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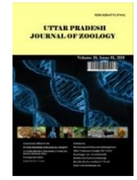

REPELLENCY EFFECT OF SOME PLANT EXTRACTS AGAINST THE DENGUE VECTOR *Aedes aegypti* LINNAEUS 1762 (DIPTERA: CULICIDAE) WITH REFERENCE TO MODE OF ACTION AND RELATED FACTORS

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors SA and GM managed the analyses of the study. Authors SS and ST managed the literature searches. Author ST performed the statistical analysis and also edited the final draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The repellent activity of *Annona squamosa*, *Ocimum basilicum* and *Piper nigrum* with *Azadirachta indica* as a standard was determined against adult *Aedes aegypti* mosquitoes. Bioassay was conducted on laboratory reared Swiss albino mice by topical application at a fixed concentration of 0.01%. Crude aqueous aerial extracts of *Annona squamosa*, *Ocimum basilicum*, *Piper nigrum* and *Azadirachta indica* repelled the test mosquitoes. No mosquito repellence was observed in the control, and all mosquitoes took feed. The number of mosquitoes repelled, landed and fed on the control and crude aerial extracts was statistically and significantly different (P=.05). The protection time offered by the tested aerial extracts against *Aedes aegypti* were 150, 210, 240 and 300 minutes for *Annona squamosa*, *Ocimum basilicum*, *Piper nigrum* and *Azadirachta indica* respectively. The maximum percent protection offered by the aerial extracts was 64, 76, 88 and 92 respectively, and its respective repellent quotient values obtained on treatment against *Aedes aegypti* were 0.6, 0.7, 0.8 and 0.9. The efficacy of the phytoextracts when tried on topical application depended upon significant factors like absorption, adsorption and evaporation, which might have certainly played a crucial role in the persistence of the phytoextract repellent. Further, extraction of bioactive compounds, and evaluation of the same with the above mentioned key factors would aid in the development and advancement of suitable phytorepellents.

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Keywords: *Aedes aegypti*; aerial extracts; repellent activity; repellent quotient.

1. INTRODUCTION

Avoidance of man mosquito contact is obligatory for fortification from mosquito-borne diseases and utilization of repellents have been advocated for the same. Consistent and indiscriminate utilization of chemical repellents have caused hostile effects on the user. Subsequently, there has been a change in outlook towards botanicals to conquer the issues related with the utilization of chemical and synthetic repellents. Compared to chemical compounds, natural products are dared to be more secure for human use [1,2]. Plants are a magnificent hotspot for mosquito repellent specialists as they establish a rich wellspring of bioactive phytochemicals [3], and their repellent properties are notable before the approach of synthetic chemicals [4]. Plant derived repellents as a rule do not present perils of harmfulness to humans and domestic animals and are easily biograded. Plant products have been utilized traditionally to repel or kill mosquitoes in many parts of the world [5], either as a fumigant or topical applicant [6-8]. Many plant species have been tested for their repellent property by topical application against mosquitoes [7,9-15]. Hence, in the current investigation, the repellent activity of crude aerial extracts of plants by topical application was evaluated against *Aedes aegypti* mosquitoes, the dengue vector.

2. MATERIALS AND METHODS

2.1 Preparation of Aerial Extract

The aerial parts of plants, *viz*., *Annona squamosa*, *Azadirachta indica*, *Ocimum basilicum* and *Piper nigrum* collected from Nagercoil, Kanyakumari district, Tamil Nadu, India were taxonomical identified and affirmed at the Department of Botany and Research Centre, Scott Christian College, Nagercoil, Tamil Nadu, India. In the laboratory, dechlorinated water was used to wash the aerial parts of each plant, and thereafter shade dried and powdered with an electric blender. Powdered aerial part (1 kg) of each plant was exposed to extraction utilizing 3 L of distilled water in a Soxhlet apparatus to acquire the crude aqueous extract of each plant [16], which was stored in air tight sterilized amber coloured bottles at 4°C for bioassay.

2.2 Repellent Bioassay

The tests were conveyed in the Vector Biology and Control Laboratory, Department of Zoology, Thiruvalluvar University, Vellore, Tamil Nadu, India against laboratory raised *Aedes aegypti* mosquitoes free of exposure to insecticides. Repellent bioassays were conducted following the technique of WHO with minor modifications [17]. Experiments were directed on laboratory reared Swiss albino mice, from 06.00 to 18.00 hours concurring with the natural feeding time of *Aedes aegypti*. Prior to the inception of the trials, healthy mice of almost equivalent size were chosen for the experimental study. During tests, the mice were each held in a mice holder that were totally covered and made inaccessible to the mosquitoes except for the tail region. The desired quantity of each plant extract (0.01%) was applied evenly on the tail. After application, the tail was taped to a wooden strip measuring 15.0 cm in length and 4.0cm in width at the base and tip to immobilize the tail. The mice holder along with the mice was kept in one feet (1.0x1.0x1.0) mosquito cage. Twenty-five healthy 24 hours starved female *Aedes aegypti* mosquitoes were released into the one feet cage. Continuous observation was carried until the first mosquito bite. Thereafter, observation was made on an hourly basis and mosquitoes that have fed were removed. A landing was described as when a mosquito landed on the test animal for at least two seconds without biting. The term bite alluded to a mosquito penetrating skin with its mouthparts and sucking blood, with resulting abdomen swelling and colour change. Hourly air temperature and relative humidity was recorded during the laboratory bioassay. Five trials were carried for assessment. The aqueous aerial extract of *Azadirachta indica* served as a standard, and the mice, which did not receive any treatment, was taken as control, and were run simultaneously. Percent protection and Repellent Quotient (RQ) [18] were calculated using the following formula.

$$\text{Percent protection} = \frac{\text{NC} - \text{NT}}{\text{NC}} \text{X } 100$$

Where

NC: Number of fed mosquitoes in control
NT: Number of fed mosquitoes in treated

$$\text{Repellent Quotient (RQ)} = \frac{\text{NR} - \text{NUR}}{\text{NR} + \text{NUR}}$$

Where

NR: Number of mosquitoes repelled
NUR: Number of mosquitoes unrepelled
Repellent quotient returns a value of 1 for complete repellency, and 0 for no effect.

Statistical significance differences between the means of the test and control group were evaluated using

Student's 't' test with the *P* values for significance set at 0.05 level.

3. RESULTS

No mosquito repellence was observed in the control, and all mosquitoes took feed. The temperature and relative humidity ranged from 22-23°C and 80-89% respectively. The total number of mosquitoes tested, repelled, landed and fed for aerial extracts and control are presented in Fig. 1. Repellency of the tested aerial extracts against *Aedes aegypti* mosquitoes was portrayed as both landings and bites, besides repellence. The mean number of mosquitoes repelled, landed and fed on topical application of the aerial extracts are presented in Table 1. The average repellency, measured in landings was relatively higher than in bites, indicating that the mosquitoes sometimes landed but did not necessarily feed, yet the impact of the aerial extracts was sufficient to prevent feeding. The mean number of mosquitoes repelled, landed and fed on the control and crude aerial extract was statistically and significantly different (*P*=.05). The protection time offered by the tested aerial extracts against *Aedes aegypti* were 150, 210, 240 and 300 minutes for *Annona squamosa*, *Ocimum basilicum*, *Piper nigrum* and *Azadirachta indica* respectively (Fig. 2). The maximum percent protection offered by the aerial extracts was 64, 76, 88 and 92 respectively, and its respective repellent quotient values obtained on treatment against *Aedes aegypti* were 0.6, 0.7, 0.8 and 0.9 (Fig. 3).

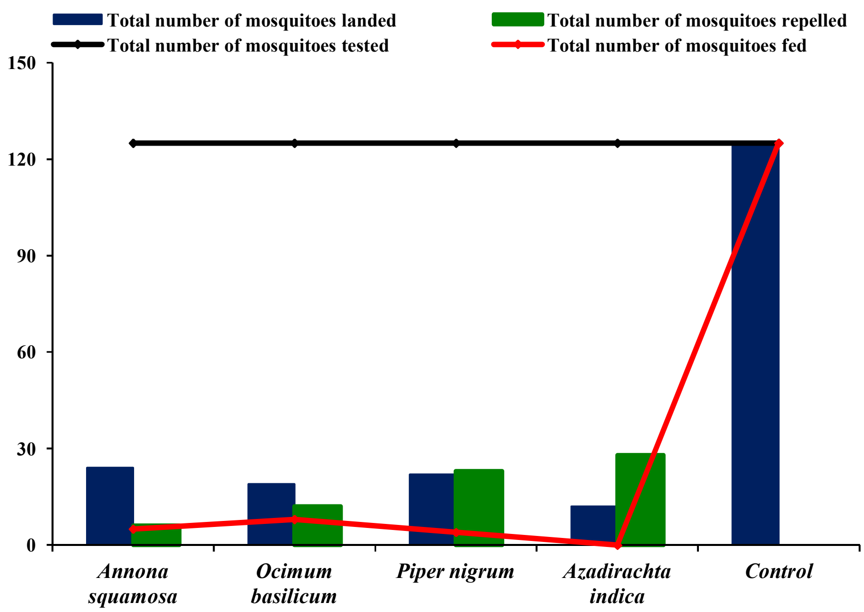

Fig. 1. Total number of mosquitoes tested, repelled, landed and fed during the repellent bioassay

Table 1. Repellent activity of aerial extracts against *Aedes aegypti*

Particulars	*Annona squamosa*	*Ocimum basilicum*	*Piper nigrum*	*Azadirachta indica* (Standard)	Control	F	*P* value
Mean number of mosquitoes repelled	1.2 ±0.4	2.4 ±0.5	4.6 ±1.5	5.6 ±1.1	0.0 ±0.0	457.301	2.41E-08*
Mean number of mosquitoes landed	4.8 ±2.9	4.4 ±1.9	3.8 ±1.9	2.4 ±1.7	25.0 ±0.0	15.853	.004*
Mean number of mosquitoes fed	1.6 ±0.5	1.0 ±0.7	0.8 ±1.6	0.0 ±0.0	25.0 ±0.0	14.629	.005*

**Student's 't' test @ P=.05.*

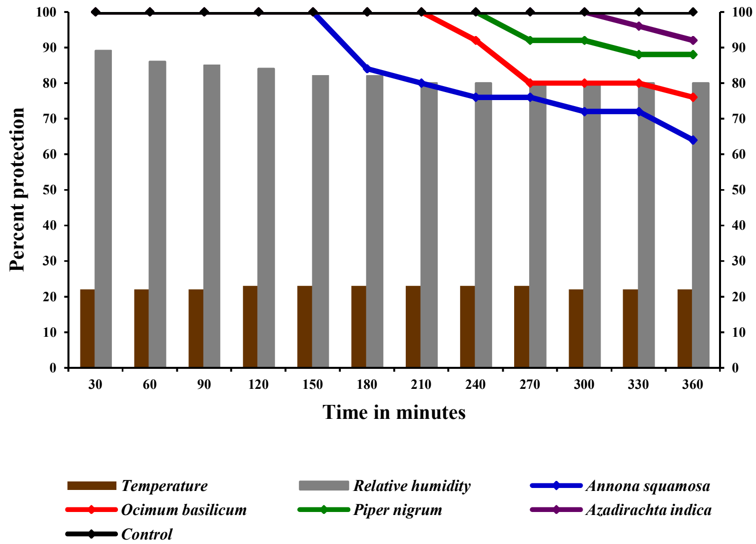

Fig. 2. Percent protection in time by crude aerial extracts against *Aedes aegypti*

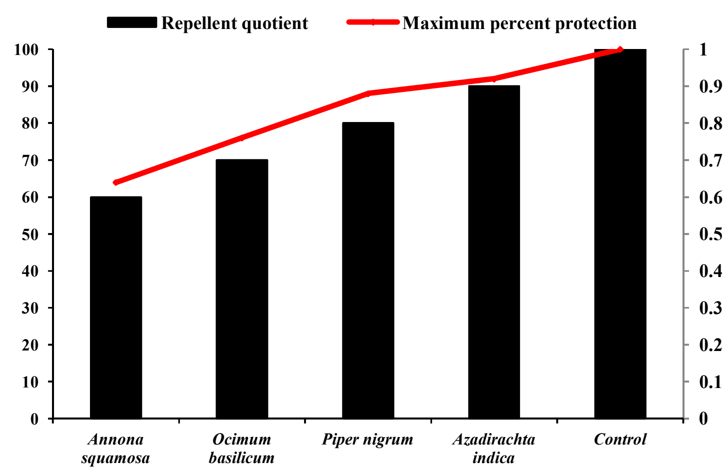

Fig. 3. Maximum percent protection and repellent quotient of aerial extracts tested against *Aedes aegypti*

4. DISCUSSION

Aedes aegypti mosquitoes are voracious and multiple feeders, and usually make surreptitious landings on exposed skin to feed. Mosquito repellency of different extracts was estimated based on the number of mosquitoes that repelled/fed within a specified time, the accurate documentation of the duration of exposure, the time of the first bite recorded, and the elapsed time to the first bite calculated and recorded as the complete-protection time [19]. The aerial extracts from different species of plants displayed repellent activity with varying degrees of time duration. Nevertheless, the interval of protection is a matter of concern, and in the current investigation, *Annona squamosa*, *Ocimum basilicum* and *Piper nigrum* aerial extracts provided a protection time for 2.5 to 4 hours, and for the standard *Azadirachta indica* it was 5 hours, after which landing endeavors and bites were experienced. This observation would support past records of repellent activity of phytoextracts, hence the duration of repellence may be certainly connected with the presence of phytochemicals.

Phytochemical particles as contact repellents find use in skin application formulations. Sustained release of the dynamic phytocomponents accounts for in terms of activity period and efficiency. Demonstration of repellent activity of the phytoaerial extracts by topical application on the tail region of Swiss albino mice in the present study produced worth referencing results. The observed variability of repellent activity amongst extracts from the different plant species may suggest that repellent activity is not just reliant on the concentration of a phytoextract, yet in addition by the active phytochemical constituents in it. Plant extracts commonly act on mosquitoes in the vapour phase [20], which are successful for a generally brief period [21], which may be compared with the present investigation. Phytoextracts from various parts of plants contains a complex of chemicals with unique biological activity due to phtyotoxins and secondary metabolites [10]. The repellent molecules intermingle with olfactory and gustatory receptors of mosquitoes. Research documented that hairs on the mosquito antennae are temperature and moisture sensitive. The repellent molecules accordingly associates with the female mosquito olfactory receptors thereby impeding the sense of smell, which consequently arises as an obstacle in the recognition of host by the mosquitoes. In the present investigation too, the repellent potential of the aerial extracts may be because of the presence of specific phytochemicals that can aggravate the olfactory senses of the mosquitoes. Active phytoingredient of most repellents go about as neurotoxins or respiratory toxins to insect [22]. The insect physiology is disturbed that affect the nervous system such as synthesis of neurotransmitter, storage, release and activation of receptors [23]. Repellents hinder the insect acetylcholinesterase enzyme that block the nerve transmission impulse in the light of the fact that acetylcholinesterase is the only resistance mechanism for insect [24].

The mechanism of repellent activity relies upon the kind of repellent particles. Phytoconstituents conveyed as vapour, fumes, or by topical application aggravate mosquitoes to move away from the source. Phytochemical compounds kindles explicit gustatory receptor neurons of the antenna in the mosquitoes evoking an aversive behavior towards the compound in *Aedes aegypti* [25]. Diminution or disguising of the discernment to host odorants by the antennal sensors is an alternative mechanism testified for the repellent activity in different vector mosquitoes like *Aedes aegypti*, *Aedes albopictus*, *Culex quinquefasciatus* and *Anopheles minimus* [26,27]. Mosquitoes do not comprehend anything revolting in the phytoextracts to repel them. Conceivably, the active phytoingredients (alkaloids, flavonoids, phenolics, saponins and tannins), present in the phytoextracts may have exercised some inhibitory effect on lactic acid receptor cells by hiding or altering the lactic acids that generally attract them, thus making the mosquitoes perplexed or befuddled [28]. Accordingly, it leads to the prevention of the blood-feeding contact or response. Subsequently, in the present investigation, with the application of the phytoextract on the tail region of Swiss albino mice, the mosquito could not bite because the active ingredients does not permit it to smell the attractant (lactic acids) and could not for that reason recognize and distinguish its source of meal. This suggests that the active phytoingredients disorganized the olfactory receptors and the mosquito could not smell the host.

Olfaction plays a significant role in host seeking mosquitoes since they identify semiochemicals with antennae and maxillary palps. Mosquitoes head straight for their blood meal attracted by the odourous cocktail of exhaled breath and sweat [29, 30]. Mosquitoes utilize extremely sensitive antennae to detect odours. These antennae are covered with fine hairs called sensilla, which can identify odour and sweat molecules, which pass via small pores in the sensilla and reach the mosquitoes sensory cells, and each scenting hair resembles a miniature nose. Sensilla in these appendages house olfactory receptor neurons that perceive a plethora of chemicals originating from skin, breath and sweat [31]. Mosquitoes utilize olfactory co-receptors to identify odour and sweat, and these coreceptors responds to carbon dioxide [32] and lactic acid, a component of sweat [30], along with ammonia in humans. Further, octenol a compound present in human perspiration too is additionally emitted in small quantities [33]. Lactic acid, a human body odour, and carbon dioxide, when presented individually, are insignificantly attractive to *Aedes* mosquitoes, yet become more appealing and attractive when blended [34]. The noteworthy attraction of mosquitoes to odours mixed with carbon dioxide proposes that carbon dioxide receptors assume a protuberant part in host-seeking behaviors. Nevertheless, *Aedes aegypti* mosquitoes lacking functional carbon dioxide receptors would be still allured to hosts [35]. It is assumed that the active ingredients in the phytoextracts when applied on the bare skin evaporate and are released with carbon dioxide from the host, thereby changing the host carbon dioxide signature to that of plants. Therefore, the host seeking mosquito perceives carbon dioxide of plants and not that of host [36,37].

Several aspects have an influence in deciding the efficiency of any repellent. These incorporate the sex and age of the host, level of activity, and biochemical attractiveness to the biting insect, sorrounding temperature, humidity, wind, and the mosquito species [38, 39]. These variables acting independently or collectively may have accounted for the varied degree of repellency observed in the current investigation, which was also reiterated by Das and Ansari [40]. Additional factors like, type of repellents, mode of application, nature of repellent, influence the effectiveness and duration of the repellent. The abiotic factors will certainly affect the outcome and interpretation of repellent bioassays. Skin-mediated effects include absorption and penetration of repellent on skin, yet evaporation, and perspiration end in repellent loss [41,42]. These physical factors are highly challenging to be controlled in a bioassay yet their involvement to experimental error can be lessened by arbitrary selection of test subjects and the utilization of appropriate sample sizes in bioassays, and by perceiving and evading quasi duplication. Light, temperature, humidity, and air quality at the testing area are significant ecological impacts in repellent bioassays [42,43]. These components can be handled to desired levels in the laboratory, nonetheless, in nature, they vary significantly and deeply affects mosquito reactions to repellent stimuli. Additional environmental sources of discrepancy in bioassays include repellent dose and exposure time [44], and structure and pattern of the test cage [19,45]. In the second instance, relationships between protection time, mosquito test population size, and the mosquito biting rate is to be noted. Notwithstanding, experiments utilizing different test cage patterns and mosquito population sizes [19,46-48] have not prompted a consent with respect to the ideal mosquito biting rate and density for repellency assessments. Further, the shape and size of test cage, and mosquito density effects differ subject to the mosquito species tested, and in the case of *Aedes aegypti*, repellent protection time is inversely correlated to cage size and not affected by mosquito density [45]. Contrariwise, the biotic/biological factors in repellent bioassays involve larval nutrition, carbohydrate accessibility to adult mosquitoes, age and uniformity in female mosquitoes, and intrinsic variances among repellent-treated test subjects [47,49], besides the timing and intensity of mosquito biting activity, which is a significant behavioral factor that affects the results of bioassay studies [50,51]. Obliviousness of temporal feeding patterns can negotiate estimates of protection time for repellents that have extended activity, as can meagre information of mosquito biting rate. Henceforth, in screened cage tests, biting patterns can differ with the size of the cage, and this factor can certainly influence the fortitude of repellency [45].

5. CONCLUSION

The efficacy of a compound when tried on topical application depends upon various factors. Amongst the most significant factors, the base used, absorption, adsorption and evaporation play a crucial role in the persistence of the compound. Further, the temperature, humidity and light play a secondary role in determining the persistence of the compound with regard to field studies. The behavior of the adult mosquito particularly its biting time, biting area and biting behaviour also play an important role, if a repellent is to be considered for protection from mosquito bite. Therefore, an extensive study on these key viewpoints mentioned above would aid in the development and advancement of suitable phytorepellents.

ETHICAL APPROVAL

The assays utilized in this study were approved by the Animal Ethics Committee of the Thiruvalluvar University in accordance with the national code for the care of animals utilized in experimentation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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