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A NOVEL PLANT-BASED SYNERGIST FOR PYRETHRUM AND PYRETHROIDS AGAINST URBAN PUBLIC HEALTH PESTS

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Abstract Natural pyrethrum and its synthetic analogues are among the most widely used insecticides in medical, veterinary and agricultural use. Low application rates, rapid knock-down and kill, range of duration of activity and good mammalian safety profile make them an active of choice in most domestic applications. The threat to continued use of these compounds is that of resistance in target organisms. To overcome resistance it is common to add a synergist, such as piperonyl butoxide. In this study we report the potential synergistic effects of a botanical substance derived from dill seed oil (*Anethum sowa*), which has been proven to enhance the biocidal activity of pyrethrins and overcome *kdr*-type resistance in malaria vector mosquitoes. With an optimal ratio of 1:3.6 pyrethrum to dill seed oil, and an enhanced biocidal activity against medically important urban pest species including *Periplaneta americana* (L.), *Musca domestica* (L.) and a pyrethroid-resistant strain of the Asian urban malaria vector *Anopheles stephensi* (Liston), we consider this combination may be suitable in a wide range of pest control situations.
Key Words dill seed oil, cockroach, house fly, permethrin, *Anopheles stephensi*

INTRODUCTION

In recent years there has been a renewed interest in the identification & development of naturally sourced, plant-based biocidal actives. A combination of factors, including rapid and widespread development of resistance to existing compounds, a slowdown in the number of new synthetic actives being registered and consumer moves towards perceived "green" alternatives have all contributed to this botanical interest. Whilst there are many naturally derived biocides relatively few have made the transition from lab to commercial use. Reasons for this include low efficacy at convenient application doses, difficulties in quality control at source and suboptimal physical characteristics such as residual activity, toxicity and stability in formulation. The multiple modes of action of many natural substances, whilst an advantage in terms of reducing likelihood of developing resistance, also makes such compounds more difficult to register with regulatory authorities (Isman, 2006). One means to overcome such inherent problems could be to investigate their synergistic properties in combination with existing actives rather than as stand-alone products. It would seem pragmatic to design such mixtures specifically to enhance key characteristics or overcome weaknesses in one or other compound. In the present study we are seeking ways to overcome issues of resistance to pyrethroids in some urban pest target species whilst improving knockdown speed of an otherwise useful botanical biocide. The natural substance investigated here, dill seed oil (*Anethum sowa*), has proven to exhibit some insecticidal activity against a wide range of insect pests including mosquitoes, houseflies and cockroaches, although speed of action is relatively slow. Studies previously undertaken on dill seed oil (DSO) and pyrethrin mixtures by CEM Analytical Services (CEMAS, 2006) in the UK demonstrate a clear synergistic effect between these substances when screened against the cereal aphid, *Rhopalosiphum padi* (L.), with an optimal ratio of 1:3.6 pyrethrin: DSO when applied to barley plants under controlled experimental conditions (Fig. 1).

Should a similar combination of DSO and a pyrethroid provide a formulation effective against resistant insects yet retain a rapid knock-down it may provide a useful product against a range of urban domestic pests.

MATERIALS AND METHODS

The following insect colonies were used in this study. All species / strains are maintained and tested under optimal conditions of 24ºC ± 2ºC, 80% RH and 12:12h photoperiod.
Anopheles stephensi "DUB-APR" Pyrethroid resistant strain from Dubai. *Periplaneta americana* standard insecticide susceptible strain in permanent culture at London School of Hygiene and Tropical Medicine. *Musca domestica* standard insecticide susceptible strain.

A proprietary formulation of DSO (*A. sowa*) referred to as LB002 and a sample of pyrethrin (50%) were supplied by Livie Biosciences, UK. Pyrethrin was used at 1% working dilution and DSO at 3.6%. The experimental mixture was used at the optimal synergist ratio of 1:3.6 pyrethrin to DSO. All working dilutions were aqueous emulsions.

Topical exposure of all insects was via direct aerosol generation using a Potter Tower laboratory insecticide sprayer (Burkard Manufacturing Ltd, Rickmansworth, UK) at 5.0 psi with a working volume of 0.5 ml of each formulation tested.

Test samples were sprayed through the Potter Tower directly onto the insects held in 9 cm diameter disposable white plastic pots with wide mesh netting covers. After exposure insects were transferred to clean pots and knockdown was recorded at 10 minutes and final mortality recorded after 24 hours in optimal conditions with a glucose meal source available. A minimum of 6 replicates on batches of 10 (cockroaches and flies) or 20 (mosquitoes) individuals was conducted for each test sample along with a corresponding negative control using 10% ethanol in water.

RESULTS AND DISCUSSION

Results in Table 1 indicate primary objectives of combining DSO and pyrethrin were met. In all species there was an observed increase in rapid knockdown in the mixture compared to DSO alone, and the mortality recorded in the resistant *An. stephensi* was much higher than with pyrethrin alone. The rapid knockdown of this mixture for household pests like cockroaches and houseflies means it would be more acceptable in a commercial flying and crawling insect products. As *An. stephensi* is the most important urban malaria vector in many parts of Asia, particularly in Indian towns and cities (Sharma, 1999), the combination of DSO and pyrethroids is likely to offer new hope in the control of this medically important vector in areas where pyrethroid resistance is appearing. We conclude that there is potential for the use of DSO as a synergist for pyrethroids and that the concept of formulating botanical compounds with synthetic actives may prove beneficial in the control of urban insect pests.

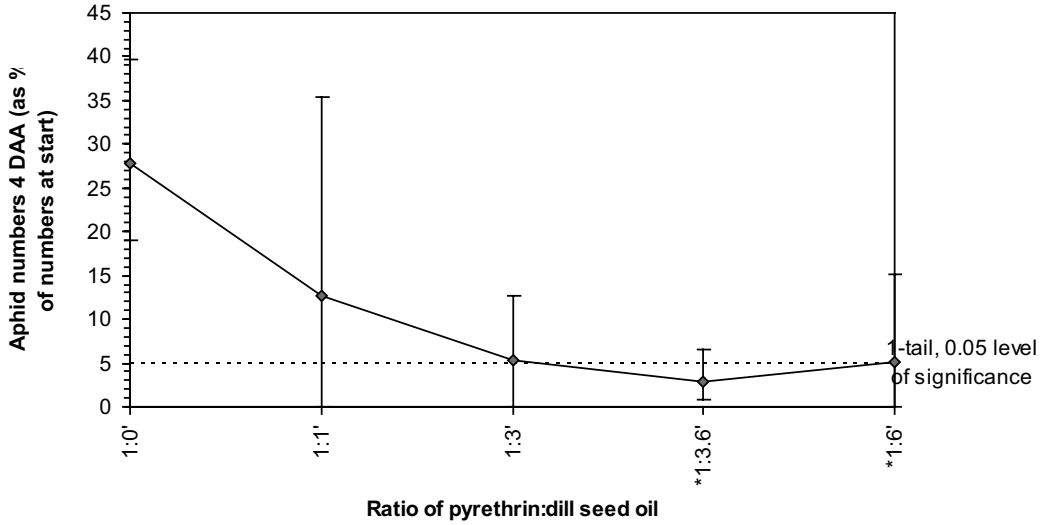

Figure 1. Synergistic Effects Between Pyrethrin and Dill Seed Oil at 1000 g product/ha on the cereal aphid. (CEMAS, 2006).

Table 1. Ten minute knockdown and 24 hour mortality (percentage) for each test sample evaluated against houseflies, cockroaches and pyreyhroid resistant mosquitoes.

	10 minute knockdown			**24 hour mortality**		
Sample	*Periplaneta americana*	*Musca domestica*	*Anopheles stephensi*	*Periplaneta americana*	*Musca domestica*	*Anopheles stephensi*
Pyrethrin	72 %	82 %	12 %	88 %	100 %	22 %
DSO	0 %	2 %	10 %	45 %	60 %	65 %
DSO + Pyrethrin	77 %	90 %	33 %	88 %	100 %	100 %

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