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ADULTICIDAL ACTION OF TEN CITRUS OILS AGAINST *AEDES ALBOPICTUS* (DIPTERA: CULICIDAE)

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The present study was carried out to evaluate the adulticidal action of ten citrus oils against *Aedes albopictus* through exposure tube method. The results showed that toxic effects of citrus oils varied with time and concentration. Jaffa (*C. sinensis*) oil was the most lethal (LC_{50} = 53.61, 11.07 and 3.41%) at all recorded times (6, 12 and 24 respectively) and concentrations (5, 10, 15 and 20%) with LT_{50} (18.70, 14.08, 10.42 and 6.59% respectively, followed by Carrizo citrange. Whereas Minneola oil (root stock) was the least effective after 6 hrs however significant change in mortality was observed after 12 and 24 hrs. Gada dehi was moderately effective after 6 hrs with its performance deteriorating at 12 and 24 hrs time intervals. Our experiments have proved that citrus oils have the potential for controlling insects. Therefore, these can be used as best alternative for controlling adults of *Ae. albopictus*.
Keywords: *Aedes albopictus*, probit analysis, citrus oils and lethal action

INTRODUCTION

Botanical products or plant derivatives serve as suitable replacement to chemical insecticides as these are safe and readily degraded in the environment (Roy and Saraf, 2006). These plant products are in use for the last two centuries and have thus minimized all the undue risks (resistance, persistence and hazards etc.) arising from chemical insecticides (Isman, 2006). Many plant families (Rutaceae, Labiatae, Asteraceae, Myrtaceae, Piperaceae, Meliaceae etc.) are in use for exploiting potential components from them against agricultural and household insects (Regnault-Roger, 1997). Family Rutaceae has been the most important in terms of its insecticidal qualities. *Citrus* a member of the said family has recently attracted noticeable interest because of its insecticidal prospects. As its oils are parts and parcel of many volatile and non-volatile compounds (Morales-Saldana *et al*., 2007). *Citrus reticulata* seeds in ethanolic extracts results in good control against larvae of *Ae. aegypti* and *Culex quinquefasciatus* (Sumroiphon *et al*., 2006). Peels of lemon oil exhibit excellent potential against test larvae (Oshaghi *et al*., 2003). In China, *Citrus sinensis* was tested as the most toxic fumigant oil among all five whereas GC-MS showed that out of 51 components identified, citral was the main contributor of lethality, causing adulticidal action in a very short time period (Yang *et al*., 2005). In 2006, adulticidal tests were performed in Thailand (Chaiyasit *et al*., 2006; Choochote *et al*., 2006) with piperaceae, umbelliferae, zingiberaceae and illiciaceae. They found that all plants have certain insecticidal value that is dose dependent.

Ae. albopictus, a well known vector of dengue, that has acquired resistance to synthetic insecticides. This scenario demands the search for an alternative that is easily biodegradable and is more target-specific. Natural products from plant origin are being tested against vectors since old ages. These products act as adulticides. In modern times, interest is focused on citrus oils as mosquitocides which thus forms the basis of present studies.

MATERIALS AND METHODS

Collection of citrus fruit: Citrus varieties viz; Hinckley (*Citrus sinensis*), Cassa grande (*C. sinensis*), Fairchild (*C. reticulata*), Sacaton citrumelo (*C. sinensis* × *Poncirus trifoliata*), Minneola (*C. reticulata* × *C. paradisi*), Gada dehi (*C. aurantium*), Carrizo citrange (*C. sinensis* × *P. trifoliata*), Jaffa orange (*C. sinensis*), Chinese lemon (*C. limon* × *C. aurantifolia*) and Citrumelo 1452 (*C. paradisi* × *P. trifoliata*), were collected at the ripening stage from Horticulture Research Area, University of Agriculture, Faisalabad.

Solvent extraction: The seeds of each variety were separated manually with knife. The seeds were rinsed in tap-water to remove the fibrous material and then, oven dried at 60°C for 48 hours. The dried seeds were powdered in an Anex Grinder and stored in capped

petri dishes. Each powdered material was put in thimble that was kept in extraction tube of Soxhlet apparatus (Bagavan *et al*., 2008). Ethanol was used as a solvent (250 ml/20g sample). The extraction time for one sample ranged four to five hours due to interrupted power supply, and replacement of solvent every time for accuracy. During normal procedure, solvent was evaporated at room temperature, leaving oil that was collected and centrifuged at 10,000 rpm for 5 minutes and supernatant was collected.

Rearing of Mosquito: Population of *Ae. albopictus* was maintained in the mosquito-rearing laboratory of the Department of Agricultural Entomology, University of Agriculture, Faisalabad. The culture was maintained at 25°C, with a relative humidity of 70-80% at L/D 12:12 hour photoperiod. Adults were kept in mesh and plastic culture cages with easy access to 10% sucrose through a cotton wick. Larvae were reared in a separate chamber (27°C, 75% RH and 12:12 L:D) in distilled water filled plastic trays (20 cm x 30 cm x 10 cm). Larvae were kept in groups of 400 per tray with free access to fish food (Min. Crude Protein 45.0%, Min. Crude Fat 5.0%, Min. Phosphorus 1.3%, Max. Crude fiber 2.0%, Max Moisture 6.0%, Min Vitamin C 183 mg/kg; Tetra, made in Germany). Pupae were collected daily in 10 ml containers and transferred to the adult cages. The female mosquitoes were fed on blood of live white rats and rarely chicks twice a week (Barnard and Xue, 2004; Shaalan *et al*., 2006). Gravid and unfed females were sorted out and kept separately for egg laying in petri dishes in the rearing boxes.

Adulticide assay: For adulticide test, the exposure tube method was used (Chaiyasit *et al*., 2006). Five tubes were used in each replication, including four tubes with different concentrations (5, 10, 15 and 20%) of extracts from seed oil, and one for control. Stock solution was prepared separately by dissolving 300, 600, 900 and 1200 µl oil in 6 ml acetone for 5, 10, 15 and 20% respectively. In each tube, 20 mosquitoes were introduced for tests. After exposure of five minutes, the mosquitoes were transferred to holding tubes and provided 10 % sucrose solution. LC_{50} was recorded after 6, 12 and 24 hrs and LT_{50} was observed after 1, 6, 12 and 24 hrs. The bioassay was repeated four times.

RESULTS AND DISCUSSION

Results showed that (Fig.1) Jaffa oil exhibited excellent effectiveness having LC_{50} after 6 hrs (53.61%), 12 hrs (11.07%) and 24 hrs (3.41%) followed by Sacaton citrumelo with LC_{50} (65.24%) after 6 hrs against *Ae. albopictus*. But this order of citrus oil effectiveness varied after 12 hrs and 24 hrs. However, Minneola oil was least effective with LC_{50} after 6 hrs as 146.22%.

Fig.2 reveals time mortality (LT_{50}) graph of *Ae. albopictus* to kill 50% of test mosquitoes. The data displayed superiority of Jaffa oil amongst the remaining citrus oils at all recorded concentrations (5, 10, 15 and 20%). Jaffa oil took remarkably less time at 20% (6.59 hrs) and this time interval increased with decrease in concentrations (Fig.2). Carrizo citrange was the 2nd most effective in killing 50% of adult *Ae. albopictus*. However, Gada dehi and Fairchild took more time to kill 50% adults, showing their least potential in terms of time duration.

Percent mortalities of oils recorded against *Ae. albopictus*, at 1, 6, 12 and 24 hrs (Fig.3) showed that effectiveness of each oils was directly proportional to concentrations and time durations. Jaffa oil exhibited its effectiveness by killing 4-5 days old adults of *Ae. albopictus* in less time and their speed of mortality increased with the increase in concentrations, followed by Carrizo citrange. However, after 1 hr same pattern of mortality was observed at all concentrations (5, 10, 15 and 20%). This pattern changed after 6, 12 and 24 hrs time intervals. Above all, Fairchild and Gada dehi was least effective, showing less mortality after 6 hrs whereas after 12 and 24 hrs Gada dehi again showed a gradual decrease in effectiveness as compared to Fairchild.

Results clearly demonstrate that comparatively Jaffa oil was the most effective in reducing / suppressing the adults of *Ae. albopictus* followed by Carrizo citrange. The variation in potential might be due to method of extraction, solvent used in extraction, age of plant and harvesting time of fruit (Shaalan *et al*., 2005). The ethanolic extracts of *Citrus reticulata* exhibited more potential than liquor and water (Sumriophon *et al*., 2006). Rutaceae oil (*Citrus sinensis*) was comparatively more effective against adults of *Culex quinquifasciatus* when Yang *et al*. (2006) while working in China and dealing with 5 different oils, found that *Citrus sinensis* oil was the best. It must contain some compound that had significant insecticidal prospects. Upon GC/MS, it was confirmed that citral was main compound and has knock down properties against adults. The same kind of work done in Thailand (Chaiyasit *et al*., 2006; Choochote *et al*., 2006) in two different labs focused on plant oils and found that plant oils can replace synthetic products like DEET. These results are inline with those of Amonkar and Reeves (1970) who tested garlic derivatives against *Aedes*

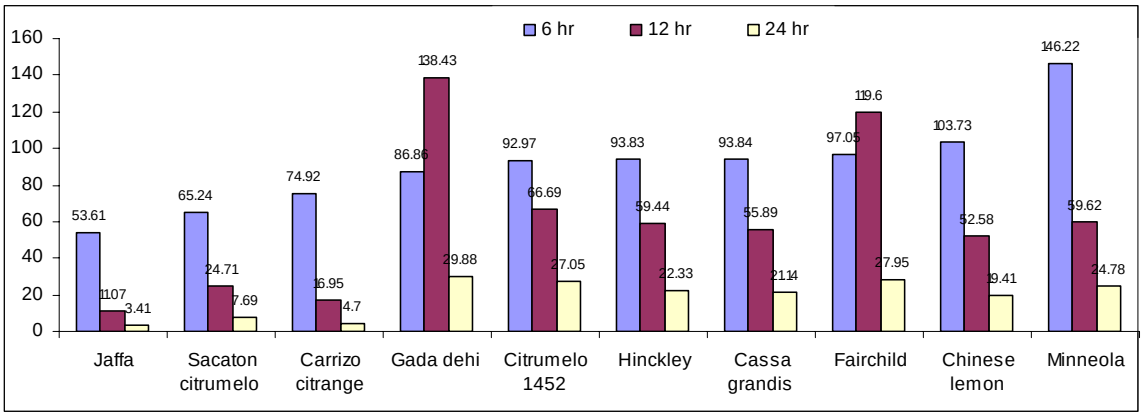

Figure 1: LC_{50} of ten citrus oils against *Ae. albopictus* at 6, 12 and 24 hrs

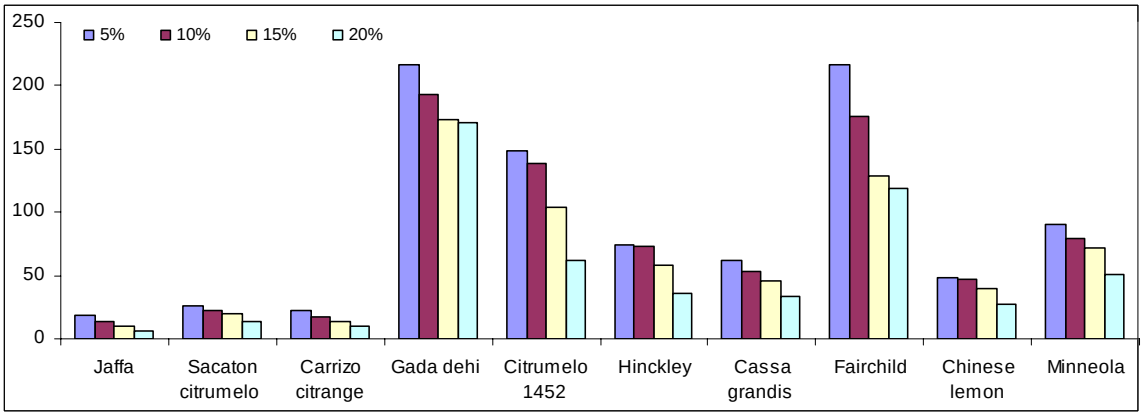

Figure 2: LT_{50} of ten citrus oils against *Ae. albopictus* at 6, 12 and 24 hrs

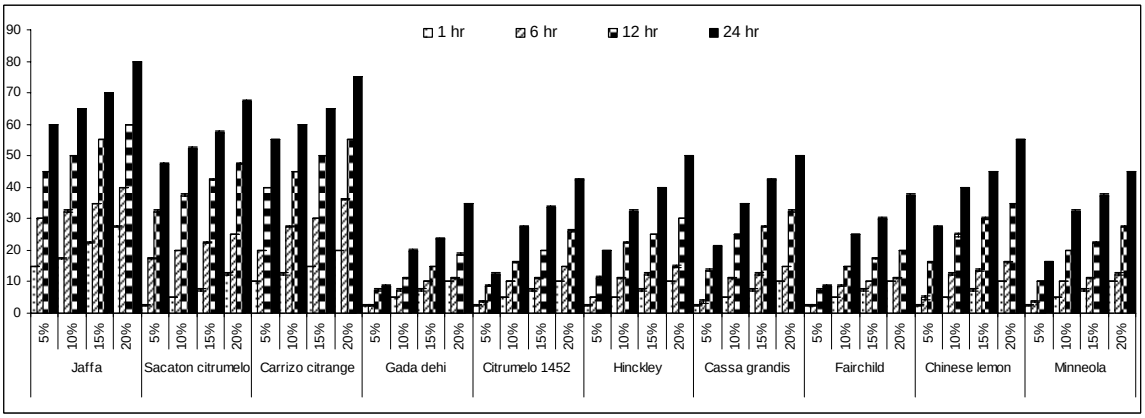

Figure 3: Percentage mortality of ten citrus oils against *Ae. albopictus* at 1, 6, 12 and 24 hrs

nigromaculis and found them highly susceptible to garlic and resistant to synthetic insecticides. Thomas and Callaghan (1999) also found the same results from garlic. Hence, plant based products not only avoid problems associated due to synthetic chemicals but also offer many benefits (Shaalan *et al*., 2005).

It was concluded that essential oils from citrus varieties possess a great potential whereas Jaffa oil had excellent potential to kill adults of *Ae. albopictus*. These oils further, need to be analyzed through more sophisticated technique to know the bio-active chemicals.

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