LARVICIDAL EFFECTS OF GRAIN SORGHUM (*SORGHUM BICOLOR*) SEEDLING EXTRACTS UPON *CULEX PIPIENS* LARVAE

F. L. C. JACKSON[1], S. S. BEHKEIT[2], S. M. EL ETR[2] AND N. K. QUACH[1]

ABSTRACT. A study of laboratory and field reared 2nd and 3rd instar *Culex pipiens* larvae suggests that extracts from 2 varieties of *Sorghum bicolor* seedlings are significant ($P < 0.05$) larvicides under laboratory conditions. These plant extracts contain the organic cyanogen dhurrin and were calibrated to produce 90% mortality in 2nd instar *Culex pipiens* larvae at 0.82 ppm and 90% mortality in 3rd instar larvae at 1.12 ppm. A preliminary behavioral assessment of late 3rd instar larvae exposed to 1.42 ppm suggests that these plant extracts produce 80% mortality after only 4–5 h of contact. Plant extracts appear stable when stored at up to 32°C in a closed container. Once the extracts are infused in water and exposed to air, however, they biodegrade after 24 h. These laboratory results emphasize the need for field tests against natural populations of *Culex pipiens* and nontarget organisms.

INTRODUCTION

In Egypt, *Culex pipiens* (Linn.) mosquitoes remain major disease vectors of bancroftian filariasis (Gad et al. 1988), Rift Valley fever virus (Bres 1978), West Nile and Sindbis viruses (Samina et al. 1986), as well as serious nusiance pests (World Health Organization 1983). The control of this mosquito vector through the use of effective, biodegradable, economically feasible botanical extracts is an important alternative control strategy. We report on laboratory toxicity tests of the application of grain sorghum (*Sorghum bicolor* [Linn.] Moench) seedling extract to 2nd and 3rd instar larvae of *Culex pipiens*.

The grain sorghum extracts evaluated as larvicides were derived from seedling varieties widely cultivated in the United States and in Egypt for local human food and animal fodder use. Our rationale for testing these extracts was the presence of the organic cyanogen dhurrin, [(S)-*p*-hydroxymandelonitrile β-D-glucopyranoside] in grain sorghum. This cyanogenic glycoside (depicted along with its hydrolysis in Fig. 1) is an endogenous biological precursor of cyanide, is present in high concentrations in the immature plant (Nartey 1981), and is the most likely toxic constituent in grain sorghum capable of larvicidal effects (Branson et al. 1969). Most discussions of the effects of plant organic cyanogens on humans and animals have focused on the potential of these toxins to initiate significant physiological modifications within the human or animal consuming a cyanogenic plant (Osuntokun 1981, Jackson et al. 1988). Our research objective in this case, however, was to test the hypothesis that extracts of this cyanogenic plant can impair the viability of immature mosquitoes and thus be an effective mosquito control agent.

MATERIALS AND METHODS

Plant extracts were prepared from 4- to 7-day-old seedlings grown from pregerminated sorghum which was cultivated at 25°C and at 55% RH in 3 cm of sterile humus soil, given 20-ml deionized water and exposed to 24-h cycles of white fluorescent light. Seedling leaves were harvested and the leaves pressed in a hand grinder to extract the liquid. A 60% yield by weight of leaves to liquid was obtained using this method. The extract was stored at 23–32°C and later centrifuged, filtered and assayed for its organic cyanogen content using a cyanogenic glycosidase specific enzymatic assay (Cooke 1979). All assays were done in triplicate.

Two sources of grain sorghum provided the basis of our extract stocks. Extract I was derived from Beefbuilder T[3] variety and contained 357.5 ppm organic cyanogen; extract II was derived from Giza 15 and Local 129[4] varieties and contained 195 ppm organic cyanogen. Serial dilutions of these extract stocks (ranging in 10% intervals from 0 to 100%) were used for mortality testing against the mosquitoes. The organic cyanogen content of these dilutions was assayed and ranged from 0 to 1.42 ppm organic cyanogen.

Second and 3rd instar larvae of *Culex pipiens* were used and represented both laboratory reared and field collected specimens. Laboratory reared larvae were maintained following a standardized protocol at the Research Institute for Medical Entomology in Dokki, Cairo, Egypt. Field reared larvae were collected from nearby untreated sites in and around Cairo. These field larvae were then transported to the laboratory, maintained in water from their original breeding

[1] Laboratory in Biological Anthropology, 1350 Turlington Hall, University of Florida, Gainesville, FL 32611.

[2] Research Institute of Medical Entomology, 1 Mathaf El Zarai Street, Dokki, Cairo, Egypt (A.R.E.).

[3] Beefbuilder T is distributed by Asgrow Seed Company, USA.

[4] Giza 15 and Local 129 were provided by the Agricultural Research Center, Giza Egypt.

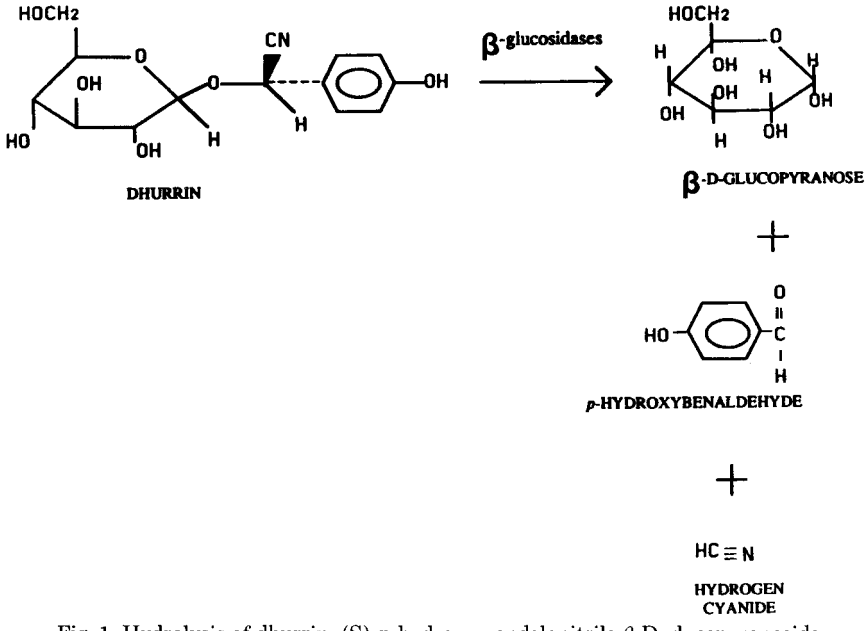

Fig. 1. Hydrolysis of dhurrin, (S)-*p*-hydroxymandelonitrile β-D-glucopyranoside.

sites and subsequently used for toxicity testing within 24 h of initial collection.

Following the World Health Organization guidelines for insecticide assessment (World Health Organization 1981), 1 ml of each serial dilution of *Sorghum bicolor* extracts was added to 225 ml of water in a 500-ml glass beaker and stirred vigorously with a glass rod. To this were added 25 *Culex pipiens* larvae (either 2nd instar or late 3rd instar) in 25 ml of water, bringing the total volume of water to 250 ml. All experimental and control waters were maintained at 23–32°C and 24-h mortality recorded. The concentrations of organic cyanogen in the treated waters was evaluated initially and again after 24 h. Since behavior is an established criterion of toxicity (Weiss 1978), to complement our studies of absolute mortality in the mosquitoes, hourly behavioral observations were made of a subsample of 50 late 3rd instar larvae exposed to 1.42 ppm organic cyanogen.

Data were initially plotted logarithmically as recommended by the World Health Organization (World Health Organization 1981). Following this, data were subjected to linear regression and *r* values calculated for the correlation of organic cyanogen concentration (independent variable) and vector mortality (dependent variable). An ANOVA was also performed and the *F* statistic evaluated relative to an alpha value of 0.05. Probability analysis was used to calibrate LC_{50}s and LC_{90}s for 2nd and 3rd instar larvae exposed to extract I and extract II of *Sorghum bicolor*.

RESULTS AND DISCUSSION

Both extracts killed 100% of both 2nd and 3rd instar *Culex pipiens* larvae under laboratory conditions of assessment. The calculated *r* and *F* values for 2nd instar larvae were significant ($r = 0.79$; $F_{(1,20)} = 33.30$). The calculated *r* and *F* values for 3rd instar larvae were also significant ($r = 0.93$; $F_{(1,20)} = 126.18$). The dilutions of plant derived organic cyanogens capable of killing 2nd instar larvae were less than those required to produce a similar level of mortality among 3rd instar larvae. Combining data from Extract I and II, the linear regression model predicted that the LC_{50} and LC_{90} for 2nd instar larvae were 0.30 ppm and 0.82 ppm, respectively. The calibrated LC_{50} and LC_{90} for 3rd instar larvae (combining data from extract I and II) were 0.67 ppm and 1.12 ppm, respectively. Figures 2 and 3 display the linear regression of mortality for 2nd and 3rd instar *Culex pipiens* larvae, respectively, exposed to sequential dilutions of extract I and II plant organic cyanogens. In both extract I and extract II, the assayed levels of organic cyanogens were well below the amounts identified as toxic for humans and other mammals (Montgomery 1965).

After 24 h of exposure to air, the water infused with the *Sorghum bicolor* seedling extract no longer contained detectable organic cyanogen and also lost its larvicidal properties. This apparent loss of toxicity was due to the degradation of the organic cyanogens and proceeded independent of the initial concentration of the ex-

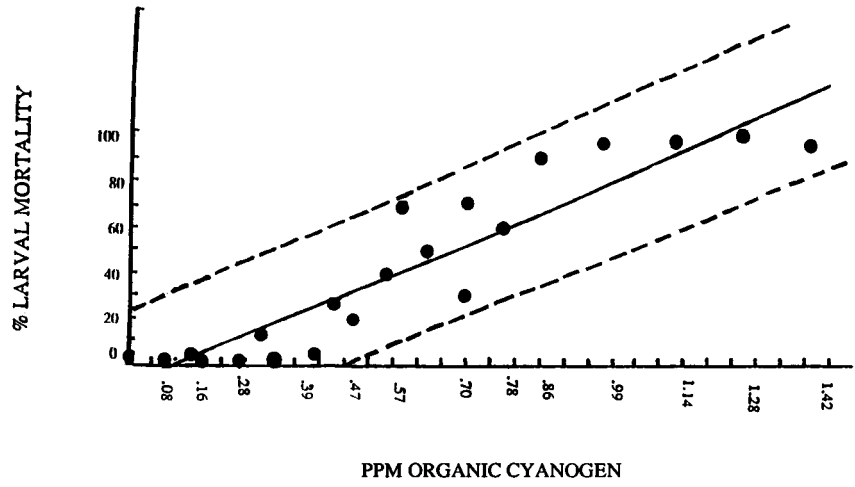

Fig. 2. Linear regression of various dilutions of plant organic cyanogens (X) on 2nd instar *Culex pipiens* larval mortality (Y) (after 24 h) with 95% confidence bands.

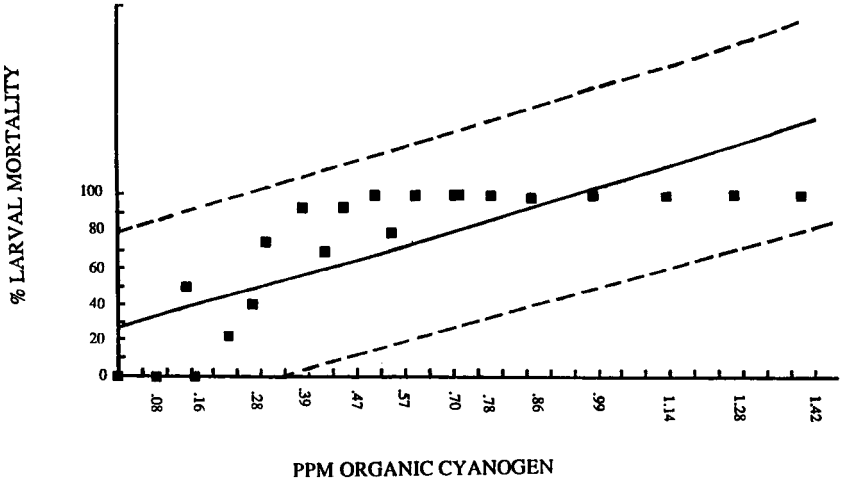

Fig. 3. Linear regression of various dilutions of plant organic cyanogens (X) on 3rd instar *Culex pipiens* larval mortality (Y) (after 24 h) with 95% confidence bands.

tract or the density of the mosquito larvae present. Dhurrin is the most likely, although perhaps not the only, insecticidal component of our plant extracts.

The results of our preliminary behavior assessments suggested that the exposure of late 3rd instar larvae to 1.42 ppm organic cyanogen initiated a rapid sequence of morbidity associated behavioral changes. Initially, all 50 larvae moved vigorously and appeared to be quite vibration sensitive. Within 17 min following their exposure, however, larvae appeared passive, resting on the surface, with no evidence of overt morphological changes. Within 1.5 h following exposure to the organic cyanogen, 2 larvae with prior siphonal distortions were dead. Prior to their deaths, they had exhibited a series of sharp, rapid turns and twists midway in the glass beaker and again on the bottom. After 2 h more of exposure to the plant extracts, larger numbers of larvae began to lie very still on the surface and then drift downward with erect siphons. These larvae remained dormant on the bottom and appeared to lack the energy to remain afloat. Four and one-half hours post-initial contact with the plant toxin, 80% of the remaining larvae appeared moribund and resisted movement in response to being gently poked in the thorax. These larvae appeared to be dead or dying; dead larvae took on a very white appearance but remained morphologically intact. Five and one-half hours following initial exposure, 82% of the larvae were dead (100% were dead after 8 h of exposure to the 1.42 ppm organic cyanogens).

Based upon these preliminary behavioral observations, we hypothesize that the organic

cyanogen in the plant extracts tested is the most important, and possibly the sole, toxic component. This organic cyanogen, dhurrin, is a well-known biological precursor of the potent metabolic toxin cyanide. In water, dhurrin hydrolyzes to D-glucose, HCN, and hydroxybenzaldehyde. HCN is further dissociated with CN^- possibly absorbed (given the rapid behavioral response observed) by the larvae. Once absorbed, it is likely that this CN^- inhibits cellular respiration through the binding and inactivation by cyanide of cytochrome oxidase (Solomonson 1981), the terminal component of the mitochondrial electron transport chain.

Our results suggest that field toxicity studies should be initiated employing these plant-derived organic cyanogens against naturally occurring *Culex pipiens* larval populations. In a field setting with the capability for continued application of these plant extracts to larvae habitats, there may be some potential for effective and sustainable mosquito control. The larvicidal merits of *Sorghum bicolor* seedling extracts are enhanced by the fact that the grain sorghums (and other plants known to contain organic cyanogens) are widely cultivated throughout the world, particularly in many economically disadvantaged countries. In many regions, their cultivation coincides with the presence of natural populations of *Culex pipens* mosquitoes. Future toxicity testing should also include evaluations of the effects of organic cyanogens on such nontarget organisms as *Dugesia dorotocephala* and *Gambusia affinis*. Several factors may contribute to the long term utility of the extracts under field conditions. The simple extraction process applied to grain sorghum seedlings does not require electricity or the addition of extensive chemicals. The extract appears stable when stored in a closed container at temperatures at least as high as 32°C. Yet the extract biodegrades in water after 24 h of exposure to air. These first results thus suggest that the use of *Sorghum bicolor* plant extracts in *Culex pipiens* control may be an important early step towards empowering certain economically developing regions to successfully and inexpensively combat this serious disease and nusiance vector.

ACKNOWLEDGMENTS

We thank Shawkat Saeid, Mohamed El Shourbagy, Rosina Hassoun and Salwa Tamer for their laboratory and field collection efforts. We are grateful to Professors M. S. A. Mostafa and O. O. El Nagoly, Agricultural Research Center, Giza, Egypt, for their contributions of *S. bicolor*. This research was conducted while one of the authors (F.L.C.J.) was a Fulbright Research Scholar in Egypt.

REFERENCES CITED

Branson, T. F., P. L. Guss and E. E. Ortman. 1969. Toxicity of sorghum roots to larvae of the western corn rootworm. J. Econ. Entom. 62:1375–1378.

Bres, P. 1978. The Rift Valley fever virus, agent of dengue-like epidemics: its geographical distribution and public health problems. J. Egypt Public Health Assoc. 53:147–152.

Cooke, R. D. 1979. Enzymatic assay for determining the cyanide content of cassava and cassava products. Cassava Information Centre. C.I.A.T., Cali, Columbia.

Gad, A. M., A. Shoukry and S. M. El Said. 1988. Vector competence to *Wuchereria bancrofti* in *Culex pipiens* collected from the Nile Delta. J. Egypt Soc. Parasitol. 18:259–272.

Jackson, L. C., M. Oseguera, S. Medrano and Y. Kim. 1988. Carbamylation of hemoglobin *in vivo* with chronic sublethal dietary cyanide: implications for hemoglobin S. Biochem. Med. 39:64–68.

Montgomery, R. D. 1965. The medical significance of cyanogens in plant food stuffs. Am. J. Clin. Nutri. 17:103–113.

Nartey, F. 1981. Cyanogenesis in tropical feeds and foodstuffs, pp. 115–132. *In:* B. Vennesland, E. E. Conn, C. J. Knowles, J. Westley, F. Wissing (eds.), Cyanide in biology. Academic Press, London.

Osuntokun, B. O. 1981. Cassava diet, chronic cyanide intoxication and neuropathy in Nigerian Africans. World Rev. Nutr. Diet. 36:141–173.

Samina, I., J. Margalit and J. Peleg. 1986. Isolation of viruses from mosquitoes of the Negev, Israel. Trans. R. Soc. Trop. Med. Hyg. 80:471–472.

Solomonson, L. P. 1981. Cyanide as metabolic inhibitor, pp. 11–28. *In:* B. Vennesland, E. E. Conn, C. J. Knowles, J. Westley, F. Wissing (eds.), Cyanide in biology. Academic Press, London.

Weiss, B. 1978. The behavioral toxicity of food additives, pp. 21–38. *In:* J. Weininger, G. M. Briggs (eds.), Nutrition update. Vol. 1. John Wiley and Sons, New York.

World Health Organization. 1981. Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. WHO/VBC/81.807.

World Health Organization. 1983. Integrated vector control. 7th report of the WHO Expert Committee on Vector Biology and Control. Tech. Rep. Ser. 688.