AQUEOUS EXTRACT OF *CODONOPSIS JAVANICA* AGAINST LARVAL AND PUPAL STAGES OF *AEDES ALBOPICTUS*

ESTRATTO ACQUOSO DI *CODONOPSIS JAVANICA* CONTRO LARVE E PUPE DI *AEDES ALBOPICTUS*

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SUMMARY

The fight against adults of *Aedes albopictus* is temporary, unsatisfied, and polluting for the environment, while larval treatment is more localized in time and space, resulting less dangerous. The most common antilarval product used is a crystal protoxin of *Bacillus thuringiensis* var. *israelensis* serum-type 14 produced during the sporulation process and considered harmless for humans, fishes, and other not dipteran insects. Unfortunately the antilarval activity of *B. thuringiensis* is lost after 24 hours, when larvae can develop undisturbed and its activity is completely absent against eggs and pupae.

Acqueous extract of roots of *Codonopsis javanica* Hook. f. & Thoms (Campanulaceae) was tested to develop new natural formulations against larval and pupal stages of *A. albopictus* showing poor or no activity against larvae; on the contrary, statistically significant activity was observed against pupae after 48 hrs with 75% of mortality both at 12.79% and 6.39% concentration of the decoction.

Key words: *Aedes albopictus*, *Codonopsis javanica*, aqueous extract.

RIASSUNTO

Il trattamento delle forme larvali rappresenta il metodo di lotta più efficace contro *Aedes albopictus* perché consente di ottenere risultati migliori nel tempo e conseguenze minori sull'ambiente rispetto alla lotta contro la forma adulta. Una tossina batterica prodotta da *Bacillus thuringiensis* var. *israelensis* sierotipo H-14 è divenuta il prodotto maggiormente utilizzato nel mondo come larvicida perché ritenuto innocuo nei confronti dell'uomo, dei pesci e di altri insetti che non siano ditteri. L'attività antilarvale di questo prodotto, però, si annulla dopo 24 ore, permettendo alle larve appena nate di svilupparsi indisturbate; inoltre, è del tutto inattivo contro uova e pupe.

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Con questa indagine sono stati studiati gli effetti dell'estratto acquoso delle radici di *Codonopsis javanica* (Campanulaceae) contro larve e pupe della zanzara. L'estratto possiede attività scarsa o nulla contro le larve, mentre è significativamente attivo nei confronti delle pupe dopo 48 ore di trattamento con mortalità del 75 % alle diluizioni del 12,79 % e del 6,39%.

Parole chiave: *Aedes albopictus*, *Codonopsis javanica*, estratto acquoso.

INTRODUCTION

Aedes albopictus, known as "tiger mosquito", is an asiatic insect, widespread in all continents and recorded in Italy for the first time in 1990 imported from China (Sabatini et al., 1990).

The "tiger mosquito" is a daytime outdoor ectoparasite, that bites with an unusual aggressiveness and it can be vector of dengue virus, encephalitis virus of men and animals, and of several arbovirus (Boromisa et al., 1987; Mitchell, 1995; Shroyer, 1986). Moreover, it is an intermediate host of filarial species such as *Dirofilaria immitis*, *D. repens*, and *Setaria labiatopapillosa* (Cancrini et al., 1995; Cancrini et al., 2003).

Aedes albopictus can survive during the cold and dried winter in temperate climates in the egg stage through embryonal diapause (Lyon & Berry, 1991), but it is active throughout the year in tropical and subtropical habitats. The females can lay 40-80 eggs after each blood taking and several hundreds of eggs during their life-time. Eggs are laid singly on the sides of water-holding containers such as tires, animal watering dishes, birdbaths, flowerpots, hollows of trees and leaves, impermeable holes of the soil, and can withstand desiccation up to one year; larval emergence occurs when rainfall raises the water level in the containers and several submersions are required before hatching; additionally, low O_2 tension is a more important factor than flooding or temperature on inducing egg hatch. Development is temperature dependent, but the larvae usually pupate after five to ten days and the pupal stage lasts two days (Hawley, 1988). Larvae are active feeders; they feed on fine particulate organic matter in water. Larvae use a breathing siphon to acquire oxygen and, for that reason, must periodically come to water surface. Larvae develop through four instars prior to pupation and, unlike many other insects, pupae are active and short-lived.

Nowadays the mosquito fight is directed against larvae and, only if it necessary, against adults. This is because the fight against adults is temporary, unsatisfied, and polluting for the environment, while larval treatment is more localized in time and space, resulting less dangerous.

Common antilarval products on market are synthetic molecules (temephos, clorpyriphos, diflubenzuron) or natural compounds such as a crystal protoxin of *Bacillus thuringiensis* var. *israelensis* produced during the sporulation process. It results the most used in the world, because it is considered to be harmless for humans, fishes, and other not dipteran insects. Unfortunately the antilarval activity of *B. thuringensis* is lost after 24 hours, when larvae can develop undisturbed and it is completely ineffective against eggs and pupae.

The aim of our work is to develop new natural antimosquitos formulations that result not toxic for men, animals, and environment and also active for all the stages of mosquitos. It's very important to develop always new antimosquitos drugs to supply the farmacoresistance of the nowadays used products.

Among various tested substances the decoction of the root of *Codonospis javanica* (Campanulaceae) has shown good activity. This species, known with the popular vietnamise name "Dang sam", has a widespread distribution in South East Asia and particularly in the region of Vietnam, where the decoction of the roots is used in traditional medicine as tonic and against leukaemia, inflammation and hepatitis (Ueda et al., 2002).

Neither phytochemical studies nor biological activity has been carried out on this plant till now.

MATERIALS AND METHODS

The dried roots of *C. javanica* of commercial use were purchased in Sapa (Vietnam) on May 2004. The voucher specimen (N° 8658) is deposited at Herbarium Horti Botanici Pisani Nuove Acquisizioni, Pisa, Italy.

The powdered dried roots (130.0 g) were heated at 100°C in 1.0 L of water to afford a decoction that was lyophilised to obtain 6.5 g of residue. Tests were performed against larvae and pupae of *A. albopictus*.

Aedes albopictus eggs were captured through ovitraps placed in selected areas where the presence of mosquitos was observed during previous inspections. Masonite strips (3x15 cm) were suspended vertically in black pots filled with 350 ml of water to provide a suitable surface for eggs deposition. Every week, pots were rinsed and filled and strips changed and checked for eggs presence. To obtain larvae and pupae successively the strips were left to dry at room temperature for 3 days, then placed individually into plastic trays containing dechlorinated tap water after which 1^{st} stage larvae were obtained, strips were left to dry again. This alternating wet and dry procedure was repeated twice (Toma et al., 2003).

In each well were placed either 6 larvae at third and early fourth instar or 6 pupae; larvae and pupae showing abnormalities were discarded. In the evaluation of the dilutions it was considered the introduction of one drop of water with each larva or pupa changing the initial dilution. For each well was added an amount of food for the larval survive. Lyophilised decoction of *C. javanica* was tested at various dilutions from 12,79 % to 0,21 %. For each well were added 20 μl of Amphotericin B (Tab. I).

Three controls were performed with deionised water, deionised water plus amphotericin B, and deionised water plus *Bacillus thuringiensis* var *israelensis* 0.25 mg finely grinded for each well respectively. The test was repeated three times for larvae and pupae.

The mobility control of larvae and pupae was performed after 24 and 48 hours of contact with the substances. To verify mortality, immobile larvae and pupae were collected one by one after 48 hours and placed in another well with deionised water without tested substances; persistence of immobility after their stimulation with a needle indicated their death.

Tab. I. Initial and final diluitions.	
Initial Dilution (%)	Final Dilution (%)
15.00	12.79
7.5	6.39
3.75	3.25
1.87	1.63
0.94	0.81
0.47	0.41
0.23	0.21

Statistical analysis

Data were subjected to ANOVA in which extract number, observation time (24 and 48 hours), and stage (pupae or larvae) with all their interactions were considered as fixed effects and percentage dead as the independent variable.

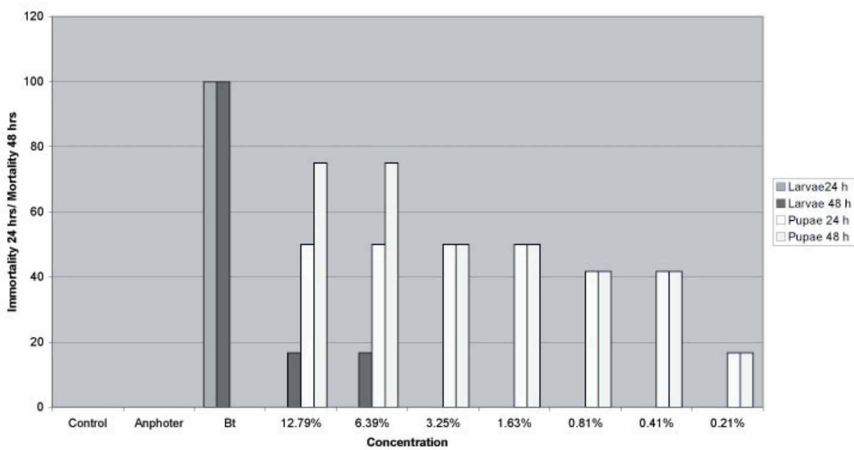
RESULTS AND DISCUSSION

Codonopsis javanica extract showed poor or no activity against larvae at each concentration tested after 24 and 48 hrs (See Tab.I, Fig1). Results against pupae showed 75% of mortality statistically significative after 48 hrs at 12.79% and 6.39% concentration, while the mortality after 24 hrs resulted not statistically significative (Tab. II).

In controls with deionized water only and with amphotericin B were observed no death of larvae and pupae both at 24 and 48 hours. *B. thuringiensis* var *israelensis* activity appeared high against larvae, determining a mortality of 100% but resulted ineffective against pupae (100% mortality of larvae vs. 0% mortality of pupae; $P<0.01$).

The results obtained may be considered interesting, because the pupicidal *C. javanica* rootsdecoction could be used in combination with the larvicidal *B. thuringensis* in the fight against *Aedes albopictus*.

Fig. 1. *Codonopsis javanica*: percentage mortality of larvae and pupae of *Aedes albopictus* exposed to aqueous of Codonopsis javanica at 24 and 48 hrs.



Tab. II. Percentage mortality differences of larvae and pupae of *Aedes albopictus* exposed to aqueous extract of *Codonopsis javanica* at different concentrations for 24 and 48 hrs. Different letters on the same column indicate significant differences; A, B: P<0.01.

	Larvae		Pupae	
	24 hrs	48 hrs	24 hrs	48 hrs
Controls	0 B	0 B	0	0 B
Amphotericin B	0 B	0 B	0	0 B
B. thuringiensis	100 A	100 A	0	0 B
12.79 %	0 B	16,67 B	50	75 A
6.39 %	0 B	16,67 B	50	75 A
3,25 %	0 B	0 B	50	50 B
1,63 %	0 B	0 B	50	50 B
0,81 %	0 B	0 B	41,66	41,66 B
0,41 %	0 B	0 B	41,66	41,66 B
0,21 %	0 B	0 B	16,66	16,66 B

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