Use of Varietal Resistance and Neem Seed Oil for the Management of Infestation of Bambara Groundnuts by *Callosobruchus maculatus* (F.) and *Callosobruchus subinnotatus* (Pic.)

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Abstract

*The efficacy of an integrated pest management strategy utilizing varietal resistance and neem (*Azadirachta indica*) seed oil (NSO) for the control of the two bruchid species,* Callosobruchus maculatus *and* Callosobruchus subinnotatus*, jointly infesting bambara groundnuts in West Africa, was evaluated under fluctuating conditions of temperature and humidity (20-27°C and 18-54% r. h.). Three local bambara groundnut cultivars (Bakingangala, Angale and Bulmono) with varying levels of susceptibility to infestation by these two bruchid species were treated with three dosages (0.5, 1.0 and 1.5 mg/20 g seed) of neem seed oil. In each of the bambara groundnut cultivars, application of NSO especially at the rates of 1.0 and 1.5 mg/20 g seed reduced egg-laying, adult progeny development, percentage of seeds damaged by* C. maculatus *and* C. subinnotatus*, severity of damage (number of adult emergence holes per seed) and susceptibility of the bambara groundnut cultivars significantly when compared to the levels of these parameters in untreated seeds. At the dosage of 1.0 mg/20 g seed, treatment with NSO reduced susceptibility of Bakingangala, Angale and Bulmono to C. maculatus infestation by 85.2, 91.3 and 100%, respectively; comparable figures for reduction of susceptibility of Bakingangala, Angale and Bulmono to* C. subinnotatus *infestation were 75.0, 85.6 and 100%, respectively. At this application rate also, the proportions of damaged seeds were 24.8, 10.6 and 0.0 for Bakingangala, Angale and Bulmono, respectively; comparable values for untreated seeds were 100, 96.3 and 82.4%, respectively. No adult progeny of either species developed and no damage occurred in seeds of Angale and Bulmono that were treated with 1.5 mg of NSO.*

Keywords: Integrated pest management, *Callosobruchus maculatus*, *Callosobruchus subinnotatus*, pest complex, host-plant resistance, botanical insecticide, neem (*Azadirachta indica*) seed oil

Introduction

Bambara groundnut, *Vigna subterranea* (L.) Verdcourt, is a major source of cheap dietary phytoprotein for both humans and livestock in the sudano-sahelian parts of tropical Africa and Asia (Sellschop 1962; Stanton *et al.* 1966; Linnemann 1994).

World annual production of bambara groundnut was estimated at 330, 000 tonnes in 1982, with about half being produced in the savanna areas of West Africa (Coudert 1984). Although accurate production figures for the last decade are not readily available (Lale and Vidal 2000), FAO, (1999) estimated current production figure for Africa to be about 43, 321 metric tons. It is widely cultivated and eaten in Nigeria, the most important producing areas being located in the savanna (Agboola 1979), but the crop is also grown in some parts of the more humid south. Nigeria is a major producer of the crop and accounts for 70% of the world's annual production (Sellschop 1962; Rachie and Silvestre 1977; Blade *et al.* 1997).

Bambara groundnut is often infested concurrently in West Africa by two species of Bruchidae, *Callosobruchus maculatus* (F.) and *Callosobruchus subinnotatus* (Pic). These bruchids commence infestation in the field once bambara groundnuts have been harvested and left to dry. Although, the level of field infestation is usually insignificant (Ofuya 2001), the bruchids expand in population rapidly in storage and cause substantial quantitative and qualitative losses manifested by seed perforation, and reductions in weight, market value and germinability of seeds (Prevett 1966; IITA 1989; Dike 1994).

Reports on the management of these bruchids in tropical storage have focused mainly on the employment of single strategies including the application of plant-derived insecticides and the use of resistant varieties/cultivars of bambara groundnut. Although some reports of the combination of these two strategies for the control of these bruchids are available in the literature (e.g. Lale and Mustapha 2000; Ajayi and Lale 2001a), they are results of work done on the use of integrated tactics for the control of single bruchid species. Most farmers in the tropics are resource-poor and the use of integrated methods of pest management or the control of a pest complex is likely to be more economical. In this study, the potential of combining available varietal resistance in local cultivars of bambara groundnut and neem (*Azadirachta indica* A. Juss) seed oil for the joint control of the two bruchids was evaluated.

Materials and Methods

Insect rearing and sources of bambara groundnut cultivars

Batches of pristine bambara groundnuts (cv. Bulmono) and cowpeas (cv. Borno brown) were obtained locally in Maiduguri in the northeastern part of Nigeria and used to rear laboratory cultures of *C. subinnotatus* and *C. maculatus*, respectively, under prevailing laboratory conditions (20-27^{o}C and 18-54% r. h.). The experiment was also conducted under these conditions.

Three cultivars (Bakingangala, Angale and Bulmono) 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*: Angale and Bulmono were identified as slightly susceptible whereas Bakingangala was identified as moderately susceptible (Ajayi and Lale 2001a).

Experimental procedure

Neem seed oil (NSO) extracted previously from dry neem seeds using the traditional kneading method, was used to treat seeds of the three bambara groundnut cultivars.

Three dosages (0.5, 1.0 and 1.5 mg) of NSO were applied separately in 0.2 ml of analytical grade of acetone to 20 g of seeds (i.e. 25, 50 and 75 mg/kg seed) of each of the three 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 completely evaporated. Control seeds were treated with 0.2 ml of pure acetone and stirred as described previously. The experiment was set up as a Randomized Complete Block Design in which treatments were replicated three times.

Two pairs of 2-day old *C. maculatus* and *C. subinnotatus* were then introduced together into each glass jar with the aid of a pooter and the female bruchids were allowed to lay eggs for 5 days. On day 5, all insects were removed and the eggs laid on seeds by the females of both species in each jar were counted together (it is not possible to distinguish the eggs of the two species). Adult progenies of both species that developed were counted separately and the number of damaged seeds in each jar was also counted. Counting of emerged adults was done daily for each of the species. Adults of the two species were distinguished by relying on their body sizes: *C. subinnotatus* is at least twice the size of *C. maculatus*. The number of damaged seeds (seeds bearing adult emergence holes) was expressed as a percentage of the total number of seeds in each replicate. Severity of damage (number of adult emergence holes per seed) was obtained by dividing the number of emergence holes by the number of damaged seeds. Susceptibility index (SI) was determined separately for each of the bruchid species and for each bambara groundnut cultivar according to the method of Dobie, (1974) and is given as:

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

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

Data obtained were subjected to two-way analysis of variance and means were compared using the Least Significant Difference (LSD) statistic at the 5% level of probability.

Results

Table 1 shows that *C. maculatus* and *C. subinnotatus* laid significantly fewer eggs on seeds of the three bambara groundnut cultivars that were treated with each of the dosages of NSO than they did on untreated seeds. Significantly fewer adult progenies of either bruchid species developed in treated than in untreated seeds (Tables 2 and 3). No adult progeny of either species developed in seeds of Angale or Bulmono that were treated with 1.5 mg of NSO; also no adult progeny of either species developed in seeds of Bulmono that were treated with 1.0 mg of NSO. In treated seeds, Bulmono supported significantly fewer adult progenies of either species than Angale or Bakingangala and in the case of *C. maculatus* (Table 2), significantly fewer adult progenies developed in seeds of Angale than in those of Bakingangala.

Treatment of the three bambara groundnut cultivars with NSO significantly reduced the percentage of seeds damaged by *C. maculatus* and *C. subinnotatus*. No damage occurred in seeds of Angale or Bulmono that were treated with 1.5 mg of NSO and in the case of Bulmono, no damage occurred in seeds treated with 1.0 mg of NSO as well (Table 4). In untreated seeds, the proportions of damaged seeds after one generation of the bruchids were 100, 96.3 and 82.4% for Bakingangala, Angale and Bulmono, respectively. Severity of seed damage was significantly higher in untreated seeds of each bambara groundnut cultivar than in seeds treated with each of the dosages of NSO (Table 5). Susceptibility of bambara groundnut cultivars that were treated with NSO to infestation by *C. maculatus* or *C. subinnotatus* was reduced significantly when compared to the level of this parameter in untreated seeds (Tables 6 and 7). Bulmono maintained consistent susceptibility to both bruchid species as indicated by the susceptibility indices (SI) values being 6.8, 0.6, 0.0 and 0.0 for untreated seeds and seeds treated with 0.5, 1.0 and 1.5 mg of NSO, respectively. At the dosage of 1.0 mg/20 g seeds, treatment with NSO reduced susceptibility of Bakingangala, Angale and Bulmono to *C. maculatus* infestation by 85.2, 91.3 and 100%, respectively; comparable figures for reduction of susceptibility of Bakingangala, Angale and Bulmono to *C. subinnotatus* infestation were 75.0, 85.6 and 100%, respectively.

Table 1. Mean number of eggs laid jointly by *Callosobruchus maculatus* and *C. subinnotatus* on seeds of three bambara groundnut cultivars treated with neem seed oil

	Dosage of neem seed oil (mg/20 g seed)			
Bambara groundnut cultivar	0	0.5	1.0	1.5
Bakingangala	172.0	75.7	60.3	50.3
Angale	111.0	75.0	60.0	33.7
Bulmono	163.3	105.7	85.0	45.7

Std. error = 12.5, LSD (0.05) = 26.0

Table 2. Mean number of *Callosobruchus maculatus* adults emerging from seeds of three bambara groundnut cultivars treated with neem seed oil and infested simultaneously with *C. subinnotatus*

	Dosage of neem seed oil (mg/20 g seed)			
Bambara groundnut cultivar	0	0.5	1.0	1.5
Bakingangala	61.0	7.3	3.7	0.7
Angale	37.0	9.7	1.0	0.0
Bulmono	15.7	0.3	0.0	0.0

Std. error = 5.3, LSD (0.05) = 11.0

Table 3. Mean number of adults emerging from seeds of three bambara groundnut cultivars treated with neem seed oil and infested simultaneously with *Callosobruchus maculatus* and *C. subinnotatus*

	Dosage of neem seed oil (mg/20 g seed)			
Bambara groundnut cultivar	0	0.5	1.0	1.5
Bakingangala	55.3	2.3	2.7	0.3
Angale	48.3	16.0	1.7	0.0
Bulmono	14.3	1.3	0.0	0.0

Std. error = 6.6, LSD (0.05) = 13.6

Table 4. Mean percentage of seed damage in three bambara groundnut cultivars treated with neem seed oil and infested simultaneously with *Callosobruchus maculatus* and *C. subinnotatus*

Bambara groundnut cultivar	Dosage of neem seed oil (mg/20 g seed)			
	0	0.5	1.0	1.5
Bakingangala	100	32.6	24.8	5.1
Angale	96.3	74.8	10.6	0.0
Bulmono	82.4	7.9	0.0	0.0

Std. error = 5.8, LSD (0.05) = 12.0

Table 5. Mean number of *Callosobruchus maculatus* and *C. subinnotatus* adult emergence holes per seed in three bambara groundnut cultivars treated with neem seed oil

Bambara groundnut cultivar	Dosage of neem seed oil (mg/20 g seed)			
	0	0.5	1.0	1.5
Bakingangala	5.5	1.3	1.2	0.7
Angale	3.8	1.5	0.7	0.0
Bulmono	1.8	1.0	0.0	0.0

Std. error = 0.5, LSD (0.05) = 1.1

Table 6. Mean susceptibility indices of three bambara groundnut cultivars treated with neem seed oil and infested with *Callosobruchus maculatus* in a mixed population of *C. subinnotatus*

Bambara groundnut cultivar	Dosage of neem seed oil (mg/20 g seed)			
	0	0.5	1.0	1.5
Bakingangala	12.8	5.4	1.9	0.0
Angale	10.4	6.4	0.9	0.0
Bulmono	6.8	0.6	0.0	0.0

Std. error = 1.0, LSD (0.05) = 2.2

Table 7. Mean susceptibility indices of three bambara groundnut cultivars treaed with neem seed oil and infested with *Callosobruchus subinnotatus* in a mixed population with *C. maculates*

Bambara groundnut cultivar	Dosage of neem seed oil (mg/20 g seed)			
	0	0.5	1.0	1.5
Bakingangala	11.2	2.6	2.8	0.0
Angale	10.4	7.3	1.5	0.0
Bulmono	6.8	0.6	0.0	0.0

Std. error = 0.7, LSD (0.05) = 1.4

Discussion

The results show that *C. maculatus* and *C. subinnotatus*, together laid higher numbers of eggs on untreated seeds of the three cultivars of bambara groundnut than on NSO-treated seeds. This may suggest that reduction of oviposition is unlikely to be a major mechanism employed by any of these cultivars to resist infestation. The application of NSO at the three rates reduced adult progeny development in these cultivars significantly and with respect to Angale and Bulmono, no adult progeny developed in seeds treated with 1.5 mg of NSO. Also no adult progeny developed in seeds of Bulmono that were treated with 1.0 mg of NSO. It has been reported previously that the population of bruchids that develops in grain legumes depends amongst other things on the number of eggs initially present (Dick and Credland 1984; 1986). The mean numbers of eggs laid jointly by the two species on Bakingangala seeds that were treated with 0.5, 1.0 and 1.5 mg of NSO were 75.7, 60.3 and 50.3, respectively; those for Angale treated with identical dosages of NSO were 75.0, 60.0 and 33.7; and those for Bulmono were 105.7, 85.0 and 45.7, respectively (Table 1). However, only small proportions of these eggs developed into adult progenies of both species. For instance, in Bakingangala, the proportions of eggs of both species that developed into adult progenies in seeds that were treated with 0.5, 1.0 and 1.5 mg of NSO were 12.7, 10.6 and 2.0%, respectively. In contrast, 67.6% of the eggs laid on untreated seeds of Bakingangala developed into adult progenies of both species. These observations show that successful infestation is also determined by the number of eggs that hatch as well as the number of first instar larvae that are able to penetrate the cotyledons. Interfering with any of these processes, as the application of NSO and the use of the inherent resistance in bambara groundnut cultivars do, leads to a reduction of the population of the bruchids and the degree of seed damage (Lale and Mustapha 2000). Application of NSO reduces infestation and damage in 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).

Furthermore, 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. Owusu-Domfeh, (1972) reported that raw bambara groundnut demonstrated a much higher trypsin inhibitory activity than raw cowpea. Trypsin inhibitor in both reduced and native forms has been identified from bambara groundnuts (Martino-Ferrer and Ferrer 1983). 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 both *C. maculatus* and *C. subinnotatus* infesting bambara groundnuts in tropical storage. In addition, their joint use in bruchid control would be likely to delay the emergence of biotypes of these bruchids that are capable of breaking down resistance in bambara groundnut cultivars or strains of *C. maculatus* and *C. subinnotatus* with resistance to NSO.

The rates of application of NSO that have provided adequate control of these two bruchid species are considerably lower than the effective rates that were reported previously for *C. maculatus* in cowpeas which ranged between 50 to 100 mg/5 g seed and 75 to 150 mg/10 g seed (Lale and Abdulrahman 1999). The smooth testate 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). Low application rates should reduce the prospects of NSO contaminating treated seeds.

Numbers of adult emergence holes per seed were 1.8, 3.8 and 5.5 for untreated seeds of Bulmono, Angale and Bakingangala, respectively. In NSO-treated seeds, the number of adult emergence holes per seed ranged from 0.0 to 1.5. At the application rate of 1.5 mg/20 g seed, however, no damage occurred in seeds of Angale and Bulmono and no damage occurred in seeds of Bulmono that were treated with 1.0 mg as well. These observations imply that bambara groundnut seeds protected with NSO especially at the rate of 1.5 mg/20 g seed are likely to be more useful sources of seed for the farmer than unprotected 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). Fewer number of holes will be required to cause total failure in seed germination in the event of infestation of bambara groundnuts by *C. subinnotatus* which is known to be at least three times the size of *C. maculatus* and, it is possible that it may consume nearly three times the amount of food that each *C. maculatus* larva is capable of eating.

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