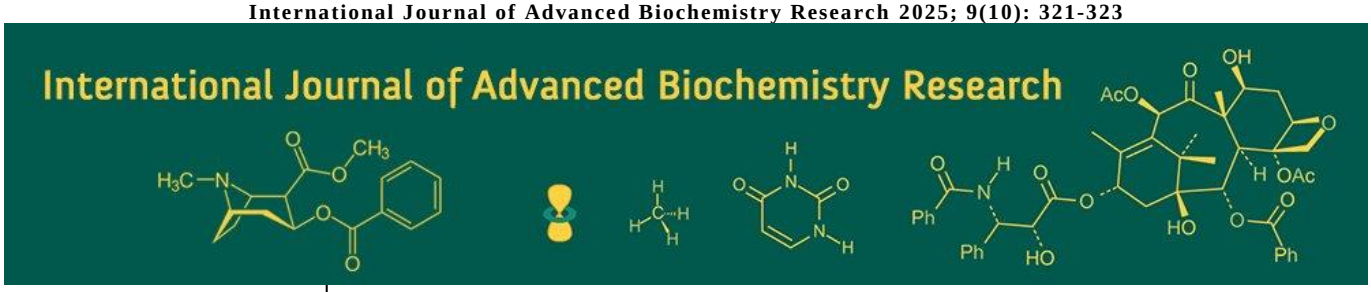

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Effect of sweet cane, neem leaf and neem chip dunks in mosquito larval control

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Abstract
Culicine mosquito larvae were collected from stagnant water bodies both on and near the College of Veterinary and Animal Sciences in Mannuthy, Thrissur. To create dunk formulations, powdered *Acorus calamus* (Sweet cane) was mixed with an appropriate natural binder. Additionally, formulations were prepared using neem leaf powder and neem chips (*Azadirachta indica*). The efficacy of various concentrations of these preparations on juvenile culicine mosquitoes was tested under laboratory conditions. A mortality rate of 98% was recorded with a 2.5% *Acorus calamus* dunk within 24 hours, with no further development observed in the remaining 2% of larvae. Comparable mortality rates for the neem leaf dunk and neem chip dunk were noted only after 72 hours.

Keywords: *Acorus calamus* dunk, neem leaf dunk, neem chip dunk, natural binder, Culicine mosquito larva

Introduction
Culicine mosquitoes prefer to lay their eggs in stagnant water. They are commonly found in ponds, marshes, swamps, wetlands, as well as bodies of water that have been still for about a week—such as puddles and streams. However, these mosquitoes can adjust to diverse environments and breed even outside their typical habitats. Various species exploit containers filled with water to deposit their eggs (Thwing *et al.*, 2023) [8].
Controlling mosquito populations is vital for global public health, particularly in tropical regions where these insects spread numerous diseases including malaria, yellow fever, West Nile virus, dengue fever, and filariasis (Kaiz *et al.*, 2021) [4].
The reliance on conventional insecticides for managing human-affected mosquito habitats can be excessively costly for management programs. Furthermore, many traditional insecticides pose environmental risks (Maple *et al.*, 2020) [5]. Thus, there is an urgent need for alternative strategies that are effective yet environmentally friendly and economical. The use of dunks to gradually release herbal formulations presents a promising alternative control approach (Mohammed *et al.*, 2021) [6]. This study aims to leverage accessible resources such as neem (*Azadirachta indica*) and sweet cane (*Acorus calamus*) to create an affordable mosquito dunk that is available to the general public.

Materials and Methods
Fresh neem leaves were thoroughly washed then dried before being ground into a fine powder using a mortar. Fresh stem bark pieces from the neem tree were also dried and powdered; finer particles were separated from larger granules through sieving. Roots from sweet cane (*Acorus calamus*) were gathered, dried out, then powdered. A binding agent consisting of gum powder (3g) was added to the powders from the neem leaves, neem bark, and acorus roots to prepare respective dunks: neem leaf dunk, neem chip dunk, and acorus dunk. Juvenile stages of culicine mosquitoes were sourced from a pool of stagnant water using a larval collection net; they were maintained in a beaker fitted with a netted cover (Shafique, 2022) [7].
In vitro trials assessed the effectiveness of various dunks at different concentrations (2.5%, 3%, 4%, and 5%) on culicine developmental stages. Since concentrations at 1% and 1.5% showed minimal effects, 2.5% was designated as the baseline concentration for all experiments.

Each trial involved introducing 100 mosquito larvae into a beaker containing 200 ml distilled water covered by netting; the dunk was suspended within this solution using permeable cloth (Adongo *et al.*, 2022) [1].

Results

Effect of *Acorus calamus* dunk on culicine larvae

The impact of varying concentrations of *Acorus calamus* dunk on juvenile culicine stages is summarized in Table-1. After six hours at a concentration of 2.5%, this formulation resulted in a larval mortality rate of 30%; lower rates—27%, 25%, and 22%—were observed at concentrations of 3%, 4%, and 5%, respectively. Ultimately within twenty-four hours at the same concentration level (2.5%), mortality reached an impressive rate of nearly all larvae—98%. No further development occurred among the remaining two percent up until twenty-four hours.

Table 1: Effect of *Acorus calamus* dunk on culicine mosquito larvae

Concentration of the dunk	Percent larval mortality			
	6 hours post exposure	12 hours post exposure	18 hours post exposure	24 hours post exposure
2.5% *Acorus calamus* dunk	30	59	78	98
3% *Acorus calamus* dunk	27	49	69	89
4% *Acorus calamus* dunk	25	37	58	88
5% *Acorus calamus* dunk	22	34	51	86
Control 1 & 2	-	-	-	-

- Control 1 (3 g binder in 200 ml water) = No mortality.
- Control 2 (200 ml water) = No mortality.

Effect of neem leaf dunk on culicine larvae

The influence exerted by different concentrations of neem leaf dunk on juvenile culicines is illustrated in Table-2. After twelve hours post-exposure with this treatment at concentrations yielding larval mortalities ranging from eleven percent to twenty-three percent across varying strengths—lower ones demonstrated progressively less efficacy over time compared to higher levels—reaching ultimately up towards ninety-eight percent mortality after seventy-two hours.

Table 2: Effect of neem leaf dunk on culicine mosquito larvae

Conc. of the dunk	Percent larval mortality					
	12 hours post exposure	24 hours post exposure	36 hours post exposure	48 hours post exposure	60 hours post exposure	72 hours post exposure
2.5% neem leaf dunk	23	40	59	75	80	98
3% neem leaf dunk	19	36	48	64	78	91
4% neem leaf dunk	15	29	41	58	71	85
5% neem leaf dunk	11	23	39	51	69	81
Control 1& 2	-	-	-	-	-	-

Effect of neem chip dunk on culicine larvae

Table-3 outlines how different concentrations affect juveniles exposed to neem chip dunks over time frames stretching between twelve hours up until seventy-two hours post-exposure; notable observations include initial mortality measures rising from twelve up toward twenty-five percent depending upon concentration levels utilized—the highest dosages eventually achieving similar ninety-eight percentile totals downrange.

Table 3: Effect of neem chip dunk on culicine mosquito larvae

Conc. of the dunk	Percent larval mortality					
	12 hours post exposure	24 hours post exposure	36 hours post exposure	48 hours post exposure	60 hours post exposure	72 hours post exposure
2.5% neem chip dunk	25	44	54	76	80	98
3% neem chip dunk	21	33	49	67	75	89
4% neem chip dunk	16	27	39	59	71	82
5% neem chip dunk	12	20	36	51	68	81
Control 1 & 2	-	-	-	-	-	-

Discussion

Larval mortality of 98% could be obtained with 2.5% of *Acorus calamus* dunk within 24 hours and a similar level of mortality was obtained with 2.5% neem leaf dunk and 2.5% neem chipping dunk only after 72 hours.

Beta-asarone is the chief constituent of sweet cane, while Azadirachtin is the prime component of neem. (Worell, 2022) [9]. Development of the female reproductive systems and ecdysis were irregular due to neem's biochemical similarity with insect moulting hormone ecdysone (Hales *et al.* 2020) [3]. Azadirachtin and beta-asarone exposed mosquitoes die prematurely. Those that survive the acorus and neem dunk exposure are formed as adult incapable of feeding, dispersing, or reproducing (Balaji and Sravan, 2021) [2].

Conclusion

This research demonstrated that *Acorus calamus* dunks exhibited superior effectiveness against mosquito larvae compared to both neem leaf dunks or neem chip dunks alone which notably required extended durations before achieving similar outcomes. It is advisable that subsequent

investigations delve deeper into their potential applications serving as agents aiming towards controlling culicine larval populations effectively.

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