the number of eggs that hatch and the number of first instar larvae that are able to penetrate the cotyledons (Lale and Mustapha, 2000). Interfering with any of these processes, as the application of NSO and the use of the resistance inherent in these cultivars do, leads to a reduction in the population of the bruchids and the degree of seed damage. Application of NSO reduces infestation and damage to pulse seeds by increasing the level of mortality of eggs and first instar larvae on the surface of the seeds (Lale and Abdulrahman, 1999; Lale and Mustapha, 2000).

In addition, partially or completely resistant cultivars of bambara groundnut are known to contain toxic chemical factors in their seed coats and/or cotyledons which confer some resistance to bruchid attack (Mbata, 1992; Ajayi and Lale, 2001a). Their cotyledons contain trypsin inhibitors, which interfere with the activity of proteolytic enzymes in the digestion of nutrients obtained by larval bruchids, and this causes their mortality. The integration of application of NSO and use of partially resistant bambara groundnut cultivars resulted in a possible synergism that may provide sustainable management of *C. maculatus*. Furthermore, their joint use in bruchid control would be likely to delay the emergence of biotypes of this bruchid that are capable of breaking down resistance in bambara groundnut cultivars or strains of *C. maculatus* with resistance to NSO.

The rates of NSO that have provided adequate control of *C. maculatus* in this study are considerably lower than the effective rates that were reported previously for this species on cowpeas (Lale and Abdulrahman, 1999). In that study, effective rates ranged between 50 to 100 mg/5 g and 75 to 150 mg/5 g seed. The smooth testae of bambara groundnut cultivars are known to contribute to the lowering of application rates by allowing a more even distribution of the oil on the surface of the seed. This provides a greater opportunity for the eggs and/or first instar larvae to make optimal contact with insecticidal plant oils than the rough or wrinkled testae of cowpea cultivars/varieties (Ajayi and Lale, 2001b).

Application of NSO at the rates being reported here is likely to maintain the germinability of protected seeds. Bambara groundnut seeds protected with NSO at this rate are likely to be more useful sources of seed for the farmer than seeds protected with NSO applied at the rate of 0.5 mg/20 g seed or untreated seeds. It has been reported, for instance, that losses in seed germination due to *C. maculatus* attack reached 100% for grains with 4 holes per seed (Santos, 1971).

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