EVALUATION OF NEEM KERNEL EXTRACT (MARGOSAN-O) AGAINST MAJOR STORED INSECT PESTS OF BEANS AND SORGHUM IN RWANDA

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Abstract

Recent studies by the authors have shown the development of resistance to actellic (pirimiphos methyl) in the major storage insect populations in Rwanda. The response of these species, Acanthoscelides obtectus Say, Rhyzopertha dominica (F.), and Sitophilus oryzae (L.) to neem kernel extract (Margosan-O) was determined in laboratory growth and development tests. Grain and beans were treated with a water-triton solution of Margosan-O at doses of 0.1, 0.05, 0.01, and 0.001 %(volume/volume). Margosan-O at all concentrations decreased survival of adult parent S. oryzae and R. dominica during their reproductive period. In the F1 generation, there was over 80% reduction in number of adults emerged. Results indicate that neem, Margosan-O, is an effective alternative to actellic for protection of stored beans and sorghum in Rwanda. Dry edible beans, Phaseolus vulgaris L., were also treated with 0.01% Margosan-O and stored. Following storage, rinsing, and preparation in the traditional Rwandan manner, the beans were presented to a 20 member sensory panel for evaluation. Seventy percent of the panel rated the neem treated beans acceptable, compared to 90% for the control (actellic treated) beans. Taste was considered one of the positive attributes of the sample.

Introduction

Neem kernel extract is produced by the neem tree, Azadiracta indica A. Juss. (= Melia azadirachta L. and Melia indica Brandis, Indian lilac or Margosa) which is native to the Indian subcontinent and is now grown in plantations and individually in many villages in Kenya and West Africa (Niger, Senegal, Mali, and Chad). Neem kernel extract (NKE) has been used effectively against bruchids infesting cowpeas and the maize weevil in maize in West Africa. The neem tree requires a hot, arid climate. Neem has not yet been grown in Rwanda.

There are many active ingredients in NKE. Some of these have been identified. Several of these, including the azadiractins, are tetranortriterpenoids. Water extracts of the whole, decorticated kernels have resulted in antifeedant responses in field insects (Radcliffe et al.1990). Areas of Rwanda could support growth of neem trees and NKE could be produced locally without importation of any material.

In the indo-subcontinent, neem leaves have long been recognized as having insecticidal properties. Traditionallly they have been used to protect stored products (Ahmed 1986). NKE is recommended as a postharvest insecticide for the protection of cowpeas, Vigna sinensis (Tourn.) Savi, against bruchids in some countries in Africa (Pereira 1983). Neem trees have been planted extensively in Niger, both in villages and in a plantation. The focus for neem research in Niger has been on prevention of postharvest loss (Ivbijaro 1986). Effective management of the maize weevil, Sitophilus zeamais Motsch., the rice weevil, Sitophilus oryzae (L.), and the cowpea weevil, Callosobruchus maculatus (F.) was achieved in laboratory experiments (Ivbijaro 1983a,b). Recent reviews discuss other applications and the environmental requirements for neem tree cultivation (Koul et al. 1990; Jacobson 1986).

At the national government warehouses of Rwanda (OPROVIA=Office National pour le Developpement et la Commercialisation des Produits viviers et des Productions Animales) a search is underway to identify preparations (Dunkel et al. 1990; Weaver et al. 1990) or procedures that will replace their sole insect protectant for grain and beans, actellic (pirimiphos methyl) which has been used prophylactically since 1983 (Dunkel et al. 1988). Populations of Acanthoscelides obtectus Say and S. oryzae with a significantly increased resistance to actellic have been identified in OPROVIA warehouses that have experienced the longest prophylactic use of actellic (Sriharan et al. 1990).

We, therefore, hypothesized that: NKE can be effective versus the primary storage insects in Rwanda, and, as such, could be an effective, economical alternative to actellic in Rwanda.

Materials and Methods

Stock cultures of A. obtectus, Rhyzopertha dominica (F.), and S. oryzae were maintained in environmentally controlled chambers at 27±1 degrees C; 65±5 % relative humidity; and 12:12::light:dark photoperiod. Acanthoscelides was reared on dry edible beans, Phaseolus vulgaris L. and R. dominica and S. oryzae on whole red spring wheat.

NKE was procured from W.R. Grace & Co., Columbia, Maryland and is sold with the label Margosan-O (a registered trademark of Grace & Co.). Margosan-O, produced from an ethanol extract of the seeds, contains 3000 ppm azadircactin and approximately 20% lipid-like solids. Wheat and beans were treated at dosages of 0, 0.001, 0.01, 0.05, 0.1, and 0.2% wt/wt (weight of extract by weight of grain or beans). Dilutions were made with water treated with

Triton, a surfactant. The grain or beans were mechanically shaken for 15 minutes and then air dried for 24 hours.

Twenty unsexed insects, 0-3 days post adult emergence from the kernel were placed in each of three replicates with 20 g of treated beans or grain. The initial observations were made at three days after inoculations, then at 7 days, and then every 7 days thereafter.

Because neem has had negative odor and taste attributed to it, a study of Rwandan consumer preference was also conducted at OPROVIA-GRENARWA II - Recherches (The National Food Quality Laboratory of OPROVIA). A trained sensory panel of 20 Rwandans (10 males and 10 females) ages 24 to 47 years were presented with beans cooked in the traditional Rwandan manner. Beans were rinsed, but not soaked and then boiled for three hours. These beans had been previously treated with 0.1 % wt/wt Margosan-O, as described for the insect bioassay, and stored for 3 weeks. Storage conditions were the same as for the insect bioassay. The control was beans that had been stored at OPROVIA. At OPROVIA all beans are treated with 1% powder preparation of Actellic. Panel members were presented with equal amounts of two samples in private booths. They were then asked to answer the three following questions: 1) Which of the two samples (of cooked beans) do you prefer?; 2) Which of these samples are you more likely to choose to eat?; and 3) For each sample, what are the positive and negative properties of each sample that you consider important?

Results

After three days, only 50% of the parent generation of S. oryzae survived at 0.2% Margosan-O (Figure 1). Even at 0.05% Margosan, a significant reduction in survival compared to the control occurred. The main effect of neem in these experiments was in the F1 generation. Even at the lowest concentration used, 0.001% wt/wt, there was a very significant reduction in the F1 generation of S. oryzae. With R. dominica there was no significant difference in parent survival after three days exposure to Margosan (Figure 1). However, the number of progeny of R. dominica reaching adulthood was significantly reduced compared to the controls, even at the lowest concentration used (Figure 1).

Figure 2 shows the effect of NKE on the parent generation in greater detail. The period shown corresponds to the period of these species peak reproductivity. At all concentrations of Margosan, there was an initial decrease in the parent generation. At 0.2% Margosan, only 38% of the adult S. oryzae survived past 7 days of oviposition. At 21 days after inoculation, all concentrations of 0.05% and above were significantly lower than the controls. With parent R. dominica, the initial decrease (0 to 7 days) was not as dramatic as with S. oryzae. At 21 days, however, the effect of Margosan on the parent generation was as strong as with S. oryzae. At 0.05% Margosan and above, a 50% reduction of the F1 population occurred.

Table I provides a number comparison of the main effect of Margosan, a reduction in the F1 generation. Even at the lowest concentration of Margosan, percent reduction of adults was over 80% of the control.

In sensory tests with a trained panel of consumers, beans stored with actellic were preferred by 70% of the panel, whereas beans treated with neem before storage were preferred by only 30% of the panel (Table IIa). When comparing acceptability, 90% of the panel found actellic treated beans acceptable, whereas 70% of the panel found the neem beans acceptable (Table IIa). The negative properties attributed to beans stored with neem were primarily

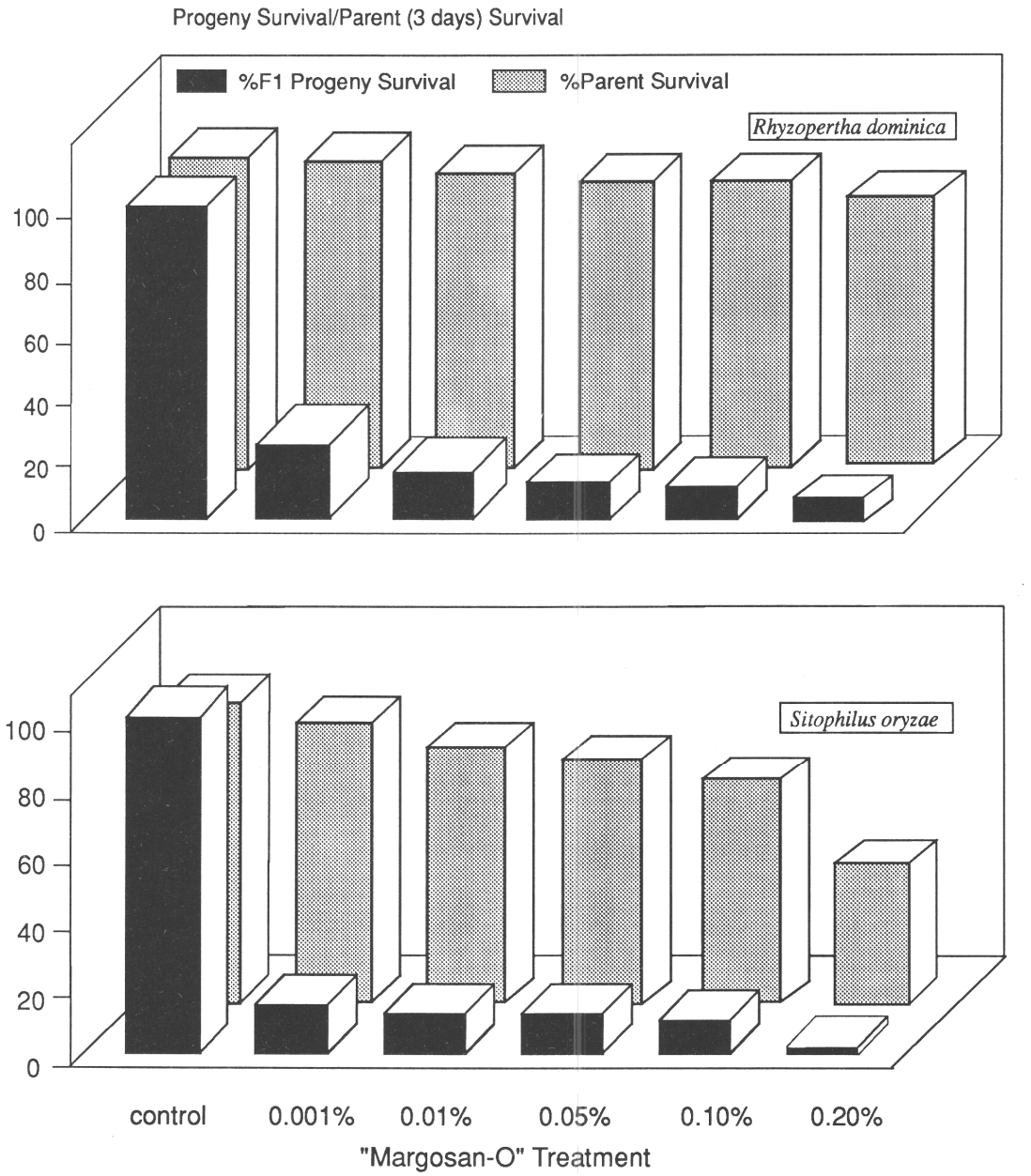

Figure 1. Mean percent survival of parent (1-3 days after emergence)(light bars) and F1 progeny (to the adult stage)(dark bars) of *Rhyzopertha dominica* (F.) and *Sitophilus oryzae* (L.) on Margosan-O (3 replicates; 20 insects per replicate)(observations of parents made 3 days after inoculation).

Table I. Mean (± standard error) of the number of F1 adults and percent reduction of F1 adults of Sitophilus oryzae L. and Rhyzopertha dominica (F). on wheat treated with Margosan-O.

Dose	No. of F_1 adults and % reduction of F_1 adults*			
	Sitophilus oryzae		Rhyzopertha dominica	
(%)	F_1 ± SE**	% Red	F_1 ± SE**	% Red
0.200	2.80 ± 1.72	98.24	11.50 ± 2.29	94.68
0.100	11.20 ± 3.65	92.94	17.75 ± 2.38	91.72
0.050	14.00 ± 2.19	91.22	20.25 ± 1.92	90.56
0.010	22.80 ± 9.74	85.71	28.00 ± 3.24	86.95
0.001	31.20 ± 7.85	80.45	36.00 ± 5.24	81.00
Control	159.60 ± 30.10	0	214.50 ± 16.69	0

[1] 20 insects in 20 g of wheat in each test sample; 3 replicates per dose.

* % Reduction = $100 - \frac{\text{No. of } F_1 \text{ adults from test sample}}{\text{No. of } F_1 \text{ adults from control}} \times 100$

** SE= standard error of the mean.

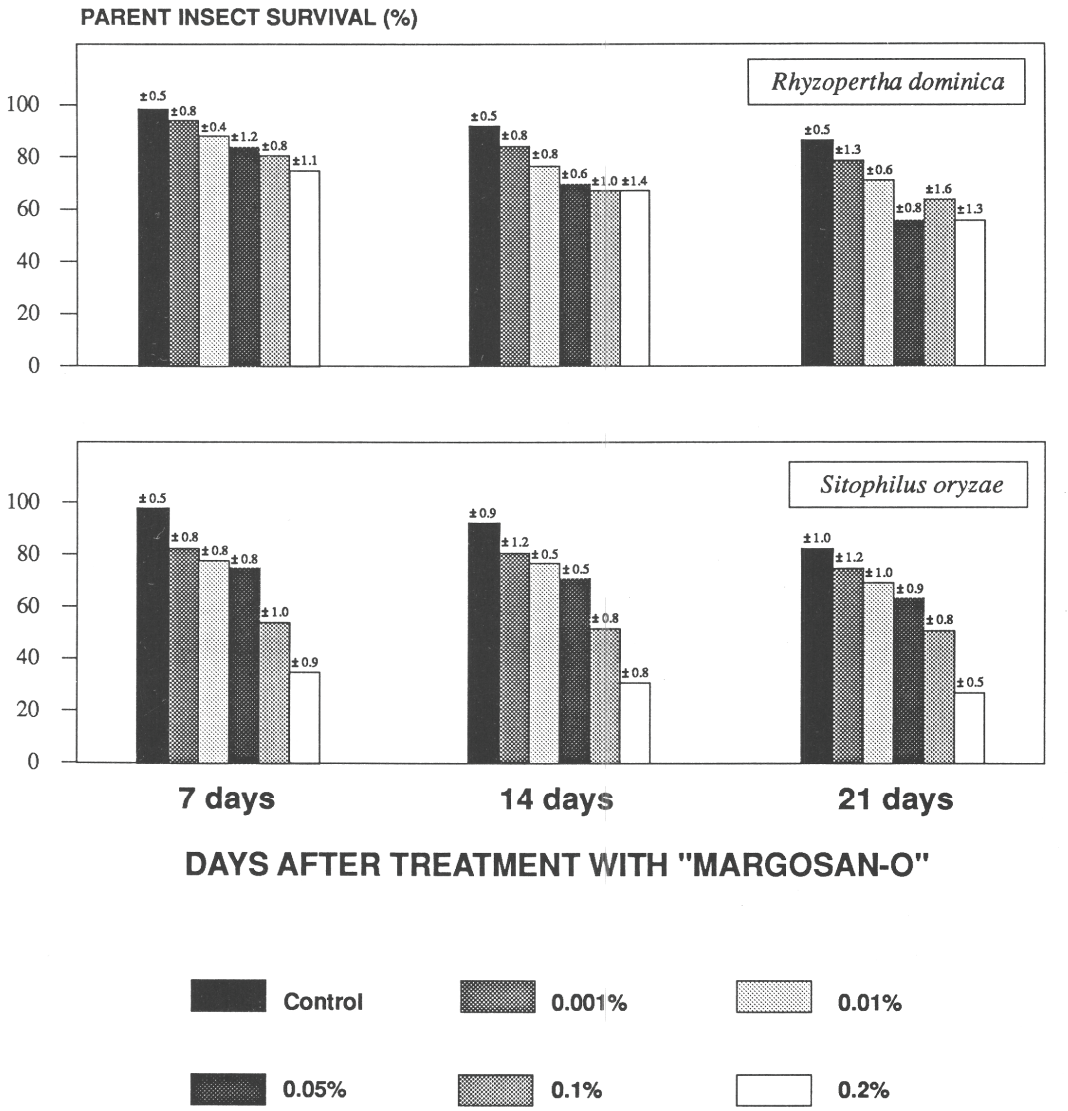

Fig 2. Mean percent parent insect survival of *Rhyzopertha dominica* (F.) and *Sitophilus oryzae* (L.) 7, 14, and 21 days after inoculation on wheat treated with Margosan-O (3 replicates; 20 insects per replicate).

Table II. Summary of sensory evaluation, measured by a trained Rwandan panel (20 men and women), of beans stored for three weeks with neem (Margosan-O) and other insecticidal plants (crushed leaves of Tetradenia riparia Hochst. CODD.; extract of flowers of Chrysanthemum cinerarium)(control= beans treated with actellic). a) Percent preference for one sample rather than the other sample and percent of panel which found each sample acceptable, b) Percent of panel which noted positive and negative attributes about the beans from the four different storage treatments.

Percent preference and percent acceptability

Sensory Comparisons

Bean Treatment	% preference for sample	% acceptability of sample
Actellic vs neem	**70–30**	**90–70**
Actellic vs *T. riparia*	45–55	85–75
Actellic vs pyrethrin	55–45	100–79

Percent appreciation of specific positive and negative properties (3 weeks storage exposure; 20 respondents).

Results of Sensorial Test[1]

	% Appreciation of certain sample properties[2]							
	Positive effects				Negative effects			
Bean Treatment	Ta	O	Te	A	Ta	O	Te	A
Actellic	54	8	23	15	15	15	23	47
Pyrethrin	41	17	25	17	0	8	42	50
T. riparia	56	11	33	0	11	11	22	56
Neem	**56**	**11**	**22**	**11**	**11**	**22**	**11**	**56**

[1] Actellic, in this experiment was considered the control because all beans sold by OPROVIA are treated with actellic.

[2] Ta = taste; O = odor; Te = texture; A = appearance

appearance and odor (Table IIb). Neem treated beans also had some positive properties, taste and texture (Table IIb).

Discussion

Neem kernel extract (Margosan-O) provides excellent suppression of the F1 generation of S. oryzae and R. dominica. Indirectly, the parent survival pattern, of S. oryzae exposed to Margosan treated wheat had a strong effect on the number of F1 progeny also.

Even though neem kernel extract has been shown to provide good insect suppression, it will not be a useful treatment if the people who must eat the commodity do not like it. Beans, the primary protein and carbohydrate source for the people of Rwanda, were almost as acceptable to Rwandan consumers when treated with neem, as were beans which are sold by the OPROVIA warehouses (= actellic treatment). In particular, taste was noted as a positive property. F1 generation insect data indicate that a lower concentration of Margosan (0.001% wt/wt) is acceptable for some storage insects. This may be an even more acceptable level for consumers.

Subsequent articles will present data on the effect of neem (Margosan) on Rwandan strains of A. obtectus, R. dominica, and S. oryzae, particularly populations which have a high resistance ratio to actellic (Sriharen et al. 1990). Sorghum is primarily used as a fermented beverage, storage with neem needs to be examined in correlation with germination studies.

Neem is an alternative that needs to be seriously considered in the Rwandan storage system. To provide favorable economics, the tree needs to be grown in Rwanda. Areas that are suitable, that is warm enough with sufficient sandy or nutrient poor soil need to be identified. Small plantings need to be started with seeds that have been screened for pathogens.

Conclusions

Neem kernel extract should be part of the Rwandan strategy for long term strategic storage of beans and sorghum in government (OPROVIA) warehouses. Although NKE is available in the USA and neem seeds are available in east central Africa, to be free from dependence on foreign exchange funds, Rwanda will need to begin nurseries of the plant for distribution within the country.

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Literature Cited

Ahmed, S. and Grainge, M. (1985) Potential of the neem tree (*Azadirachta indica*) for pest control and rural development. *Economic Botany.* 40:201-209.

Dunkel, F.V., Wittenberger, T.P., Read, N.R., and Munyarshoka, E. (1988) National survey of the storage of beans and sorghum in Rwanda. *Misc. Publ. No. 46. Minnesota Experiment Station, University of Minnesota, St. Paul.* 186p.

Dunkel, F.V., Weaver, D., VanPuyvelde, L. and Serugendo, A. (1990) Growth regulatory effects of a Rwandan medicinal plant *Tetradenia riparia* (Lamiaciae) on stored grain and bean insects. *Proc. fifth Internat. Conf. on Stored Product Protection* in press.

Hanegreefs, P., Kayinamura, P., Dunkel, F.V. and Clarke, S.A. Underground storage of dry edible beans (*Phaseolus vulgaris* L.) in Rwanda. *Tropical Science.* in press.

Ivbijaro, M.F. (1983a) Preservation of cowpea, *Vigna unguculata* (L.) Walp, with the neem seed, *Azadirachta indica* A. Juss. *Protec. Ecol. 5:177-182.*

Ivbijaro, M. F. (1983b) Toxicity of neem seed, *Azadirachta indica* .Juss. to *Sitophilus oryzae* (L.) in stored maize. *Protec. Ecol.* 5:353-357.

Ivbijaro, M.F. (1986) Prospects for neem in Nigerian agriculture. *Proc. 3rd Internat. Neem Conf.* Nairobi, Kenya pp 525-533.

Koul, O., Isman, M.B. and Ketkar, C.M. (1990) Properties and uses of neem, Azadirachta indica ***Can. J. Botany.* 68,2-11.**

Jacobson, M. (1986) Role of allelochemicals. in Green, M.V. and Hedin, P.A. (eds) *Natural resistance to plants and pests. American Chemical Society Symposium Series No. 296.* pp. 220-232.

Pereira, J. (1983) The effectiveness of six vegetable oils as protectants of cowpeas and bambara groundnuts against infestation by *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *J. Stored Products Research.* 19:57-62.

Sriharen, S., Dunkel, F.V., and Nizeyimana, E. (1990) Status of actellic resistance in three stored product insects infesting sorghum and beans in Rwanda. *Proc. Fifth Internat. Working Conf. on Stored Product Protection.* in press.

Weaver, D.K., Dunkel, F.V., and Jackson, L.L. (1990) Toxicity of R,S-linalool to four species of storage Coleoptera as influenced by degradation and volatilization. *Proc. Fifth Internat. Working conf. on Stored Product Protection.* in press.

EVALUATION DE L'EFFET DES EXTRAITS DE NEEM (MARGOSAN-O) SUR LES PRINCIPAUX INSECTES PARASITES DES STOCKS DE HARICOTS ET DE SORGHO AU RWANDA

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RESUME

Des études récentes entreprises au Rwanda par les auteurs montrent le développement de la résistance à l'actellic (pirimiphos-méthyl) des principaux insectes des stocks du pays. La réponse de populations d'*Acanthoscelides obtectus* Say, de *Rhyzopertha dominica* F. et de *Sitophilus orizae* L. aux extraits de noix de Neem (Margosan-O) a été déterminée par des tests de croissance et de développement en laboratoire. Des graines de sorgho et de haricots (*Phaseolus vulgaris* L) ont été traitées par une solution eau/triton de Margosan-O à 0,1, 0,05, 0,01 et 0,001 % (v/v). A aucune concentration le Margosan-O n'a modifié la survie des insectes adultes. A 0,1 et 0,05 %, l'émergence des adultes issus de F1 a été retardée de 7 et 10 jours pour *R. dominica* et *S. orysae*, respectivement. Le pourcentage de survivants de la génération F1 ayant donné des adultes a été fortement diminué. Les résultats indiquent que le Margosan-O constitue une alternative efficace à l'actellic dans la protection des stocks de sorgho et de haricots au Rwanda.