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REPELLENCY AND SMOKE TOXIC EFFECT OF FIVE COMMON INDIGENOUS WEEDS AGAINSTANOPHELES SUBPICTUS, AEDES AEGYPTI,AND CULEX QUINQUEFASCIATUS

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Abstract:

Transmitted by mosquitoes, vector-borne diseases such as Malaria, Filariosis and Dengue had an enhance in the global mortality rate due to increase in resistance of mosquitoes towards the commercial insecticides.Leaf extracts can produce the knock-down effect and smoke toxicity, the aim of the study is to evaluate such effects of five common indigenous weeds, i.e. Lantanacamara Linn., Cassia alata Linn, Zephyranthes minuta (Kunth) D.Dietr, Ocimum gratissimum Linn., & Parthenium hysterophorus Linn. against three mosquito species, namely of Anopheles subpictus, Aedes aegypti and Culex quinquefasciatus.The plants extract were formulated individually at concentration range 50mg,100mg &150mg and in combination with supportive ingredients such as filler, binder, burning agent, adhesives, smell enhancer etc.This aspect of the mortality rate is depending on the amount of dose administered. Efficacy against the mosquitoes tested for mosquito repellent activity with ethanol solvent extracts was found to be statistically significant. The results of the smoke toxicity and repellency tests were quite positive.Results of analysis showed that formulation containing extract of L.Camara, O. gratissimum, & P.hysterophorus have the most promising activity exhibiting 79-95% repellency as compared to other repellent preparation that made of individual extract of plant leave. The combinations showed prolonged burning and repellency both as compared to other formulations. The Mosquito coils prepared from the combination extract of all plantsexhibited 89%-100% mortality for 60 min time intervals. As the coils were prepared with combination of different ingredients, the results obtained in control 2 and commercial coil containing d-Trans Allethrin are almost equivalent. In this study, almost all the formulations used herbal and environmentally friendly ingredients. Since, there is no inhalation side effect. For texture, effectiveness and stability, etc., the same formulations were assessed.

Keywords: Mosquito repellent, mosquito coil smoke, Weeds,

1. Introduction

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Mosquitoes are vectors of insects with significant morbidity and mortality that transmit parasite and viral disease to millions of people around the world. Mosque-borne infections include paludism, yellow fever, chikungunya, filariasis and other arbovirus[1]. Dengue and yellow fever can be transmitted by Aedees aegypti (Ae. aegypti) while malaria is transmitted by Anopheles species. A primary Malaria mosquito vector in urban India is Anophelessubpictus (An. subpictus). Aedes aegypti(Ae. aegypti) and Culex quinquefasciatus(Cx. quinquefasciatus),these mosquitoes are human transmitters of malaria parasites[2]. More than 1.2 million deaths in both children and adults worldwide were recorded in 2010[3]. Dengue is one of the lethal human viral diseases with mosquitoes. Ae. aegypti is the dengue fever propagation vector.In Asia, dengue hemorrhagic fever is the cause of the majority of deaths of infants. Every year, dengue is affected by 50,000,000 people and around 20,000 deaths[4]. The largest reemerging arboviral disease, dengue fever, causes approximately 390 million infections annually in the world, with almost 100 million of them requiring treatment and more than 500000 among them requires hospitalisation to recover. Repellents are one of the major ways to protect humans from the bites of the insects.[5]. An efficient repellent is useful to reduce the interaction with the human vector and stop the transmission of diseases. Toxic, unpleasant, and long-lasting should be a repellent compound. Amides, imides, esters and others are considered to be acceptable repellents.Plants synthesise bioactive chemicals which can act as mosquito repellents and these chemicals are usually harmless[6]. Plant products have historically been used against pests and vectors by human populations in many parts of the world. Plant-based phytochemicals can function as larvicides, insect growth regulators and repellents. Plant sources of bioactive chemicals are considered rich, and can be an alternate source of moustache control agents. The plant-based phytochemicals have a chemical complex with unique biological activity[7].

2. Materials and methods

2.1. Collection and rearing of test mosquitoes

The present study was conducted in the department of Pharmacognosy,Rungta college of pharmaceutical sciences and research, Bhilai, Chhattisgarh, India during October-December 2019. Mosquito larvae of Anopheles subpictus, Aedes aegypti and Culex quinquefasciatuswere collected in the glass pots from cemented drainage surrounding the college. The different species of mosquitoes used in the present study were physically verified by Prof. Prashant Kumar Kanoje, HOD, Department of Zoology, Government Nagreek Kalyan Mahavidyalaya, Nandini Nagar (Ahiwara), Durg 490036, Chhattisgarh, India. Collected larvae were kept with artificial food (mixture of dog biscuits and yeast powder in the ratio of 3:1)and aquatic weed was held at 31-33°C and 85 percent relative humidity free and exposed to pathogens, insecticides or repellents (RH) and allowed to hatch. Some of the larvae described previously have also been conditioned to affirm their identification during the adult phases[8].

2.2. Collection and authentication of plants materials

The leave part of plants ofLantanacamara Linn., Cassia alata Linn, Zephyranthes minuta (Kunth) D.Dietr,Ocimum gratissimum Linn.& Parthenium hysterophorus Linn were collected from Semariya, Village, Baghdumar, Bhilai-Durg region of Chhattisgarh state India, in the month of September 2019 and authenticated by Dr. Sunita Garg, Emeritus Scientist, CSIR-NISCAIR, Ministry of Science and Technology, Government of India, New Delhi, India. The plant name has been confirmed with the data in the website http://www.theplantlist.org mentioning the data of accessing that website.

2.3. Preparation of extracts.

Leaves of plants are kept in shade drying after washing with tap water which is followed by powdering with electrical blender. Extraction of the active compounds were processed using 300mL ethanol in Soxhlet apparatus for 8h followed by evaporation under vacuum. The final extract was then diluted to various concentration to perform the bioassays. [9].

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2.4.Bio-efficacy evaluation for mosquitocidal activity by Smoke toxicity test using a glass chamber method.

Smoke toxicity test method was adapted from Koyel Mallick et al (2014), with some minor modifications. 50mg, 100mg& 150mg of solid extract residue is mixed with 0.5g sawdust (as binding material) and 0.5g charcoal powder (burning material) along with 1-2 drops of adhesive to form semisolid paste. From the paste, 0.4cm thick and 1.0cm long mosquito coils were prepared and kept for shade drying. In control 1, the coils were prepared in the same procedure vomiting the plant extract[10]. In control 2, Commercial mosquito coil (containing d-Trans Allethrin, Allout® coil) of same dimension was taken. The prepare coils were burned inside the glass chamber (measuring 70 cm x 70 cm x 70 cm, with a window measuring 30 cm x 15 cm) containing 100 blood fed adult An.subpictus, Ae. aegypti and Cx. quinquefasciatusmosquitoes (age-3 or 4 days) and exposed to the smoke from the coils for 1h. The ethanol extract of leaf ofL.camara, C. Alata, Z. minuta, O. gratissimum, & P. hysterophorus were used. Similarly, control 1 and 2 groups were also treated with the smoke from coil. After exposing to the smoke, the number of dropped down and dead mosquitos were counted after 10, 20, 40 and 60 min of exposure. The experiments were repeated three times on three successive days and the means were calculated[11].

2.5 Safety assessment of mosquito coil smoke (MCS) Preparations

Female Sprague-Dawley rats (~250 gm) were used to evaluate toxicological effects of the investigational- and standard-preparations. The rats were kept at 25 ± 2 °C, with a relative humidity of 70 ± 5%, 12:12h light-dark cycle, with chow diet and drinking water ad libitum till the end of the experiment. The experiments were approved by the Institutional Animal Ethical Committee, Rungta College of Pharmaceutical Sciences and Research, Bhilai, India (Approval No. 1189/PO/Re/S/08/CPCSEA/2021/15). Since the most likely human exposure route is inhalation, the inhalation toxicity tests were performed. To simulate the human exposure, 3 identical rooms (room-1, 2, 3) with a floor area of ceramic tile of 13.6 m^2 and a volume of 43 m^3were used (Islam et al., 2018). The rats were acclimatized for at least a week before the experiments began. Animals were divided randomly into different groups (n = 6 per group): (I) without any exposure i.e. control, (II) exposure to MCSs preparations of L.camara, O. gratissimum, & P. hysterophorus(50 mg) and (III) exposure to standard preparation i.e. All Out® coil, and held in identical ventilation cages. The inhalation toxicity was observed for 14 days according to the OECD guideline 403. Animals were observed for clinical signs of intoxication like salivation, lacrimation, diarrhea, hyperactivity, choreoathetosis, incoordination, piloerection, Straub reaction, tremors, seizures, lethargy, sleep and death on day 1 and 14 of the study. Moreover, the changes in food intake and body weight were measured.

3. Result

Table 01 to 06 represents the effect of smoke toxicity of ethanol extract of L.camara, C. Alata, Z. minuta , O. gratissimum, & P. hysterophorus leave at the concentration range of 50mg, 100mg &150mg each. Only 4-6% of mortality was observed in the control 1 group, i.e. coils without extracts within 1hr of exposure of An.subpictus, Ae. aegypti and Cx. quinquefasciatusrespectively. Mosquito coils prepared from the extract of L.camara exhibited 89%-93% (Table 01) C. Alata 29%-38%((Table 02)), Z. minuta 19%-21% ((Table 03))O. Gratissimum92%-96%((Table 04)), & P. hysterophorus92%-96%(Table 05)mortality for 60 min time intervals. The result showed for same time intervals which were the extract of L.camara 79%-88% O. Gratissimum 86%-91%, C. Alata 24%-33%, Z. minuta 14%-16% & P. hysterophorus87%-95% additional mortality over the control coil 1. Moreover the Mosquito coils prepared from the combination extract of L.camara, C. Alata, Z. minuta , O. gratissimum, & P. hysterophorus leaveexhibited 89%-100%(Table 06) mortality for 60 min time intervals. The interesting result is seen between control group 2 and commercial coils containing d-trans Allethrin where the results were almost similar with the combination preparation of extract of all plants.Mosquito coils prepared from the combinationofextract exhibited 70.67% and 77.00% mortality for same time intervals which were 65.34% and 71.00% (table 06).

3.1 Safety assessment

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The inhalation toxicity of MCSs preparations of L.camara, O. Gratissimum& P. hysterophorus(50 mg each) were tested in rats to ensure their safety (Table 1). None of the rats died following exposure to lab prepared coil and standard preparation (All Out® mosquito coil) during 14 days of the study period. Mild increase in physical activity was observed in the animals exposed to standard preparation only on day 1 of the exposure. No other treatment-related clinical signs of intoxication including salivation, lacrimation, diarrhea, choreoathetosis, incoordination, piloerection, Straub reaction, tremors, seizures, lethargy and sleep were observed following exposure to lab prepared coil and standard preparation. Moreover, food intake and body weight of rats exposed to lab prepared coil and standard preparation were similar to that of control ($p>0.05$).

4. Discussion

The main pest control in ancient era, agriculture and veterinary was based on crude plant materials and botanical derivatives. Chemically synthesized insecticides replaced prominence at medium time because of their magical effectiveness [12]. The value of insecticides of green origin has recently been reaffirmed by the unfair long-term application of synthetic insecticides causing numerous damages to the ecosystem [13]. Various plants have various complicated chemicals with specific biological activities. A good amount of documentation on the effectiveness of plant materials extracted from mosquitoes is provided through the literature[14].The result showed that smoke from plant leave of L.camara, O. Gratissimum& P. hysterophorushad more toxic effect on An.subpictus, Ae. aegypti and Cx. quinquefasciatusthan C.alata, & Z. minuta. Therefore, plant leaves of L.camara, O. Gratissimum& P. hysterophorus can be a potential candidate for development of commercial repellents whichmay be an alternative tosynthetic chemical repellents. On further analysis, after purification the ethanol extract might give increased efficacy.

5. Conclusion

Based on the results, it can be concluded that ethanol extracts of leaf could also be used as smoke toxic material against those two mosquitoes. From the above results, authors are encouraged to investigate the active ingredient responsible for the mentioned activityof L.Camara, C.alata, Z. minuta ,O. gratissimum, & P.hysterophorus extracts against the mosquito species of An.subpictus, Ae. aegypti and Cx. quinquefasciatuswhich are under progress.This study shows the ability to use mosquito coil smoke (MCS) Preparations in mosquito repellent formulations from medicinal herbs.Crude extract of all the weeds plants showed mosquitocidal activity. The extracts at the studied concentration (100 mg) and at different time intervals did not target non-target organisms. Based on the results, crude extract of L.Camara, C.alata, Z. minuta ,O. gratissimum, & P.hysterophorus indiduals and combination were an excellent potential for making mosquito repellent.

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Table-01 Effect of smoke toxicity of mosquito coil smoke (ethanol extract) of Lantana Camara leaves against Anopheles subpictus, Aedes aegypti and Culex quinquefasciatus adult mosquitoes

Mosquito species	Observation time (min)	Doses of Extracts									control 1	control 2
		50mg			100 mg			150mg				
		% Dropped down mosquitoes in treated	% Dead mosquitoes in treated	% Dead mosquito over control 1	% Dropped down mosquitoes in treated	% Dead mosquitoes in treated	% Dead mosquito over control 1	% Dropped down mosquitoes in treated	% Dead mosquitoes in treated	% Dead mosquito over control 1	% Dead mosquitoes in control 1	% Dead mosquitoes in control 2
Aedes aegypti	10	10±1.63	06±2.45	6	16±0.00	10±2.45	10	22±1.33	19±0.82	19	00±0.00	45±0.33
	20	28±1.63	21±3.27	21	38±0.82	30±3.27	30	43±0.33	36±3.27	36	00±0.00	80±1.20
	40	66±1.63	51±3.27	47	80±0.41	67±0.82	63	81±0.33	72±0.82	68	04±1.63	100±0.33
	60	82±00	69±3.27	63	99±0.41	89±1.63	83	96±2.96	88±3.27	82	06±0.82	
Anopheles subpictus	10	09±2.45	07±2.45	6	14±0.82	11±1.63	10	23±1.15	19±1.63	18	01±0.82	41±1.73
	20	28±3.27	22±0.82	18	40±0.82	32±1.63	28	43±2.96	33±2.45	29	04±2.45	78±0.33
	40	64±3.27	52±0.41	50	81±2.86	69±0.82	67	83±1.20	71±3.27	69	02±0.00	98±1.15
	60	78±0.82	71±1.63	66	99±0.41	92±0.00	87	92±2.33	90±0.82	85	05±3.27	99±0.33
Culex. Quinquefasciatus	10	8±2.45	07±2.45	6	12±4.08	09±2.04	8	15±0.67	13±0.41	12	01±0.82	41±2.40
	20	26±1.22	22±1.63	20	36±1.63	28±1.22	26	42±1.76	27±2.04	25	02±2.04	78±2.33
	40	67±0.82	53±0.00	49	77±2.45	66±0.00	62	82±1.15	61±1.63	57	04±0.82	100±0.00
	60	84±0.41	73±0.82	67	99±0.82	86±0.82	80	99±0.67	83±0.82	77	06±0.41	

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Table -02 Effect of smoke toxicity of mosquito coil smoke (ethanol extract) of Cassia alata leaves against Anopheles subpictus, Aedes aegypti and Culex quinquefasciatus adult mosquitoes

Mosquito species	Observation time (min)	Doses of Extracts									control 1	control 2
		50mg			100 mg			150mg				
		% Dropped down mosquitoes in treated	% Dead mosquitoes in treated	% Dead mosquito over control 1	% Dropped down mosquitoes in treated	% Dead mosquitoes in treated	% Dead mosquito over control 1	% Dropped down mosquitoes in treated	% Dead mosquitoes in treated	% Dead mosquito over control 1	% Dead mosquitoes in control 1	% Dead mosquitoes in control 2
Aedes aegypti	10	00±0.00	00±0.00	0	07±2.45	00±0.00	0	9±1.76	05±0.82	5	00±0.00	45±0.33
	20	10±0.82	06±2.45	6	17±3.27	06±0.41	6	24±1.33	18±1.63	18	00±0.00	80±1.20
	40	18±0.41	09±2.04	6	32±2.45	18±1.63	15	39±0.00	29±0.82	26	03±0.82	99±0.67
	60	31±1.22	18±0.00	14	43±0.82	29±1.63	25	46±0.88	41±1.63	37	04±0.82	
Anopheles subpictus	10	0±0.00	00±0.00	-2	08±1.63	03±1.22	1	10±2.33	05±1.22	3	02±0.41	41±0.33
	20	11±1.22	06±0.41	3	21±0.82	08±0.00	5	20±1.33	17±2.45	14	03±0.82	78±1.76
	40	16±2.04	10±2.04	6	37±1.63	22±1.63	18	41±0.58	27±2.45	23	04±2.04	98±1.86
	60	32±2.04	21±1.63	16	53±2.04	38±0.83	33	61±1.20	43±2.45	38	05±1.63	98±0.88
Culex. Quinquefasciatus	10	00±0.00	00±0.00	-3	06±0.82	00±0.00	-3	12±0.88	06±0.82	3	03±1.22	42±2.31
	20	08±0.41	7±0.41	5	15±2.45	07±0.82	5	19±1.76	19±4.08	17	02±1.22	78±2.89
	40	14±0.00	13±1.22	10	33±1.22	16±3.27	13	39±0.67	28±4.08	25	03±0.82	99±0.88
	60	28±0.82	22±0.41	16	41±1.22	27±3.27	21	49±2.31	43±3.27	37	06±1.63	

Table -03 Effect of smoke toxicity of mosquito coil smoke (ethanol extract) of Zephyranthes minuta leaves against Anopheles subpictus, Aedes aegypti and Culex quinquefasciatus adult mosquitoes

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Mosquito species	Observation time (min)	Doses of Extracts									control 1	control 2
		50mg			100 mg			150mg				
		% Dropped down mosquitoes in treated	% Dead mosquitoes in treated	% Dead mosquito over control 1	% Dropped down mosquitoes in treated	% Dead mosquitoes in treated	% Dead mosquito over control 1	% Dropped down mosquitoes in treated	% Dead mosquitoes in treated	% Dead mosquito over control 1	% Dead mosquitoes in control 1	% Dead mosquitoes in control 2
Aedes aegypti	10	00±0.00	00±0.00	0	08±0.41	00±0.00	0	11±1.15	05±0.00	5	00±0.00	45±0.67
	20	03±0.00	00±0.00	0	10±1.22	04±2.86	4	16±2.33	09±0.41	9	00±0.00	81±2.40
	40	10±0.82	06±0.82	2	19±0.41	08±0.82	4	29±1.15	21±1.63	17	04±1.22	99±1.33
	60	16±3.27	08±1.22	3	31±0.82	18±2.45	13	41±0.33	32±0.82	27	05±2.45	
Anopheles subpictus	10	00±0.00	02±1.22	0	06±3.27	03±0.82	1	12±0.67	05±0.41	3	02±1.22	41±2.33
	20	04±3.27	03±2.45	0	09±2.45	02±2.04	-1	17±0.58	12±2.04	9	03±2.86	78±0.58
	40	11±0.82	06±1.22	3	16±0.82	07±1.63	4	28±1.76	19±1.63	16	03±2.45	98±1.20
	60	18±1.63	09±1.22	4	27±2.45	21±2.04	16	40±2.31	31±1.63	26	05±2.45	98±1.20
Culex. Quinquefasciatus	10	00±0.00	03±0.82	0	09±1.63	03±0.41	0	13±1.73	05±0.82	2	03±1.63	43±2.40
	20	04±2.04	03±1.22	0	12±2.45	05±0.41	2	16±0.58	08±4.08	5	03±0.82	78±1.15
	40	09±2.45	06±2.45	2	18±3.27	11±0.41	7	27±1.76	20±2.45	16	04±1.63	100±0.33
	60	15±0.82	10±0.00	4	30±0.41	21±0.82	15	42±2.40	32±0.00	26	06±2.45	

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Table -04 Effect of smoke toxicity of mosquito coil smoke (ethanol extract) of Ocimum gratissimum leaves against Anopheles subpictus, Aedes aegypti and Culex quinquefasciatus adult mosquitoes

Mosquito species	Observation time (min)	Doses of Extracts									control 1	control 2
		50mg			100 mg			150mg				
		% Dropped down mosquitoes in treated	% Dead mosquitoes in treated	% Dead mosquito over control 1	% Dropped down mosquitoes in treated	% Dead mosquitoes in treated	% Dead mosquito over control 1	% Dropped down mosquitoes in treated	% Dead mosquitoes in treated	% Dead mosquito over control 1	% Dead mosquitoes in control 1	% Dead mosquitoes in control 2
Aedes aegypti	10	12±1.22	08±0.41	8	19±0.00	13±0.82	13	31±2.33	28±1.63	28	00±0.00	45±1.76
	20	28±2.04	24±1.22	24	42±1.22	33±1.22	33	60±1.33	57±0.00	57	00±0.00	79±2.96
	40	64±0.41	53±2.04	49	84±2.86	75±1.22	71	95±0.67	85±1.63	81	04±2.86	99±0.67
	60	80±0.00	66±2.04	60	99±0.82	97±0.41	91	99±1.33	100±0.41	94	06±2.45	
Anopheles subpictus	10	14±0.82	08±2.45	6	18±1.22	13±2.04	11	30±1.73	28±0.41	26	02±0.82	41±0.67
	20	29±1.22	23±2.45	20	43±1.22	32±1.22	29	54±1.20	54±1.22	51	03±1.22	78±2.33
	40	66±0.41	54±2.45	51	89±0.82	76±0.41	73	81±0.67	80±0.82	77	03±0.82	98±1.20
	60	80±0.00	67±0.82	62	98±1.63	93±1.22	88	98±1.00	95±0.00	90	05±1.63	99±0.67
Culex. Quinquefasciatus	10	14±1.63	09±0.82	7	21±2.45	17±0.82	15	29±0.88	27±2.45	25	02±1.22	41±1.33
	20	28±1.22	25±0.82	22	41±1.63	29±0.41	26	60±2.40	61±1.22	58	03±0.82	78±0.33
	40	67±0.00	57±1.63	54	82±0.82	69±0.41	66	80±0.88	82±1.22	79	03±1.22	99±0.67
	60	79±0.41	69±1.63	63	99±1.22	92±2.04	86	98±1.45	99±0.00	93	06±0.00	

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Table -05 Effect of smoke toxicity of mosquito coil smoke (ethanol extract) of Parthenium hysterophorus leaves against Anopheles subpictus, Aedes aegypti and Culex quinquefasciatus adult mosquitoes

Mosquito species	Observation time (min)	Doses of Extracts									control 1	control 2
		50mg			100 mg			150mg				
		% Dropped down mosquitoes in treated	% Dead mosquitoes in treated	% Dead mosquito over control 1	% Dropped down mosquitoes in treated	% Dead mosquitoes in treated	% Dead mosquito over control 1	% Dropped down mosquitoes in treated	% Dead mosquitoes in treated	% Dead mosquito over control 1	% Dead mosquitoes in control 1	% Dead mosquitoes in control 2
Aedes aegypti	10	18±2.04	14±0.41	14	21±2.45	18±2.04	18	39±2.91	33±0.41	33	00±0.00	45±2.89
	20	38±0.41	31±0.82	31	54±1.22	44±1.22	44	75±1.20	62±2.04	62	00±0.00	80±0.33
	40	73±1.22	60±0.41	57	89±2.04	78±0.82	75	93±2.91	81±1.22	78	03±0.82	99±0.67
	60	89±1.22	76±0.82	71	100±0.41	100±0.41	95	100±0.00	98±0.82	93	05±3.27	
Anopheles subpictus	10	19±2.45	12±0.00	11	19±0.41	14±1.63	13	37±1.33	35±4.08	34	01±1.63	41±1.15
	20	41±0.41	30±0.82	27	32±0.82	29±3.27	26	71±2.33	65±2.45	62	03±1.63	78±0.33
	40	70±1.63	54±2.04	51	78±0.82	64±2.45	61	90±0.88	84±2.45	81	03±0.82	98±0.67
	60	88±0.82	81±1.22	76	94±1.63	87±0.82	82	99±0.67	99±0.82	94	05±0.41	100±0.00
Culex. Quinquefasciatus	10	20±2.04	15±1.22	14	23±0.00	16±3.27	15	33±1.15	36±4.08	35	01±1.22	41±1.86
	20	37±0.41	29±2.45	27	43±2.45	31±1.22	29	68±2.33	64±1.63	62	02±1.22	78±1.20
	40	72±0.41	62±0.00	58	84±2.45	71±0.82	67	92±1.33	89±1.63	85	04±1.63	100±0.33
	60	88±2.45	81±0.82	75	99±0.82	93±0.82	87	99±1.00	98±0.41	92	06±0.41	

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Table 06-Effect of smoke toxicity of mosquito coil smoke (ethanol extract) of combination preparation against Anopheles subpictus, Aedes aegypti and Culex quinquefasciatus adult mosquitoes

Mosquito species	Observation time (min)	Doses of Extracts									control 1	control 2
		50mg			100 mg			150mg				
		% Dropped down mosquitoes in treated	% Dead mosquitoes in treated	% Dead mosquito over control 1	% Dropped down mosquitoes in treated	% Dead mosquitoes in treated	% Dead mosquito over control 1	% Dropped down mosquitoes in treated	% Dead mosquitoes in treated	% Dead mosquito over control 1	% Dead mosquitoes in control 1	% Dead mosquitoes in control 2
Aedes aegypti	10	24±3.27	21±0.82	21	39±4.08	34±0.41	34	55±0.88	51±2.04	51	00±0.00	45±2.33
	20	49±0.00	43±0.82	43	69±1.63	66±0.41	66	85±0.00	81±1.63	81	00±0.00	80±1.73
	40	79±0.00	69±1.63	65	86±0.82	76±0.82	72	99±1.00	99±0.00	95	04±2.45	99±0.67
	60	97±0.82	88±1.63	83	99±0.82	99±1.63	94	99±0.58	99±0.41	94	05±0.82	
Anopheles subpictus	10	26±3.27	19±1.22	17	42±1.63	34±0.00	32	61±0.58	53±2.04	51	02±1.63	41±1.76
	20	51±1.63	42±0.82	39	75±2.45	63±2.45	60	92±1.73	86±1.63	83	03±2.45	78±0.33
	40	77±0.82	66±0.82	62	92±0.82	80±0.82	76	99±0.67	100±0.41	96	04±3.27	98±1.86
	60	92±2.45	86±1.63	81	99±0.82	89±1.63	84	99±0.33	99±0.00	94	05±2.04	100±0.33
Culex. Quinquefasciatus	10	23±3.27	23±3.27	22	40±0.41	38±0.00	37	57±1.20	57±1.63	56	01±0.82	42±1.20
	20	48±3.27	46±0.82	44	70±1.22	71±0.82	69	82±2.33	87±1.63	85	02±0.41	77±1.86
	40	82±1.63	71±1.63	67	90±0.41	90±1.63	86	100±0.00	100±0.41	96	04±1.22	99±0.67
	60	95±0.82	96±2.45	90	99±0.00	98±2.04	92	100±0.00	99±0.82	93	06±1.22	

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