PharmacologyOnLine December 30, 2013
Archives • 2013 • vol.3 • 137 - 140

Larvicidal activities of a neem cake fractions on *Aedes albopictus*

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Abstract

There is an urgent need for ecologically sound, equitable and ethical pest management, based on control agents that are pest-specific, nontoxic to humans and other biota, biodegradable, less prone to pest resistance and resurgence and relatively less expensive. The last aspect is fundamental for a large scale use in emerging countries. Among various options concerning botanical bio-pesticides, neem has been identified as a source of eco-friendly "soft" natural pesticides. We report our results in scientific validation of neem cake, a by-product of seed neem oil production.

Neem cake was selected on the basis of low cost, sustainability, availability of enormous quantities, other uses in agriculture and possible direct utilization. However, the proposal had to face the possibility of different compositions of marketed neem cakes and their corresponding activities. Furthermore, neem cakes compositions resulted so different from the neem oil ones to make necessary a re-writing of the chemistry chapter. The last episode of this research is here reported with new data concerning the larvicidal activities of neem cake extracts at different compositions.

KEY WORDS: NEEM CAKE, LARVICIDAL ACTIVITIES, AEDES ALBOPICTUS

Communication to the conference "Neem: multipurpose plant of the XXI"
conducted at the Department of Environmental Biology, University La Sapienza in Rome June 21, 2013

Introduction

In September 1962 Rachel Carson's *Silent Spring* was published[1]. The book inspired widespread public concerns with pesticides and pollution of the environment.

Silent Spring documented detrimental effects of pesticides on the environment, particularly on birds[2]. Carson accused the chemical industry of spreading disinformation and public officials of accepting industry claims uncritically.

In 1972 in the United States banned the use of pesticide DDT[3] for agricultural use. Synthetic organochloride insecticides (like DDT) had been worldwide largely used and were fundamental to eradicate malaria in developing countries, but poisoned our world. Carson first highlighted environmental effects, including permanent toxicity to non targeted organisms, resistance development in insect populations, long term damages[4].

Therefore other considerations and approaches emerged, as well as a general interest for new materials in pest management, in particular in urban area. Attention was shifted from synthetic to natural substances. First efficient replay came from pyretroids, that nowadays represent 80% of the total market of global botanical insecticides. Pyretroids are efficient and rapid, but their limits are the high cost and low stability in sunlight. In the past 15 years several other natural products with different modes of action have been proposed, but only two new types of botanical bioinsecticides have been commercialized: essential oils and neem seed extracts, the last ones based on nortriterpenoids azadirachtins[5-9].

Neem oil products repeated in part the Chrysanthemum experience. However, in this case the high cost, due to the complex method of production, and easy degradation could be economically in part compensated by the renewable raw material and the highly increasing of the distribution of neem trees.

For these reasons we decided to explore the insecticide potentiality of neem cake against selected target pest insects. Neem cake is obtained as final by-product during the extraction process of seeds to obtain the oil. The extraction gives two main products, the neem oil and the oiled neem cake, which can be re-extracted by *n*-hexane giving raise to the de-oiled neem cake. During the process the oil content in neem cakes decreases to 6-1,5%, as well as azadirachtins content.

Neem cake have been reported to have at least eight types of activities (a) antifeedant (b) attractant (c) repellent (d) insecticide (e) nematicide (f) growth disruptor (g) antimicrobial. This wide range of activities is the direct consequence of the complex chemical composition. Only in the seed oil, hundreds of constituents have been identified and others are waiting to be discovered.

Methods

Mosquito colony handling

A population of *Aedes albopictus,* coming from larvae collected in the field in summer 2013 in the C.R. Casaccia area (Rome), were reared in culture rooms at 26° ± 1°C, 50-80% relative humidity (RH), and a 14L:10D photoperiod. Adults were placed in screen cages (50x50x50cm) continuously supplied 10% sucrose solution in 10 mL plastic jar provided with a cotton wick, to allow comfortable landing on the moist cottons for the assumption of the sugar solution. On day 5 post emergence, the adults were deprived of sucrose feeding for 12 h, then supplied with artificial blood feeding. The blood meal was furnished, by means of a professional heating blood (lamb blood), at fixed temperature of 38°C, provided with a membrane of caw gut. After 30 minutes the blood meal was removed, due to the drying phenomena of gut and blood and substitute with a new fresh blood meal for following utilization. On day 5 after blood feeding the gravid females were allowed to lay eggs inside 50 ml plastic pot, with walls covered of paper (white creped papers Industrialfiltro srl.), filled with 10 ml of raining water. Week old eggs were allowed to hutch in raining water and larvae were reared every day with

a mixture of cat food powdered at the doses 30, 60, 120 and 90 mg/l for 1°, 2° , 3° and 4° instar until the pupae stage. Water level was controlled every twice day and replayed to counterbalance the loss due to evaporation.

Toxic and growth retarding effects tests

Biological tests were carried out in rearing room kept at 26 ± 1°C, 50-60% relative humidity (RH) and a 14L:10D light: dark photoperiod. Toxic effects were measured using a rainwater solution 100 ppm of extract - 100 ppm of Tween 80. The control solution was 100 ppm of Tween 80.

The experiments were carried out starting from a week old eggs, still laying on paper material, coming from the mosquito colony. 50 eggs of *A. albopictus* were exposed to 16 ml of extract solutions. Each treatment was replicate four times. Observations on the mortality of the larvae were made daily and the results noted. The new hatched larvae in treated and control cups were not fed during the experiments first 24 h, then larvae still alive were fed every day with cat powder. Water level was controlled every day and replayed to counterbalance the loss due to evaporation. Was not taken into account the % of eggs hatching, as verified not reliable results in previous experiences, only larval mortality and abnormal effects on larval development were recorded.

Preparation of extracts

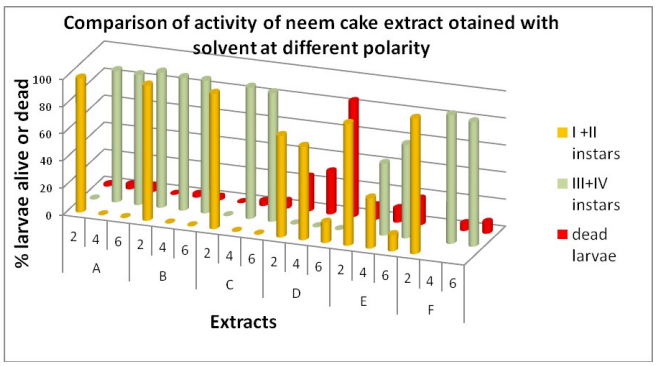
Four extracts (A-D) of neem cake were obtained by different preparations. Extract **D** was obtained by direct extraction of neem cake by ethyl acetate at room temp; extract **A** by defatting methanol extract; extract **B** by direct extraction of methanol extract of neem cake by H_2O at room temp; extract **C** by repartition of the methanol extract between H_2O and ethyl acetate (1:1) and taking the aqueous phase **E**; **D** was obtained by direct extraction of neem cake by AcOEt; finally extract **E** was obtained by dilution of Tween 80.

Results

Biological tests

A set of bioassays experiments was performed to evaluate the biological activity of several neem cake extracts obtained by mean of solvent extraction. The aim of the reported experiment was the checking of the activity of polar against apolar constituents of neem cake. For this reason five samples were appositely prepared: **A**, AcOEt obtained by treatment with hexane of the methanol extract to separate the great part of fatty acids; **B** and **C**, two aqueous extracts, the first one directly obtained from neem cake and the second after by repartition from neem cake methanol extract; **D**, the ethyl acetate extract directly obtained form neem cake; **E** was the EtOAc extract obtained by repartition between AcOEt and water; Tween solution used as the standard.

The highest biological activity will be found for the extract **D**. Fig. 1 shows the mortality and the different larval instars distribution at day 2-4-6-days in the solutions tested. **D** was the most effective in terms of larval mortality, in particular in **D** no larval moulting occurred. Practically no activity was observed for the aqueous extracts, albeit obtained with different procedures.



Discussion

The experiments confirm that the activity is concentrated in the apolar fraction. As a matter of fact, the aqueous fractions did not show any antilarvicidal activity. On the contrary, the two ethyl acetate extracts were very effective. It is notewor-

thy the difference in the activities, showing a relative importance of the fatty component.

The HPTLC analyses showed for this fraction a very complicated composition, having a great prevalence of fatty acids[9-10]. For this reason a defatting treatment of performed and the resulting extract, **A**, compared with the previous one, **D**. The resulting decreasing of the activity evidenced that also the fatty acids part could be important for the whole activity. Thus first we demonstrated that the activity was not totally to be attributed to the nortriterpenoids and now confirm the necessity of further research to assign the active constituents for the neem cake larvicidal activity.

References

1. McLaughlin D. "Fooling with Nature: Silent Spring Revisited". Frontline. PBS. 2010.
2. Glausiusz J. (2007), Better Planet: Can A Maligned Pesticide Save Lives? Discover Magazine. EPA reference: DDT. 2007.
3. Peakall DB, Van Emden HF, ed. (1996). Beyond silent spring: integrated pest management and chemical safety. London: Chapman & Hall.
4. Schmutterer H (2002) The Neem Tree: Source of Unique Natural Products for Integrated Pest Management, Medicine, Industry And Other Purposes (Hardcover), 2nd Edition, Weinheim,Germany: VCH Verlagsgesellschaft.
5. Tewari DN (1992) Monograph on neem (Azadirachta indica A. Juss.). Dehra Dun, India: International Book Distributors. pp.123-128.
6. Vietmeyer ND (1992) Neem: A Tree for Solving Global Problems. Report of an ad hoc panel of the Board on Science and Technology for International Development, National Research Council, Washington, DC, USA: National Academy Press. pp.74-75.
7. Puri HS (1999) Neem: The Divine Tree. Azadirachta indica. Harwood Academic Publishers, Amsterdam. ISBN 90-5702-348-2.
8. Rossi Forim M, da Silva MF das Gracas F, Fernandes JB. Secondary Metabolism as a Measurement of Efficacy of Botanical Extracts: The Use of Azadirachta indica (Neem) as a Model in Insecticides - Advances in Integrated Pest Management. Farzana Perveen (Ed.). Intech. Rijeka, Croatia. 2011, p. 367-390.
9. Nicoletti M, Maccioni O, Coccioletti T, Mariani S, Vitali F. Neem Tree (Azadirachta indica A. Juss) as source of Bioinsecticides. in Insecticides - Advances in Integrated Pest Management. Farzana Perveen (Ed.). Intech. Rijeka, Croatia, 2011; 411-428.
10. Nicoletti M, Serafini M, Aliboni A, D'Andrea A, Mariani S (2010) Toxic effects of neem cake extracts on Aedes albopictus larvae. Parasitol Res 107, 89-94.