PN-ACM-579

IPM CRSP Working Paper 97-1

Storage of Neem Kernel Extract: Differential Effects of Temperature on Oviposition Deterrency and Larval Toxicity

By
David A. Jenkins and Florence V. Dunkel
Department of Entomology
Montana State University

IPM CRSP
Office of International Research and Development
1060 Litton Reaves Hall
Virginia Tech
Blacksburg, VA 24061-0334

This publication was made possible through support provided by the US Agency for International Development (USAID), under the terms of Grant No. LAG-4196-G-00-3053-00 made to Virginial Polytechnic Institute and State University (Virginia Tech) for the Integrated Pest Management Collaborative Research Support Program (IPM CRSP).

The opinions expressed herein are those of the authors and do not necessarily reflect the view of USAID.

Working papers are intended to stimulate discussion and elicit comments from interested professionals, both within and outside the IPM CRSP. They are not reviewed publications. They may be cited with due acknowledgement.

A

Contact Address for the Management Entity

IPM CRSP
Office of International Research and Development
1060 Litton Reaves Hall
Virginia Tech
Blacksburg, VA 24061-0334

Telephone (540) 231-3513
FAX (540) 231-3519

E-mail: BRHANE@vt.edu

IPM CRSP US Institutions

Lincoln University
Montana State University
Ohio State University
Penn State University
Purdue University
Rodale Inst. Res. Ctr.
Univ of CA/Berkeley
University of Georgia
USDA Veg Lab
Virginia Tech

Host Country Institutions

Prime Sites

Guatemala - Agri-lab, ALTERTEC, CARE, ICTA
Jamaica - CARDI, Ministry of Agriculture
Mali - IER
Philippines - NCPC/UPLB, PhilRice

Satellite Sites

Ecuador - INIAP

Honduras - EAP
Thailand - Dept of Agriculture
Uganda - Makerere University

International Centers

AVRDC - Taiwan
IRRI - Philippines
CIAT - Columbia
CIP - Peru

Private Sector

The Kroger Company
Caito Foods
PICO

NGOs/PVOs

CLADES

B

Send Galley Proofs to:
Florence V. Dunkel
Department of Entomology
324 Leon Johnson Hall
Montana State University
Bozeman, Montana 59717
phone: 406-994-5065
fax: 406-994-6029
e-mail:
ueyfd@oscs.msu.montana.edu

Storage of Neem Kernel Extract: Differential Effects of Temperature on Oviposition Deterrency and Larval Toxicity

David A. Jenkins and Florence V. Dunkel

Department of Entomology

Montana State University

Bozeman, Montana 59717

to be submitted to: Environmental Entomology

ABSTRACT: Neem has been established as an effective postharvest protectant of many crops, especially against the cowpea weevil, *Callosobruchus maculatus* (F.), a major pest on cowpeas. Although it is well known that neem breaks down rapidly in light, the information concerning neem kernel extract's stability under higher temperatures has not been conclusively investigated. This is an important question to West African farmers who may wish to make an extract at their convenience and store it for use later. Our investigations using a destructive storage insect model indicate that neem kernel extract (NKE) is effective at reducing infestations of *C. maculatus*, even when stored at high temperatures, including 2 weeks at 50°C followed by up to five months storage at 28°C. High pressure liquid chromatography (HPLC) revealed that many of the compounds present in unheated or fresh NKE dissipate when stored at the temperature regime described above. Heated NKE also loses a substantial amount of oviposition deterrent effect; however, larval toxicity, or the number of eggs that do not survive to become adults, is not significantly reduced by the heat regime we imposed. We hypothesized that larval toxicity, which is maintained even when the chemistry of NKE is drastically altered, may be a physical manifestation of the toxicity through the action of the oil. We do note that cowpeas on which eggs have been oviposited and that were treated (50% and 25% NKE, stored for one week at 50°C) up to one week after oviposition produced no F_1 adults and that adults placed on cowpeas treated one month prior produced no progeny. Treating cowpeas with 25% NKE while the insects are in the pupal stage (5 days prior to adult emergence) did not significantly affect the adult emergence, a further indication that NKE may exert its toxicity on the embryo and very young larvae at

the doses we assayed. We conclude that NKE can be stored at high temperatures for up to at least five months without a significant reduction in its overall effectiveness.

KEY WORDS neem, cowpeas, *Callosobruchus maculatus*, cowpea weevil, postharvest, high pressure liquid chromatography, heat degradation

Introduction

The neem tree, *Azadirachta indica* A. Juss (Meliaceae), has long been studied as a potential source of medicines and insecticides (Mordue (Luntz) and Blackwell, 1993; Ascher, 1993). Neem's greatest potential is in the subsistence agricultural systems that predominate in the tropics. In these regions neem grows readily, is abundant as a resource and, if efficiently used, can be an effective crop protectant; however, neem's rapid photodegradation (Schmutterer, 1988) relegates its most effective use to the postharvest storage system where light is usually of negligible importance. Many scientists have demonstrated neem's effectiveness at protecting stored products, especially cowpeas, *Vigna unguiculata* Walpers, an important source of protein in many West African countries (Makanjoula,1989; Sowunmi and Akinnusi,1983; Tanzibul, 1991; Xie et al, 1995; Echindu, 1991; Pierrard, 1986; Zehrer, 1983).

Although the neem seed kernel, where most of the active components in neem trees are concentrated, is continually available in some areas, fruiting is restricted to once or twice a year in other areas. Storing the seeds or fruit is a questionable practice due to the build-up of aflatoxin B_1 (Chourasia and Roy 1991). This brings up the important question of the storage of neem kernel extracts themselves: Do they lose potency over time under predominating storage conditions? The subsistence farmer will be interested in whether he/she can prepare NKE at their convenience and store it for extended periods without severe degradation occurring. Stark and Walter (1995) have shown that heat does degrade azadirachtins A and B in neem seed oil (Margosan-O) but have not demonstrated that this

degradation necessarily reduces neem's effectiveness at protecting crops. There are a number of compounds in neem kernel extract that have demonstrated bioactivity at or above the level shown by the single compound, azadirachtin (Lin-er et al. 1995). Therefore, we suggest it is a mistake to attribute all of neem's effectiveness to azadirachtin. Furthermore, the physical properties of the NKE may contribute substantially to NKE's effectiveness as an ovicide or a larvicide, as evidenced by the findings of Pacheco et al. (1995), Rajapakse and Van Emden (1997), and Don-Pedro (1989). If the protective capacity of the neem kernel extract is indeed not dependant on the constitutive chemistry of the tree then concerns of chemical variation of the trees over geography or season can be discarded. No published studies have addressed the efficacy of degraded NKE.

We hypothesized that prolonged high temperatures, as are commonly encountered in the Sahel region of West Africa, will decrease the bioactivity of NKE as well as chemically alter the extract. The ultimate goal of our research is to examine the feasibility of prolonged storage of neem kernel extract for later treatment of stored products, particularly cowpeas. The most destructive pest in the cowpea system is the cowpea weevil, *Callosobruchus maculatus* (F.) (Ofuya, 1991). For this reason we have selected the cowpea/cowpea weevil system to monitor neem kernel extract's break down under storage conditions, in particular, those conditions of temperature and humidity predominating in the Sahel region of West Africa. These bioassays have been strengthened by concomitant chemical analysis of the NKE over time using high-pressure liquid chromatography (HPLC).

Materials and Methods

Neem kernels used for extraction in this experiment were gathered in Sept 1995 and 1996, from trees in the Sirakorola arrondissment in southwest Mali, West Africa. Another exctract prepared in Sept 1996 in Benin was also obtained by our lab for purposes of comparison. Extractions were performed using a hand press (GRAT 1993; Dunkel 1996). The oil was filtered through a cloth mesh with an aperture diameter of 300-400 micra. Once the extracts were obtained by our lab they were stored at 5°C until use in bioassays. *Callosobruchus maculatus* individuals were obtained from the University of Wisconsin, Madison, WI, Department of Entomology, USDA-ARS Stored Product Insect Laboratory. The cowpea weevils were reared on cowpeas in 1025cc jars in a controlled environment at 28±0.5°C; 65±5% R.H.; 12:12::L:D.

Bioassay Procedure. Approximately 20 grams (±0.05g) of cowpeas equilibrated at 38°C and 60% (±10%) relative humidity (RH) were apportioned to each 40ml multiple-entry vial, obtained from Supelco, Inc., Bellefonte, PA. NKE, stored at 5°C, was mixed with deionized water in the appropriate proportion in a 15ml multiple-entry vial, obtained from Supelco, Bellefonte, PA. Three vials of cowpeas were assigned to received a particular concentration of aqueous NKE; either 10%, 25%, 40%, 55%, 70%, 85%, or 100%. Untreated controls and controls treated with deionized water were used. Seven-hundred-fifty microliters of the treatment were added, using a p-1000 Pipetman, to the 20 grams of cowpeas in a Pyrex petri dish. The petri dish was covered and shaken vigorously for 10

seconds to thoroughly coat the cowpeas. The cowpeas were allowed to dry for 10 seconds on a Whatman No 1 filter paper. The cowpeas were transferred to an appropriately labelled 40ml multiple-entry vial and 5 adult female and 3 adult male *C. maculatus* individuals (0-24 hrs after adult emergence) were placed in each vial. Each vial (replicate) in a treatment received NKE from a separate preparation of the appropriate concentration to ensure that each replicate received a similar amount of the NKE.

After a period of three days, the adult *C. maculatus* individuals were removed from each vial and the number of eggs oviposited on each cowpea were counted. The cowpeas were monitored each day for F_1 adult emergence. Eleven days after the first F_1 emergence the number of emerging adults were totalled and divided by the number of parent females per vial (5) to estimate the number of progeny per parent female.

Estimation of the stage of *C. maculatus* effected by NKE: Three sets of cowpeas were also treated that had *C. maculatus* eggs oviposited on them 1 day, 2 days, 3 days and 1 week prior to treatment with 50% NKE. These cowpeas were subject to infestation in the same manner described above. Another treatment consisted of putting 5 adult female and 3 adult male *C. maculatus* (0-24 hrs after adult emergence) on cowpeas treated with 50% NKE one month earlier. Finally, a group of cowpeas infested with *C. maculatus* in the above manner (5 females and three males for three days) were treated with 25% of the heat-treated NKE 25 days after commencement of oviposition. This subjected pupae to the NKE so that we could ascertain whether they were affected by heat-treated NKE as much as the eggs and early stage larvae.

Heat-degradation experiments: To examine the effect of heat on village-produced NKE's capacity as a protectant for cowpeas, sealed 15ml multiple-entry vials of NKE were stored under the following temperature regimes prior to the treatment of the cowpeas: Treatment I - continuous storage for 5 months at 28°C; Treatment II - 2 weeks at 50°C followed by continuous storage for 5 months at 28°C; Treatment III - continuous storage for 5 months at 5°C. All 28°C treatments were stored in a walk-in incubator (28°C$\pm$0.5°C, 65%$\pm$10% RH). All 50°C treatments were stored in an oven set on "warm" (50°C$\pm$0.5°C). The 5°C treatment, as well as all stock NKE, were stored in a refrigerator (5°C). Treatments of 50% NKE (the concentration typically used by Malian farmers, albeit diluted in kerosene) from each of the above temperature regimes were applied to three groups of cowpeas, 20 grams each, after 5 months. To heighten the sensitivity of our bioassay to the chemical breakdown the treatments were applied at 25% NKE after 5 months. In early trials, not reported here, high variability in oviposition was encountered due to NKE's low miscibility in water. To counter this high variability, each replicate received its NKE solution from a single preparation specifically prepared for that replicate. By preparing the NKE solutions for each replicate separately, we insured that each replicate received a similar dose.

HPLC Procedure: Azadirachtin Standard. Five point forty-nine milligrams of 95% pure azadirachtin A were dissolved into 5ml of HPLC-grade methanol. Ninety-five percent pure Azadirachtin A was obtained from the laboratory of Dr. W. Kraus (Department of Chemistry, University of Stuttgart-Hohenheim, Germany). Ten microliters of this solution were injected into the column using a 25 microliter syringe, obtained from Supelco, Inc.,

Bellefonte, PA. (The flow rate was set at 1ml/min; UV absorbance = to 214nm) An isocratic 50:35:15 H_2O:MeOH:Acetonitrile (ACN) mobile phase was used for analysis and pure ACN was used for 20 minutes to clean the column between runs. A C-8 reverse phase HPLC column was obtained from Alltech Associates, Inc., Deerfield, IL. The Bakerbond C-18 solid phase extraction (SPE) tubes were obtained from J.T. Baker. The HPLC machine used was a Shimadzu LC-6A with a Spectroflow 757 absorbance detector = to 214 nm and a Chromatopac C-R68 integrator from Shimadzu (Kyoto, Japan).

Raw Neem Kernel Extract. Raw NKE (0.5ml) was mixed with 5ml of HPLC grade methanol and placed on an equilibrated solid phase extraction (SPE) tube. No pressure was used to extract the NKE. The one hour filtrate was collected for analysis and run on the HPLC according to the settings for the pure azadirachtin run.

Data Analyses. One-way analysis of variance and standard deviations of all oviposition and adult emergence data were obtained using the SAS statistical program (ANOVA)(SAS Institute, 1988). Student-Neuman-Keuls on the SAS program was used for multiple comparison tests of the different treatments with an alpha level of 0.05.

Results

Bioassays. NKE had a significant effect on F_1 adult emergence at all of the concentrations used (Table 1.). None of the F_1 adults emerging in the lower doses (10% and 25%: concentrations above 25% produced no adults) exhibited any malformations, indicating

that the growth regulatory effects of the NKE were probably not involved in the toxicity. All treatments above 40% NKE provided full protection, killing even parent beetles. Neem formulations sold in the United States are typically 3 to 4% azadirachtin and 30 to 40% neem kernel extract, but these formulations contain petroleum-based solvents at a rate of 60 to 65% (Align-XL EPA#62552 and Azatin-XL EPA# 62552 registration). These inert materials alone have been shown to be lethal to non-target insects and other macroinvertebrates (Dunkel and Richards in review).

All NKE from the various storage regimes were fully effective at the 50% concentration after five months (no eggs and, obviously, no F_1 emergence); however, the 50% concentration bioassay may have been too high to be sensitive to chemical break down. The 25% NKE applied five months after heat treatment and continuous storage at 28°C was still fully effective at keeping adults from emerging, although oviposition was increased considerably.

All of the post oviposition treatments (1 day, 2 days, 3 days and 1 week after oviposition) were effective at keeping progeny from developing. No F_1 adults emerged from any of these cowpeas. Also, cowpeas treated one month prior to infestation by adults were effective at deterring development, as no adult progeny emerged from these cowpeas, even though they were treated with the heat-stored NKE (Treatment II). Cowpeas treated during the pupation period (5 days prior to estimated emergence) did not have a substantial effect at reducing F_1 emergence, leading us to believe that the toxic effect of the oil is exerted on the embryo or early larval stages. (It is important to note that the NKE used to treat pupae had

been heat treated and was devoid of terpenoids detectable by our protocol. NKE with its chemistry intact may have significant effects on the emergence of treated pupae.) This was corroborated by examining the stage of development attained by treated eggs. It was revealed that approximately 80% of the eggs, when treated with NKE, only got to the advanced embryo stage, the stage where the larvae is visible in the egg. Removing eggs from the surface of the cowpea rarely (15% of those checked; sample of 200 eggs) revealed an entrance hole, indicating that the larvae were not able to enter the testa of the cowpea to commence damage.

HPLC. HPLC analysis revealed that many chemical components in the NKE, including azadirachtin, are dissipated by the temperature regimes to which we subjected the NKE (Figure 1).

Discussion and Conclusion

The NKE used in these trials proved to be effective at reducing infestation by *C. maculatus* at all concentrations tested. Storage at high temperatures did not seem to negate NKE effectiveness, even after 5 months. The high sensitivity bioassay (25% NKE) still demonstrated no deterioration of toxicity to F_1 progeny, although oviposition was substantially increased, indicating that an oviposition deterrent in the NKE had been reduced by heat. It is important to note that many vegetable oils were effective ovicides for *Callosobruchus maculatus* (Don-Pedro 1989; Pacheco et al 1995; Rajapakse and Van Emden 1997) and that it may be the oil portion of the NKE that is lethal to the embryos. Analysis

using HPLC should be able to establish whether it is the oil or the component chemicals that are exerting the lethal effect. Our timing of application trials reveal that heat degraded NKE is effective at reducing progeny if applied up to a month prior to infestation by *C. maculatus* or up to a week following infestation by *C. maculatus* but is not effective when applied during the pupal period. This indicates that NKE is probably more active against eggs and against early stage larvae that have penetrated the testa of the cowpea. Also, NKE treatments appear to lower oviposition by females, though not as substantially as the effect exerted on fertility (number of F_1 adults emerging from the cowpeas). It would be interesting to examine how females choose whether a treated cowpea is acceptable as a host; do they use antennae or palpi to detect malevolent chemicals, a possibility suggested by the work of Blaney et al. (1994)?

In summary, high temperatures do significantly reduce the oviposition deterrency and alter the chemistry of NKE, but do not significantly affect the larval toxicity of the NKE. Because most of the eggs do not survive to become destructive larvae, the decrease in oviposition deterrency is of little or no consequence to the Malian farmer. The net result, then, is that NKE is just as effective at protecting cowpeas from *C. maculatus* after storage at high temperatures as it is when it is fresh. What is important to the Malian farmer and other subsistence farmers of the Sahel, where neem trees are readily available, is that the NKE is available and efficacious whenever it is needed in the post-harvest situation.

AKNOWLEDGEMENTS

The authors wish to acknowledge the essential collaboration of Dr. K. Gamby, Institut d'Economie Rurale (IER), Bamako, Mali and her assistant, Justin Diabate, for providing raw NKE and other materials. Appreciation is expressed for the technical assistance of Dr. L.J. Sears, Department of Chemistry and Biochemistry, Montana State University-Bozeman. The authors wish to acknowledge the participation of Dr. Wilhelm Kraus, Department of Chemistry, University of Stuttgart-Hohenheim, Germany. Partial support for this project was received from the Montana Agricultural Experiment Station (#161)(F. Dunkel) and The Integrated Pest Management Collaborative Research Program (IPM CRSP)(F. Dunkel). The IPM CRSP is an initiative of the US Agency for International Development (USAID), Grant No. LAG-4196-G-00-3053-00, Title XII and the Board for International Food and Agricultural Development and Economic Cooperation (BIFADEC), the participating US Universities and other collaborating institutions. This article is a contribution to the NC-213 Committee on "Marketing of Quality Grain to Foreign and Domestic Markets" and number J-___ of the Montana Agricultural Experiment Station.

LITERATURE CITED

Ascher, K.R.S., 1993. Nonconventional insecticidal effects of pesticides available from the neem tree, *Azadirachta indica*. Arch. Insect Biochem. and Physiol. 22:433-449.

Blaney, W.M., M.S.J. Simmonds, S.V. Ley, J.C. Anderson, S.C. Smith, and A. Wood,1994. Effect of azadirachtin-derived decalin (perhydronaphthalene) and dihydrofuranacetal (furo[2,3-b]pyran) fragments on the feeding behaviour of *Spodoptera littoralis*. Pestic. Sci. 40:169-173.

Chourasia, H.K. and A.K. Roy, 1991. Effect of temperature, relative humidity and light on aflatoxin B_1 production in neem and *Datura* seeds. Int. J. Pharmacognosy. 29(3):197-202.

Don-Pedro, K.N. 1989. Mode of action of fixed oils against eggs of *Callosobruchus maculatus* (F.) Pestic. Sci. 26:107-115.

Dunkel, F.V. 1996. Integrated Pest Management Cooperative Research Support Program (IPM CRSP): Mali site (Bamako, Sotuba, and Sirakorola). Video filmed 8-16 November 1996. 20 Minutes.

Dunkel, F.V. and D.C. Richards. The effect of an azadirachtin formulation on six non-target aquatic macroinvertebrates. Environ. Entom. in review.

Echindu, T.N.C., 1991. Ginger, cashew and neem as surface protectants of cowpeas against infestation and damage by *Callosobruchus maculatus* (Fab.). Trop. Sci. 31: 209-211.

Edwards, C.R. and J.S. Caldwell. 1996. Assessment of on-farm IPM trial implementation

and insect pest monitoring. Rept. for IPM CRSP USAID Grant No. LAG-4196-g-00-3053-00. 14pp.

GRAT (Groupe de Recherches et d'Applications Techniques). 1993. Une ONG Malienne au service du developement. GRAT, Bamako, Mali. 6 pp.

Lin-er, L., J.J.A. van Loon, and L.M. Schoonhoven, 1995. Behavioral and sensory responses to some neem compounds by *Pieris brassicae* larvae. Physiol. Entom. 20:134-140.

Makanjuola, W.A., 1989. Evaluation of extracts of neem (*Azadirachta indica* A. Juss) for the control of some stored product pests. J. Stored Prod. Res. 25(4):231-237.

Mordue (Luntz), A.J. and A. Blackwell, 1993. Azadirachtin: an update. Insect Physiol. 39(11):903-924.

Ofuya, T.I. and K. A. Bambigbola, 1990. Damage potential, growth and development of the seed beetle, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae), on some tropical legumes. Trop. Agric. 68(1):33-36.

Pacheco, I.A., M.F.P.P.M. De Castro, D.C. De Paula, A.L. Lourencao, S. Bolonhezi, and M.K. Barbieri, 1995. Efficacy of soybean and castor oils in the control of *Callosobruchus maculatus* (F.) and *Callosobruchus phaseoli* (Gyllenhal) in stored chick-peas (*Cicer arietinum* L.). J. Stored Prod. Res. 31(3):221-228.

Pierrard, G., 1986. Control of the cowpea weevil *Callosobruchus maculatus*, at the farmer level in Senegal. Trop. Pest. Man. 32(3):197-200.

Radcliffe, E.B., F.V. Dunkel, P.P. Strzok, and S. Adam. 1991. Antifeedant effect of neem, *Azadirachta indica* A. Juss., kernel extracts on *Kraussaria angulifera* (Krauss)(Orthoptera: Acrididae), a Sahelian grasshopper. Trop. Agric. 68:95-101.

Rajapakse, R., and H.F. Van Emden, 1997. Potential of four vegetable oils and ten botanical powders for reducing infestation of cowpeas by *Callosobruchus maculatus*, *C. chinensis* and *C. rhodesianus*. J. Stored Prod. Res. 33(1):59-68.

SAS Institute. 1988. SAS/STAT user's guide, release 6.03 ed. SAS Institute. Cary, NC.

Schmutterer, H. 1988. Potential of azadirachtin containing pesticides for integrated control in developing and industrial countries. J. Insect Physiol. 34:713-719.

Sowunmi, O.E. and O.A. Akinnusi, 1983. Studies on the use of neem kernel in the control of stored cowpea beetle (*Callosobruchus maculatus* F.) Trop. Grain Leg. Bull. 27:28-31.

Stark, J.D. and J.F. Walter, 1995. Persistence of Azadirachtin A and B in the soil: effects of temperature and microbial activity. J. Environ. Sci. Health 30(5):685-698.

Tanzubil, P.B., 1991. Control of some insect pests of cowpea (*Vigna unguiculata*) with neem (*Azadirachta indica* A Juss.) in Northern Ghana. Trop. Pest Man. 37(3):216-217.

Xie, Y.S., P.G. Fields, and M.B. Isman, 1995. Repellency and toxicity of azadirachtin and neem concentrates to three stored product beetles. Submitted to J. Econ. Entom.

Zehrer, W., 1983. The effect of the traditional preservatives used in northern Togo and of neem oil for control of storage pests. Proc. 2nd Int. Neem Conf. Rauischolzhausen,

pp. 453-460.

Table 1. Baseline dose-response of the cowpea weevil, *Callosobruchus maculatus* (F)[1] to cowpeas, *Vigna unguiculata*, treated with aqueous extracts of neem kernel extract (NKE) grown and produced in Mali.

TREATMENT (n=3)	**MEAN[2] NO. OF EGGS LAID PER ADULT FEMALE ± STANDARD ERROR (R-squared=.976)**	**MEAN[2] NO. ADULTS EMERGED PER ADULT FEMALE ± STANDARD ERROR (R-squared=.917)**
UNTREATED	42.47±.88 A	23.93±.24 B
WATER TREATED	42.53±1.11 A	29.13±1.27 A
10% NKE[3]	28.73±.35 B	8.93±3.86 C
25% NKE[3]	26.2±1.89 B	1.93±1.93 D
40% NKE[3]	1.67±.64 C	0 D
55% NKE[3]	3.2±3.2 C	0 D

[1] five adult females and three adult males (0-24 hrs post emergence) placed in vial with 20±0.05g cowpeas and removed after three days.

[2] Means followed by the same letters are not significantly different (Student-Neuman-Kuels, alpha=0.05).

[3] % NKE refers to the concentration of raw neem kernel extract dissolved in deionized water. Seven hundred fifty microliters of each solution were applied in each replicate.

Table 2. Effect of heat on NKE's oviposition deterrence and larval toxicity of *Callosobruchus maculatus* (F)[1].

TREATMENT (n=5)	MEAN[2] NO. OF EGGS LAID PER ADULT FEMALE ± STANDARD ERROR (R-squared for 1995 acq. = .822) (R-squared for 1996 acqs. = .894)	MEAN[2] NO. OF ADULTS EMERGED PER ADULT FEMALE ± STANDARD ERROR (R-squared for 1995 acq. = .005) (R-squared for 1996 acqs. = .040)
NKE[3] STORED AT 5°C		
(Mali 1995 acq.)	24.92±1.62 A	5±3.02 A
(Mali 1996 acq.)	30.40±0.59 B	6±.55 A
(Benin 1996 acq.)	29.84±1.10 B	5±.42 A
NKE[3] STORED AT 28°C		
(Mali 1995 acq.)	30.00±1.18 B	5±3.82 A
NKE[3] STORED AT 50°C		
(Mali 1995 acq.)	38.60±1.07 C	6±3.30 A
(Mali 1996 acq.)	39.48±.65 C	6±.96 A
(Benin 1996 acq.)	38.48±.62 C	5±.62 A

[1] five adult females and three adult males (0-24 hrs post emergence) placed in vial with 20±0.05g cowpeas and removed after three days.

[2] Means followed by the same letters are not significantly different (Student-Neuman-Kuels, alpha=0.05).

[3] Seven hundred fifty microliters of a 25% NKE solution stored at the indicated temperature regime.

Fig. 1 HPLC Chromatograms of Mali 1995 acquisition of NKE: A) Solid phase extraction of raw NKE (in methanol) stored at 5°C **B)** Solid phase extraction of raw NKE (in methanol) 5 months after heat treatment at 50°C for two weeks and stored at 28°C for the rest of the time.

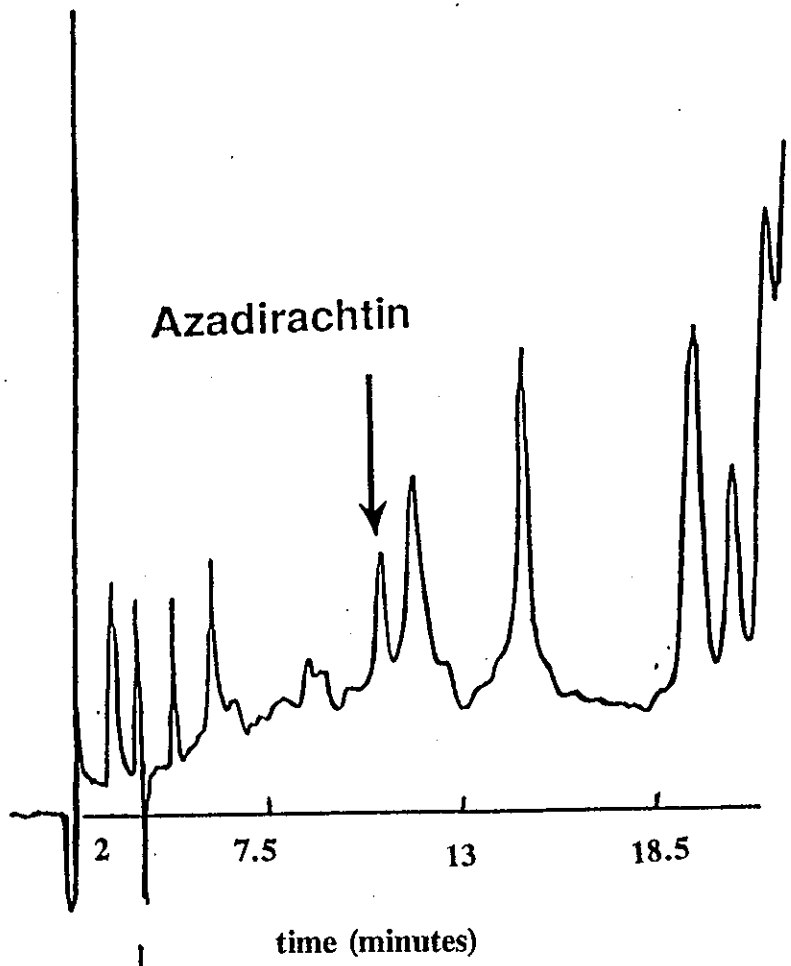
Fig. 2 HPLC Chromatograms of Mali and Benin 1996 acquisitions of NKE and pure azadirachtin: A) Solid phase extraction of raw NKE (in methanol); Mali 1996 acquisition. **B)** Solid phase extraction of raw NKE (in methanol); Benin 1996 acquisition. **C)** 95% pure azadirachtin A.

Fig. 1

High-Pressure Liquid Chromatograms (HPLC) of Neem Kernel Extract

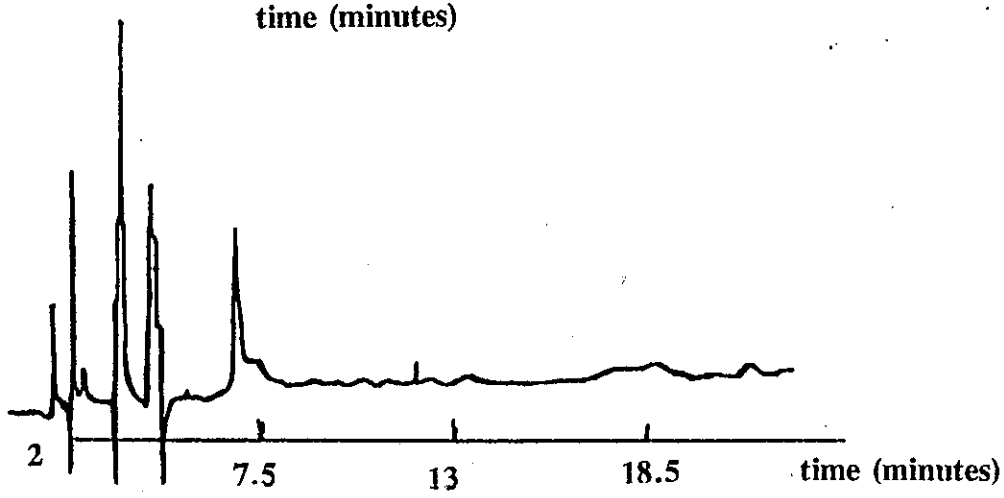
A

Untreated



B

Heated
(2 Weeks at 50°C)



21-