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Repellent activity of *N, N*-diethylphenylacetamide (DEPA) with essential oils against *Aedes aegypti*, vector of dengue and chikungunya

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Abstract
N, N-diethylphenylacetamide (DEPA) is known multi-insect repellent against haematophagous insects and essential oils also having repellent property. Efforts have been made to develop combination of DEPA with essential oils for better and effective repellent activity. Ten essential oils were used separately (at 1%) along with DEPA20% and 22 essential oils (at 20%) were tested for their repellent activity. It is found that protection time of essential oils when tested individually was found to be upto 4hrs. DEPA 20% alone was more effective than that of combination with essential oils and essential oils alone.

Keywords: DEPA, essential oils, repellent, *Aedes aegypti*

1. Introduction
Aedes aegypti is a major vector in India transmitting dengue and chikungunya with more than 2.5 million people are at risk in tropical and subtropical regions every year [1]. Besides various vector control measures personal protection is one of the methods to reduce the man-mosquito contact and prevent mosquito biting there by lowering down the disease transmission [2, 3]. *N-N*-diethyl phenyl acetamide is known to have multi-insect repellent property against various haematophagous insects [4, 5].
Various plant based essential oils are known to have insect repellent property and ample literature is available on repellent property of essential oils viz. catnip oil [6-9], lemon grass oil [10], andiroba oil [11], citronella oil, dill, basil, galbanum and lavender [12, 13], piper essential oil [14, 15]. The information on traditional plants based repellent is available and documented for their safety and use as repellents and has been extensively reviewed [16, 17].
Various formulations of essential oil combination and synthetic repellents have been tested in laboratory against *Aedes* and *Culex* mosquito species [18, 19]. Further Lawal *et al* investigated comparative study of blend of essential oils of lemon grass, eucalyptus, sweet basil, neem, sweet orange and scent leaf commercial repellent cream showing repellency for two hours almost similar to commercial repellent cream [20].
Over the years various insecticides are being used as mosquito repellents in various forms such as coils, mats, vaporizers. However, continuous and prolonged use has ill effect on the health of human [21] and indiscriminate use of insecticides has led to development of resistance [22-24]. Considering this the use of repellents is as an alternative for prevention of mosquito biting and disease transmission. Efforts are being made to develop safer and effective repellents though a daunting task to synthesize molecules to get the desired repellency against mosquito vectors. The essential oils are the choice to formulate better repellent for prevention of mosquito biting with longer protection.

2. Material and methods
Ae. aegypti female mosquitoes 200 in number were drawn from the stock colony maintained for more than two decades in the laboratory at 27°C ± 2°C and 75% ± 5% RH. Five to seven days old blood unfed (starved) female mosquitoes were released in the test chamber of size 75cmX60cmX60cm having wooden frames and wire mesh to prevent mosquito from escape. The tests were performed during day time. A series of ten formulations of DEPA 20% with

essential oils 1% in isopropanol and 23 essential oils 20% (prepared at FFDC, Kanauji, India) were taken for the repellent evaluation and each test compound of 150 microliter was applied on the fist on the area of 150sqcm. The DEPA 20% in isopropanol was taken as standard for comparison. Prior to the testing of repellency bare fist was inserted to ensure that the mosquitoes are hungry enough to take blood meal. There were two volunteers for each testing. The treated hand was inserted for five minutes and observations were taken for every 30min interval. The testing was ended after five bites received in 5 minutes and considered as the protection time [4, 25].

3. Results & Discussion

Among the various formulations tested DEPA 20% showed about 5.30 Hrs mean protection where as the DEPA 20% with essential oil 1% of tagetes, lemon grass and peppermint individually exhibited protection of 3.30hrs followed by DEPA 20% + basil oil 1% and DEPA + catnip oil 1% provided three hours protection against mosquito bites. Rest three formulations of DEPA 20% with citronella oil 1%, chamomile oil 1% and galbanum oil 1% were found to give protection of 2.30hrs. In order to find out the repellency of essential oils alone all the 23 test oils at 20% concentrations were evaluated for their repellency against *Ae aegypti*. Among all the essential oils the geranium oil has shown maximum protection of 4 hrs, while rest of the four essential oils namely citronella, chamomile, LM, and lavender were found to have repellency of 2 to 2.30 hrs followed by another four oils LS, lemongrass, cinnamon and galbanum showing 1 to 1.30 hrs protection. Rest of the essential oils did not show appreciable protection against mosquito bites for more than one hour. It was found that DEPA 20% alone provided maximum protection time than in combination with essential oils, with no compounding repellent effect against *Ae. aegypti*. Essential oils alone at 20 percent concentration did not show encouraging protection as compare to DEPA 20%.

4. Discussion

Insecticide or chemical control is the best weapon in the armoury of vector control however; it has certain merits and demerits. In various conditions of the day to day life in order to get rid from mosquito vectors the use of insect repellent is first choice because it can be applied at any time anywhere depending on the user's requirement. During travel, outdoor activities, picnic, schooling, the use of insecticides is less preferable rather avoided and in such situations repellent is the good option to protect from mosquito biting. In India various insect repellent formulations are available which are mostly synthetic chemical based and or in combinations of essential oils. Apart from the commercial repellents in India, DEPA based multi-insect repellent formulations are widely used in the Armed Forces and give better protection or at par with DEET based repellent [4]. Apart from DEPA the DEET based commercial formulations are available, however more than 20 percent concentrations are being used for longer protection [19]. Plant based insect repellents containing essential oils of citronella, peppermint, lemongrass, geranium are considered to be safe being of natural origin.

In present study we have made efforts for better and safer repellent formulation in terms of application and protection against mosquitoes. All other essential oils we tested in our studies, regardless of their active ingredients gave a mean protection ranging from 30 minutes to 3hrs 30mins against *Ae. Aegypti* [18]. Tawatsin *et al* so reported the repellency of 18 plant species belonging to 11 families, against *Ae. aegypti*, *Ae. albopictus*, *Anopheles dirus* and *Culex quinquefasciatus* where among these the protection time was found to be less than the standard DEET and IR3535 repellent ranging from 30 minutes to 2hrs 30 minutes for three minutes exposure period [26]. Various studies have been conducted on different plant based essential oils at different concentrations showing varying degree of protection against mosquito biting. Studies conducted in *Ae. aegypti* have shown that essential oils citronella, geranium, lemongrass, dill, basil, galbanum and lavender provided protection ranging from 120-180 minutes, while juniper, chamomile, jasmine gave protection from 210 - 270 minutes while maximum protection of 330 to 480 minutes was obtained from rosemary, cinnamon, catnip and litsea oil [12]. Almost corroborating results were obtained in present study of essential oils at 20% concentration resulting protection period ranging from 30mins -1hr, 1hr - 2hrs, 2hrs – 2.30 hrs and further up to 4hrs against *Ae. aegypti* where as DEPA 20% alone provided protection ranging from 5-6 hrs in laboratory conditions. The commercial DEET 23.8% (OFF) and 20% (Sawyer) provided mean protection of 301.5 and 234.4 minutes respectively while the citronella oil 5% (Natrapel) and 25% (Gone Plus) wrist band exhibited 13.5 and 0.2 minutes protection [27].

The efficacy of essential oils depends on the formulation and concentration. We have found that incorporation of essential oils did not enhance the protection time of DEPA-20% when used alone. Essential oil based DEET formulations along with geranium oil, citronella oil were tried but were not found to prolong the protection as compared to DEET alone [8, 29] which is more or less similar to observations of present study in case of DEPA and various essential oil combinations. Petroleum jelly based lemongrass oil has shown 3–5 hrs protection against *Ae.* Aegypti [11]. Various reports available have shown that combination of essential oils also did not resulted promising protection against mosquito bite [19]. The repellent action is due to the avoidance behaviour of insects [30] and keeps them away from the treatment source [31] however, the varying degree of repellency in different mosquito species may be due to the number of sensory receptors responding [32], volatility and chemical constituents of essential oils, relatively short lived and effective till oils volatilized [33].

Table 1: MPT of DEPA with essential oil against *Aedes aegypti*

S.N	Formulation (DEPA20% + essential oil 1% + 79% isopropanol)	Mean Protection Time (MPT)
1	Citro I/7 (Citronella Oil) *Cymbopogon winterianus*	2 Hours 30 mins
2	TGT I/7 (Tagetes oil) *Tagetes minuta*	3 Hours 30 mins
3	CNM I/7 (Cinnamon oil) *Cinnamomum zeylanicum*	3 Hours
4	CHMO I/7 (Chamomile oil) *Anthemis nobilis*	2 Hours 45 mins
5	BSL I/7 (Basil oil) *Ocimum basilicum*	3 Hours
6	DL I/7 (Dill Oil) *Anethum graveolens*	2 Hours
7	LMNG I/7 (Lemon grass oil) *Cymbopogon citrates*	3 Hours 30 mins
8	PMNT I/7 (Peppermint oil) *Mentha piperita*	3 Hours 30 mins
9	CTN I/7 (Catnip oil) *Nepeta cataria*	3 Hours
10	GLB I/7 (Galbanum oil) *Ferula galbaniflua*	2 Hours 30 mins
11	DEPA 20%	5Hrs 30 mins

Table 2: MPT essential oils against *Aedes aegypti*

S. No.	Essential oils	Mean Protection Time MPT
1	NIRF/ FFDC/Kannauj/ CITRO20	2 Hrs 30 min
2	NIRF/ FFDC/Kannauj/ CHMO20	02 Hrs
3	NIRF/ FFDC/Kannauj/ LS20	1 Hr 30 min
4	NIRF/ FFDC/Kannauj/ JP20	10 mins
5	NIRF/ FFDC/Kannauj/ GRN20	04 Hrs
6	NIRF/ FFDC/Kannauj/ LM-SN20	02 Hrs
7	NIRF/ FFDC/Kannauj/ LMNG20	1 Hr 30 min
8	NIRF/ FFDC/Kannauj/ RS20	30 min
9	NIRF/ FFDC/Kannauj/ CMP20	2min
10	NIRF/ FFDC/Kannauj/ LVD20	02 Hrs
11	NIRF/ FFDC/Kannauj/ BLK20	2min
12	NIRF/ FFDC/Kannauj/ BSL20	30 min
13	NIRF/ FFDC/Kannauj/ PMNT20	30 min
14	NIRF/ FFDC/Kannauj/ CNM20	1 Hr 30 min
15	NIRF/ FFDC/Kannauj/ TY20	30 min
16	NIRF/ FFDC/Kannauj/ FKC20	2min
17	NIRF/ FFDC/Kannauj/ DL20	0 Hrs
18	NIRF/ FFDC/Kannauj/ JM20	30 min
19	NIRF/ FFDC/Kannauj/ AR20	30 min
20	NIRF/ FFDC/Kannauj/ CTN20	30 min
21	NIRF/ FFDC/Kannauj/ GLB20	1 Hr
22	NIRF/ FFDC/Kannauj/ RM20	30 min

5. Conclusions

It is evident from the available literature and present study that the use of essential oils alone and in combination with synthetic chemical repellent considered for use as natural and safer for the application as insect repellents. However, it did not provide promising protection for longer duration. The use of synthetic DEPA based repellents an economically viable option when used alone, however efforts have been made for better and safer repellent formulations for protection from mosquito bites thus reducing disease transmission.

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