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Eco-friendly Control Strategies Against the Asian Tiger Mosquito, *Aedes albopictus* (Diptera: Culicidae): Repellency and Toxic Activity of Plant Essential Oils and Extracts

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Abstract

The economic and health problems related to the Asian tiger mosquito, *Aedes albopictus* (Diptera: Culicidae), are due to its ectoparasitic behaviour and to the transmission of many diseases, particularly arbovirus and parasites. The difficulty to control the larval instars of *A. albopictus*, the reduced effectiveness and high environmental impact of adulticide treatments, highlight that the most effective solution to protect against *A. albopictus* is the use of repellent products for personal use such as DEET (N, N-diethyl-3-methylbenzamide). This compound showed a high repellent power in time but also some disadvantages including toxic effects on humans, especially on children and elderly. On this purpose, natural substances acting as repellent, such as plant essential oils and extracts, are considered very promising. Here we reported a critical review of our researches on repellence exerted by six different essential oils. The most effective essential oil was *C. sativum*. Furthermore, we discussed our research work conducted on ten essential oils tested as alternative larvicidal compounds. Following the dedicated WHO method, we proved that *R. chalepensis* EO was the most effective larvicidal among ten tested essential oils. Moreover, as regards to plant extracts, here we conducted bioassays to evaluate if the methanolic neem cake extract and its fractions of increasing polarity exhibit good mortality rates against *A. albopictus* larvae. We believe that the chance to use natural products such as essential oils and neem cake extracts, effective at lower doses when compared to synthetic products currently marketed, could be an advantageous alternative to build newer and safer mosquito control tools.

Key words- Arbovirus and Filariasis Vector; Plant-borne Molecules; Mosquitocidal Compounds.

Introduction

In tropical countries, mosquitoes are vectors of very dangerous diseases which contribute consistently to poverty and social debility (1). Mosquitoes are important human pests also in Europe, since their bites causes local skin reactions, as well as serious allergic and systemic responses such as angioderma and urticaria (2).

Among Culicidae, *Aedes albopictus*, commonly known as the Asian tiger mosquito, is currently retained the most invasive mosquito species in the world (3), since it is able to rapidly adapt to different anthropogenic environments thanks to its ecological and physiological plasticity (4). Recently, the Asian tiger mosquito has invaded many countries, spreading rapidly to Europe, North and South America, the Caribbean, Africa and the Middle East (5;6). The Asian tiger mosquito is both a nuisance and a disease vector. Its medical importance is mainly due to the aggressive daytime human-biting behaviour (7) and to its ability to transmit many diseases. In fact it serve as a vector for many viruses, including dengue, yellow fever, West Nile, Japanese encephalitis, St. Louis, encephalitis virus (Flaviridae, genus *Flavivirus*); chikungunya, Eastern Equine encephalitis, Venezuelan Equine encephalitis, Western Equine encephalitis, Ross River, Sindbis, Mayaro, Getah (Togaviridae, genus *Alphavirus*); Potosi, San Angelo, La Crosse, Jamestown Canyon (Bunyaviridae, genus *Bunyavirus*); Rift Valley fever (Bunyaviridae, genus *Phlebovirus*) and Orungo virus (Reoviridae, genus *Orbivirus*) (3; 5). *A. albopictus* is also the vector of different filariasis, such as *Dirofilaria immitis* Leidy, *Dirofilaria repens* Railliet & Henry and *Setaria labiatopapillosa* Perroncito (5).

Since there are no vaccines or drugs against some of the main pathogens and parasites transmitted by *A. albopictus*, the vector control remains the crucial tool for the prevention of these problems. Against adult mosquitoes, the application of repellents on the human skin is one of the oldest and commonest tools for bite's protection (8). Among repellent molecules, the efficacy of DEET (N,N-diethyl-3-methylbenzamide) in providing a long-lasting protection against many mosquito species has been documented in several studies (9). However, DEET is neurotoxic, irritating for mucous membranes and it was well known that, in some cases, concentrated formulations dissolve plastic. Moreover, toxic effects have been reported although infrequently and generally associated with over application of this product (9; 10).

One way to reduce the *A. albopictus* populations is targeting larvae with organophosphates and insect growth regulators (e.g. diflubenzuron and methoprene) (11; 12). Among the most commonly used organophosphates adulticides or larvicides, populations of *A. albopictus* from Singapore and Vietnam showed resistance to malathion, thoose from Malaysia to fenthion, and those from Madagascar to fenitrothion (13). Strains of *A. albopictus* from Texas showed resistance to malathion and tolerance to bendiocarb and resmethrin (11). Moreover, treatments with *Bacillus thuringiensis* (var. *israeliensis*) can be a solution. However, due to peculiar reproductive traits of *A. albopictus*, *B. thuringiensis* (var. *israeliensis*) is not suitable against this pest (14). Biological control strategies based on the release of larvivorous organisms require further research (15). For these reasons, there is a worldwide need to find functional alternatives concerning *A. albopictus* control strategies.

Recently, great efforts have been carried out in order to investigate the effectiveness of plant-borne compounds (e.g. essential oils and plant extracts) against a wide range of arthropod pests, including tephritid pests (16; 17), foodstuff beetles (18) and parasites of medical and veterinary importance (19), including mosquitoes (20; 21; 22).

This paper offers a review about the research activity carried out by our group on bioactivity of natural compounds against the Asian tiger mosquito. In this researches, (*i*) the repellent properties against *A. albopictus* adults were compared among six different essential oils extracted from wild or cultivated aromatic plants; (*ii*) the toxicity on larval instars were compared among ten plant essential oils; (*iii*) as regards to plant extracts, larvicidal toxicity of neem cake methanol extract and fractions of increasing polarity was firstly assessed against *A. albopictus* larvae.

Effectiveness of six plant essential oils as repellent against *Aedes albopictus* adults

Among natural compounds, plant essential oils are reported as toxic against Culicidae, acting as adulticidal (23), larvicidal (24; 25), ovicidal (26), oviposition deterrents, growth and/or reproduction inhibitors (27) and/or adult repellents (28). Here, we review the repellent properties against *A. albopictus* of six essential oils extracted from wild or cultivated aromatic plants of *Hyptis suaveolens* Poit., *Salvia dorisiana* Standl., *S. longifolia* Nutt., *S. sclarea* L. (Lamiaceae), *Ruta chalepensis* L. (Rutaceae) and *Coriandrum sativum* L. (Apiaceae). The most effective essential oil to repel *A. albopictus* adults was *C. sativum* (29) (Fig-1). This oil was also effective as DEET tested at the same dosages (B. Conti, unpublished data). The chemical composition of *C. sativum* EO was investigated by GC-EIMS analysis. Coriander EO was mainly composed by monoterpene hydrocarbons and oxygenated monoterpenes, with linalool (83.6%) as the major constituent (29). Repellence bioassays highlighted that *C. sativum* EO was an excellent repellent against *A. albopictus*, also at lower dosages. Indeed, RD_{50} was 0.0001565 µL/cm^2 of skin, while RD_{90} was 0.002004 µL/cm^2. At the highest dosage (0.2 µL/cm^2 of skin) the protection time achieved with *C. sativum* essential oil was about 60 min (29).

Effectiveness of ten plant essential oils against *Aedes albopictus* larvae

Here, we reviewed the biotoxicity of essential oils from *Achillea millefolium* L., *Helichrysum italicum* (Roth) Don (Asteraceae), *C. sativum*, *Foeniculum vulgare* Mill. (Apiaceae), *H. suaveolens*, *Lavandula angustifolia* Mill., *Rosmarinus officinalis* L. (Lamiaceae), *Melaleuca alternifolia* (Maiden & Betche) Cheel, *Myrtus communis* L. (Myrtaceae), and *R. chalepensis* against fourth instar larvae of the Asian tiger mosquito.

We pointed out that *R. chalepensis* essential oil was the most effective as larvicidal among ten tested essential oils (Fig-2). GC and GC-MS analyses of essential oils from cultivated and wild *R. chalepensis* plants showed only quantitative differences, in particular relatively to the amounts of ketones derivatives, while the qualitative profile evidenced a similar chemical composition (30). Both essential oils from wild and cultivated *R. chalepensis* plants were able to exert a very good toxic activity against *A. albopictus* larvae (wild plants, LC_{50} = 35.66 ppm; cultivated plants: LC_{50} = 33.18 ppm), and mortality was dosage dependent (30).

Larvicidal efficacy of neem cake extracts against *Aedes albopictus* larvae

Azadirachta indica (Meliaceae), commonly known as neem tree, is a fast growing evergreen tree native of India (32). Its seeds contain about one hundred biologically active compounds. Major constituents are azadirachtin, nimbin, nimbidin and nimbolides. Many formulations deriving from neem's seeds show antifeedancy, fecundity suppression, ovicidal and larvicidal activity besides insect growth regulation and repellence against insects, also at very low dosages (33; 34). Furthermore, neem-borne products rarely induce resistance since their multiple mode of action against insects. Moreover, insect growth regulating activity of neem-borne molecules weakens the cuticle defence system of the young instars causing easy penetration of pathogenic organisms. Only low toxicity rates have been detected against vertebrates. Overall, the insecticidal properties, environmental safety and public acceptability of neem and its products for control of insect pests has led to its adoption into several Diptera control programs (35; 36; 37). Emulsified formulations of *A. indica* oil showed an excellent larvicidal potential against different mosquito genera, including *Aedes, Anopheles* and *Culex*, also under field conditions (33). However, the commercial success of neem-borne compounds has been limited by the relatively high cost of refined products and the low persistence on treated surfaces exposed to sunlight (38). Recently, several attempts were carried out to evaluate the biotoxicity of neem cake extracts against mosquitoes (39; 40), allowing the chance to exploit this low-cost by-product of neem oil production in eco-friendly Culicidae control strategies.

Here, the larvicidal efficacy of neem cake methanol extract was assessed against *A. albopictus* larvae. Furthermore, we tested the larvicidal toxicity of the following extract fractions of increasing polarity: hexane, ethyl acetate, *n*-butanol and aqueous fractions.

Methods and Methods

Larvae of *A. albopictus* originated from field-collected eggs, deposited by wild females on bars of masonite placed outdoors in dark vases containing water. Eggs batches were daily collected and kept moist for 24 hours. Then they were placed in laboratory conditions (24 ± 1°C; 50 ± 5% R.H.; natural photoperiod) in 250 mL beakers and submerged in mineral water for hatching (29). Ten first-instar larvae were isolated in 250 mL beakers and exposed for 20 days to 50 and 100 ppm of neem cake methanol extract. Furthermore, ten first-instar larvae were isolated in 250 mL beakers and exposed for 20 days to 100 ppm of the following neem cake extract fractions of increasing polarity: hexane, ethyl acetate, *n*-butanol and aqueous fractions. All fractions were obtained following the method described by Nicoletti et al. (39).

Each tested product was dissolved in mineral water containing 0.1% of Tween® 80. Mineral water with 0.1% of Tween® 80 was used as control. Mortality and developmental stage of each tested larva were checked daily. Each two days, a small amount of cat food was given to the larvae. Larval mortality was reported as an average of four replicates, with the only exceptions of aqueous fraction, in which two replicates were conducted, and control, in which five replicates were *carried out*.

Both larval mortality and developmental stage data were transformed into arcsine√proportion values, before statistical analysis. Data were processed with JMP®, using a General Linear Model (GLM) (JMP® SAS, 1999) with two factors, the tested compound and the exposure time: $y_j = \mu + C_j + T_j + C_j{*}T_j + e_j$, in which y_j is the observation, μ is the overall mean, C_j the compound (j = 1-7), T_j the exposure time (j = 1-4 for mortality rates; 1-5 for developmental stages), $C_j{*}T_j$ the interaction compound*exposure time and e_j the residual error. Averages were separated by Tukey-Kramer HSD test. Only probability level $P < 0.05$ was used for the significance of differences between means.

Results and Discussion

In *A. albopictus* larval toxicity trials, significant effects of tested compound (*F* = 29.998; *d.f.* = 6; *P* < 0.001), exposure time (*F* = 98.054; *d.f.* = 3; *P* < 0.001) and interaction compound*exposure time (*F* = 1.196; *d.f.* = 18; *P* = 0.285) were detected (Fig-3).

Our results confirm previous evidences about the larvicidal activity of neem cake against both the Asian tiger mosquito (39; 40) and *Culex quinquefasciatus* Say. Concerning this latter mosquito, it was observed that neem cake powder applied in rice fields at the dose of 500 kg/ha, either alone or coated over urea, was able to exert a striking reduction in the abundance of late instars larvae and pupae (41). However, after ten days, we found that larval mortality rates did not strongly differ among the tested neem cake extracts, while other researches reported that the ethyl acetate fraction of neem cake extract exhibited the most relevant larvicide effect (39). We suppose that these differences could be due to discrepancies in method of bioassays. Indeed, in our trials, *A. albopictus* larvae were not starved.

We hypothesized that the bioactivity of neem cake extracts could be mainly related to salannin and/or limonoid contents, even if also minor constituents could play a pivotal role synergizing the insecticidal effect of major molecules. The huge quantity of different molecules allows the chance of increasing the presence of selected constituents by chemical treatment (40). Furthermore, we observed that the treatment with neem cake fractions did not slow down the development of *A. albopictus* larvae (Fig-4). Indeed, the mean duration of *A. albopictus* young larval instars development was affected by the tested compound (*F* = 3.000; *d.f.* = 6; *P* = 0.009) and the exposure time (*F* = 284.046; *d.f.* = 4; *P* < 0.001), while it is not influenced by the interaction compound*time (*F* = 2.212; *d.f.* = 24; *P* = 0.003).

Conclusions

We believe that the tested plant essential oils and extracts can be considered very promising against insects of medical and veterinary importance such as mosquitoes. As regards to their repellent properties, we highlighted that the essential oil from *C. sativum* plants was the most effective among the tested oils. Concerning larvicidal tools, we proved the high efficacy of *R. chalepensis* essential oil, recognized the most effective larvicidal among ten tested oils. As regards to plant extracts, larvicidal efficacy of neem cake methanol extract and its fractions of increasing polarity was demonstrated against *A. albopictus* larvae.

Overall, the chance to use natural products such as essential oils and neem cake extracts, effective at lower doses when compared to synthetic products currently marketed, appears very promisingly and we believe that it could be an advantageous alternative to build newer and safer mosquito control tools.

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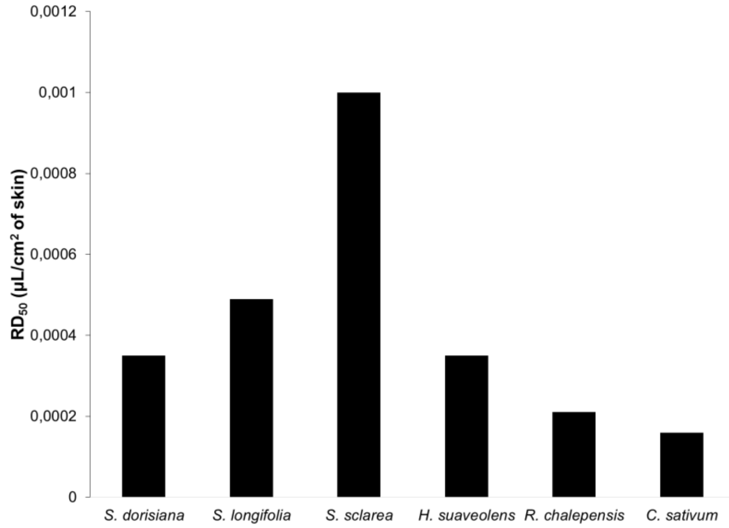

Fig-1 Repellent properties against *Aedes albopictus* adults of six essential oils extracted from wild or cultivated plants of *Hyptis suaveolens*, *Salvia dorisiana*, *S. longifolia*, *S. sclarea*, *Ruta chalepensis* and *Coriandrum sativum*. RD_{50} = dose that repelled 50% of *A. albopictus* adults (modified from 21; 22; 29; 30).

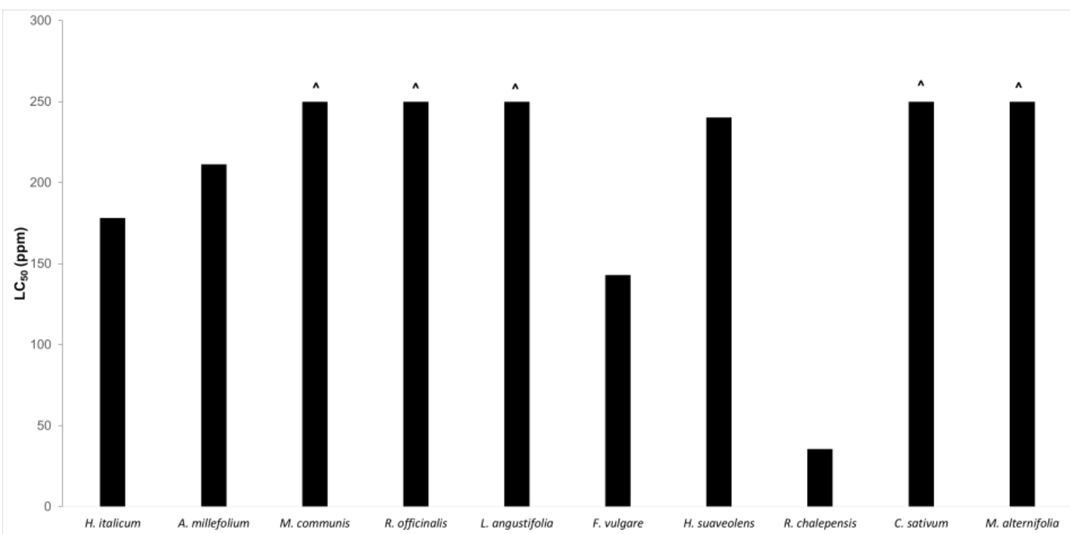

Fig-2 Larvicidal properties against *Aedes albopictus* larvae of ten essential oils extracted from *Helichrysum italicum*, *Achillea millefolium*, *Myrtus communis*, *Rosmarinus officinalis Lavandula angustifolia*, *Foeniculum vulgare*, *Hyptis suaveolens*, wild *Ruta chalepensis, Coriandrum sativum* and *Melaleuca alternifolia*. LC_{50} = dose that killed 50% of *A. albopictus* fourth instar larvae. ^ = $DL_{50} \geq 250$ ppm (modified from 21; 29; 30; 31; Benelli et al., unpublished data).

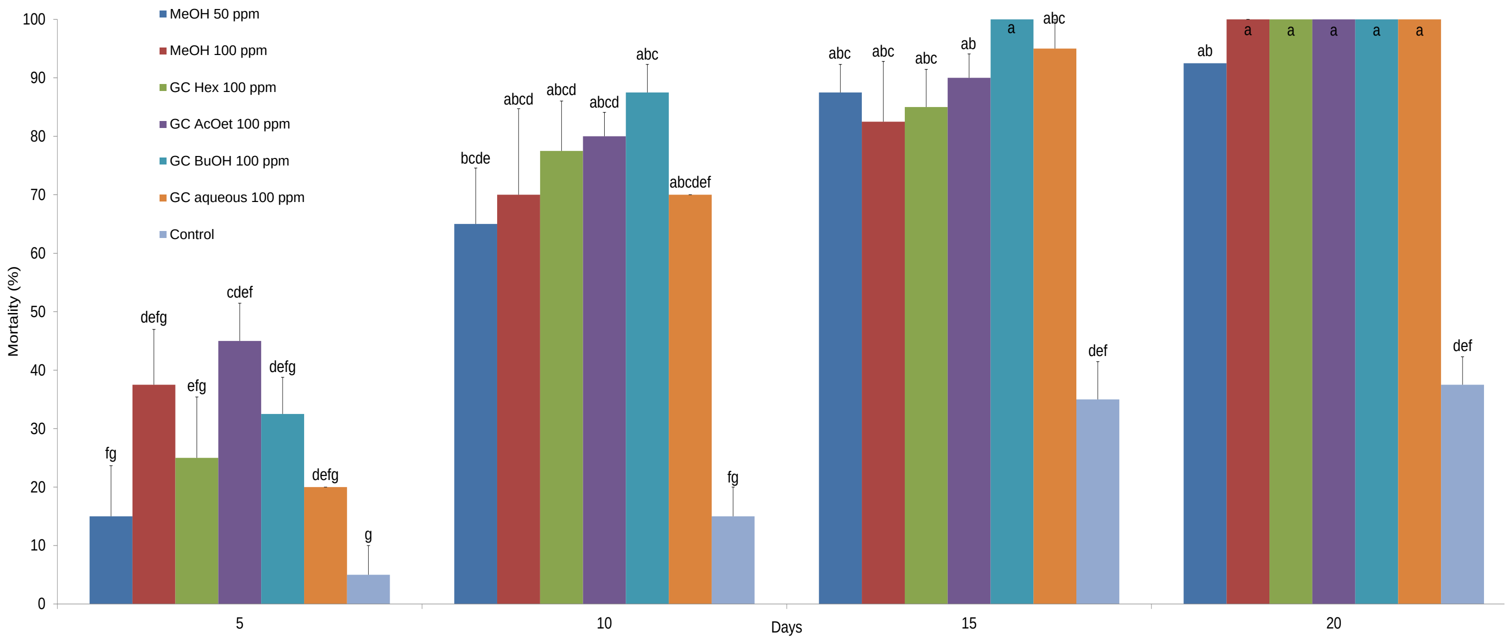

Fig-3 Biotoxicity of neem cake methanol extract and fractions of increasing polarity against *A. albopictus* larvae over time. MeOH = neem cake methanol extract; GC hex = hexane fraction; GC AcOet = ethyl acetate fraction; GC BuOH = *n*-butanol fraction; GC aqueous = aqueous fraction. T-bars indicated standard errors. Different letters above each bar indicated significant differences among treatments at P = 0.05 (General Linear Model followed by Tukey HSD Test).

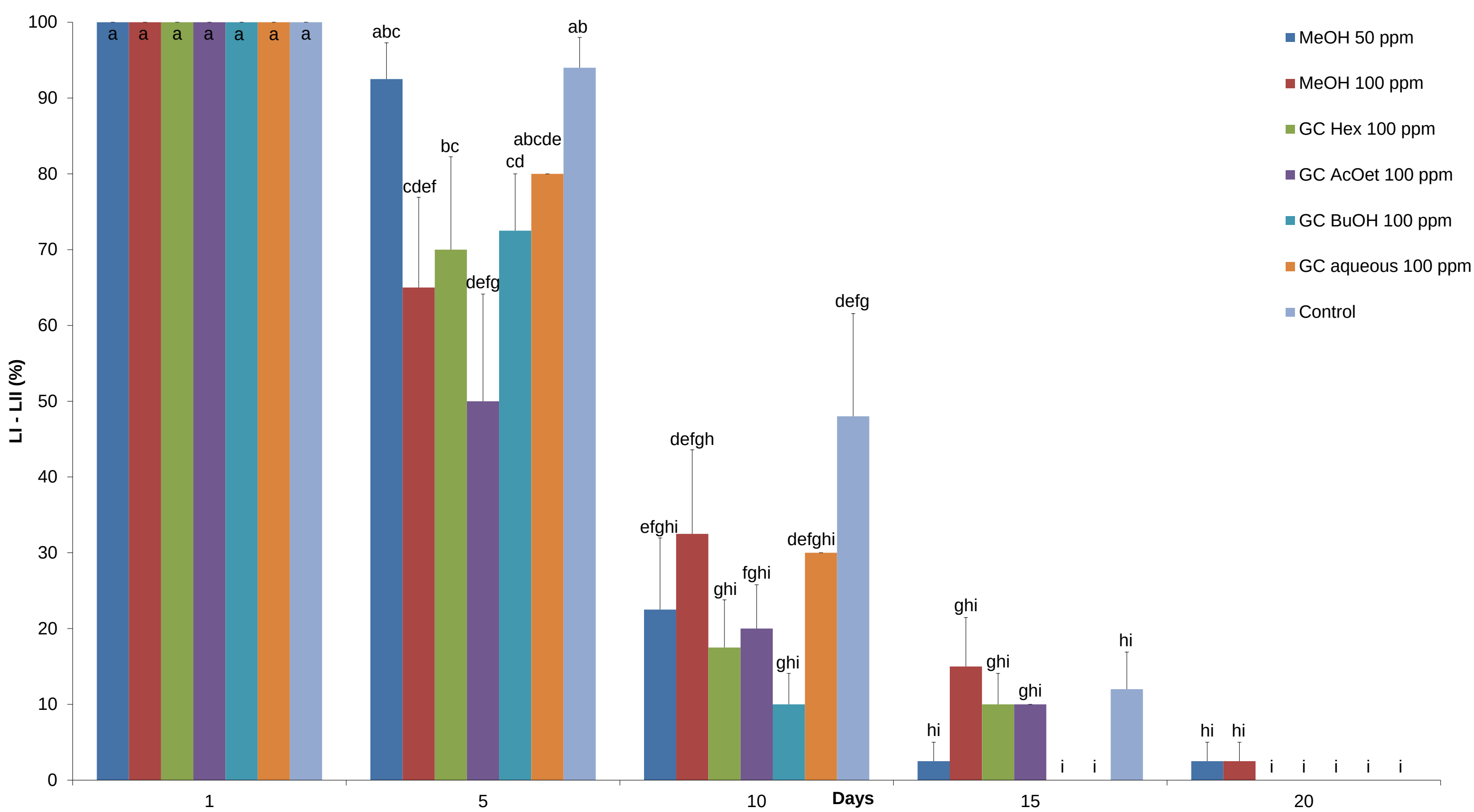

Fig-4 Effect of neem cake methanol extract and fractions of increasing polarity on developmental time of *A. albopictus larvae* after 5, 10, 15 and 20 days. LI – LII (%) = mean percentages of first and second instar *A. albopictus* larvae. MeOH = neem cake methanol extract; GC hex = hexane fraction; GC AcOet = ethyl acetate fraction; GC BuOH = *n*-butanol fraction; GC aqueous = aqueous fraction. T-bars indicated standard errors. Different letters above each bar indicated significant differences among treatments at P = 0.05 (General Linear Model followed by Tukey HSD Test).