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BIO-EFFICACY OF INDIGENOUS *Nicotiana Tabacum* EXTRACTS AT DIFFERENT SOLVENTS AGAINST THE DENGUE VECTOR *AEDES AEGYPTI* (DIPTERA: CULICIDAE)

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ABSTRACT:
Dengue fever remains a persistent health issue in various regions of Pakistan, particularly in urban areas where the Aedes aegypti vector is prevalent. This mosquito is a key target for reducing the disease. Chemical insecticides have long been employed, but they face formidable hurdles, including pollution, damage to beneficial insects, and health risks, as well as growing resistance in the host mosquitoes. This study investigates a more sustainable and readily available alternative, Nicotiana tabacum (tobacco) leaves, a plant widely used in traditional medicine. We have also tested the effects of different solvents: methanol, hexane, n-butanol, and water (over 24, 48, and 72 h; 200–600 ppm) on mosquito larvae, pupae, and eggs after the extraction of bioactive compounds from the leaves. Mosquitoes were reared at the NIFA Medical Entomology lab in Peshawar under controlled conditions. According to the results summarized in Tables 4.2.1, 4.2.2, and 4.2.3, *Nicotiana tabacum* extracts demonstrated a highly significant, dose- and time-dependent biocidal effect against all developmental stages of *Aedes aegypti*, with methanol consistently proving to be the most potent solvent. At 600 ppm, methanolic extracts achieved complete larval mortality (100%) by 48 hours, drastically reduced egg hatching to 5%, and induced up to 95% pupal mortality by 72 hours, outperforming hexane, butanol, and aqueous extracts. The pronounced efficacy observed in methanolbased formulations, statistically supported by Tukey HSD analysis ($p \leq 0.05$), highlights its superior ability to extract active phytochemicals with strong ovicidal, larvicidal, and pupicidal properties. These findings position methanolic *N. tabacum* extract at 600 ppm as a highly promising**,** ecocompatible biopesticide for integrated mosquito control strategies targeting multiple life stages of *Aedes aegypti*. Hexane and n-

butanol extracts were also effective, whereas water extracts appeared to be less effective. These results suggest that N. tabacum, which is locally available in Pakistan, may play a significant role in eco-friendly mosquito control. If used as a botanical insecticide, it could mean less dependence on harmful chemicals and a local tool for communities to fight dengue. This strategy aligns with the IVMM strategy and promotes sustainable public health practices in Pakistan.

Keywords: Aedes Mosquito, *Nicotiana tabacum*, larvicidal, Ovicidal, suicidal toxicity

INTRODUCTION:

Mosquito control remains a cornerstone of public health. Of all arthropod vectors causing disease, mosquitoes are the worst offenders (Mdoe et al., 2014). The mosquito is Public Enemy Number One, according to the World Health Organization (WHO, 2006). They are vectors for a variety of deadly diseases, such as malaria, yellow fever, dengue fever, chikungunya, filariasis, encephalitis, and West Nile virus cells. In over 100 countries worldwide, mosquito-borne diseases are a significant cause of illness, affecting more than 700 million people annually (Ghosh & Dash, 2012). On a worldwide basis, there are greater than 3,500 species of mosquitos that are classified into 41 genera and 135 subgenera (Reinert, 2000; CDC, 2004). However, the intensive use of chemical insecticides as the primary weapon for controlling mosquitoes has caused several serious problems, including resistance in mosquito populations, environmental pollution, toxicity toward non-target organisms, and persistence due to non-biodegradability (Shakeel et al., 2017; Ahmed et al., 2018). Prior to the 1940s, when plant-derived materials were widely used, pest resistance to pesticides did not pose a significant problem (Bakkali et al., 2008). Recent years have seen an increasing demand for new, environmentally friendly, specific, and biodegradable mosquito control agents (Ohia & Ana, 2015). Some of these plant products are found to be effective as insecticides, repellents, feeding deterrents, attractants, sterilants, or growth regulators. Tobacco, Nicotiana tabacum L. (Solanaceae), a widely used annual herb, has been investigated for mosquitocidal activity. It is a highly available vegetable in countries such as Nigeria, growing to a height of approximately 9–12 cm (Olofintoye et al., 2011). The efficacy of extracts from the leaves of N. tabacum against several species of mosquitoes has been demonstrated in various reports. The current work has been conducted to assess the larvicidal potential of potential of N. tabacum in controlling Aedes mosquito larvae as an alternative to synthetic insecticides. However, the use of traditional insecticides is also associated with several drawbacks, including insect resurgence, resistance, mammalian toxicity, environmental residue, and nonspecificity in action (Singhi et al., 2004). Synthetic insecticides have induced environmental and health issues, creating public awareness (St. Leger et al., 1996). The overuse of these compounds in agriculture and public health schemes has contributed to their environmental contamination and toxicity to humans and non-target animals (Severini et al., 1993). In response, the WHO has been promoting the transition to safer alternatives 13, 15 by advocating for the standardization and registration of microbial and plant-derived insecticides. In recent years, growing interest has been developed in focusing on the ability of plant extracts and essential oils as alternatives that provide biodegradable, bioactive compounds useful for mosquito control. Within these, N. tabacum possesses insecticidal principles, which may cause larvicidal death due to certain active ingredients, such as limonoids (Govindarajan et al., 2011). The primary objective of the present investigation was to assess the larvicidal, ovicidal, and repellent efficacy of N. tabacum extracts, which could contribute to the development of an eco-friendly approach to mosquito control.

MATERIALS AND METHODS Study Setting Area

The experiment was conducted at the Division of Plant Protection, Medical Entomology Mosquito Rearing Laboratory (NIFA), Peshawar, during the years 2020-2021.

Collection of N. tabacum
The plant was gathered from their local area in the district of Nowshera (Risalpur) and was found to possess insecticidal activity against dengue vector control.

Plant Material & Aqueous Extract Preparation:
The harvested plant materials were thoroughly washed with tap water and shade-dried to prevent degradation of the active principles. The dried material was ground to a fine powder in a motoroperated blender. Extraction: The powdered material (200 g) was extracted with solvents of different polarities (ethanol, hexane, n-butanol, and water) separately in a Soxhlet apparatus (Make: Borosil). The extractions were performed for 8 hours at 50–80°C (boiling point) using 500 mL of each solvent. The filtrates obtained were filtered using Whatman No. 1 filter paper under a vacuum in a Buchner funnel. The crude extract was concentrated under vacuum and dried using a bowl-type rotary evaporator then transferred into glass vials for analysis. Stock solutions of each plant extract were prepared in their respective solvents, except for the dried hexane extract, using the method described by WHO (2015) with slight modifications.
Tested organism: Aedes aegypti mosquito. These extracts were tested using the larvae, eggs, and pupae of A. aegypti for toxicity. The tests met the conditions established by the World Health Organization (WHO) for evaluating pesticides (WHO, 2013).

Rearing/ culture of Aedes Mosquitoes
The mosquito larvae stage–eggs, larvae, and pupae used in the present study were received from the Division of Plant Protection, Medical Entomology Mosquito Rearing Laboratory, NIFA, Peshawar. Specimens were kept free from exposure to pathogens, insecticides, and repellents. The rearing environment was maintained at a temperature of 28°C, 70–75% relative humidity (RH), and an 11±0.5-hour photoperiod (Reegan et al., 2013).

Larval and Pupal Effectiveness Test
The WHO (2015) procedures were used to determine the larvicidal and pupicidal effects of the plant extracts. Thirty-third-instar Aedes aegypti larvae were held in plastic containers, each containing 100 mL of dechlorinated water. Larvae were treated with concentrations of extracts at 200, 300, 400, 500, and 600 ppm, whereas control groups were kept in dechlorinated water only. Mortality data (%) were recorded at 24, 48, and 72 hours after treatment. Three replicates of each concentration were analyzed. Mortality rates The mortality rates were estimated as (Elango et al., 2009).

$$\text{Percent Mortality (\%)} = \frac{\text{Number of dead larvae}}{\text{The number of larvae introduces}} \times 100$$

Assessment of Ovicidal Effects
Freshly laid eggs (0–3 hours old) of Aedes aegypti were exposed to varying concentrations of plant extracts (200, 300, 400, 500, and 600 ppm) for four days. Each treatment was replicated three times. The non-hatchability of eggs was calculated using Abbott's formula (Abbott, 1925):

$$\text{Percent egg hatchability} = \frac{\text{Number of eggs hatched}}{\text{Number of eggs released.}} \times 100$$

Corrected Ovicidal activity (%) =

$$\frac{\text{Larvae hatched in control} - \text{larvae hatched in treatment}}{100 - \text{Larvae hatched in control (\%)}} \times 100$$

Statistical Analyses:

Mortality percentage was estimated based on Abbott's (1925) In: % mortality = more line/more control × 100. For whole mortality, statistical analysis was conducted using probit analysis (SPSS, version 16). Differences between groups were tested by one-way ANOVA and Tukey's HSD posthoc test. Differences were defined as significant if P ≤ 0.05. Statistical analyses were performed us ing IBM SPSS Statistics v22 at a 95% significance level (SPSS, 2010).

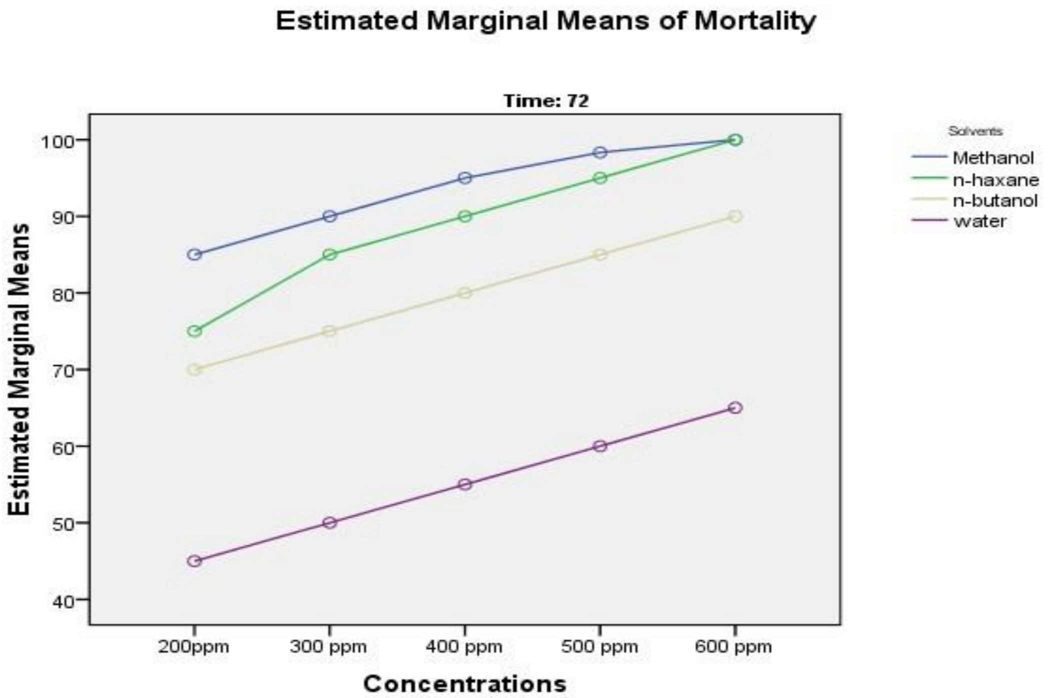
RESULTS:

The results presented in Table 4.2.1 demonstrate a significant concentration- and time-dependent increase in larval mortality of *Aedes aegypti* when exposed to *Nicotiana tabacum* extracts prepared using different solvents. Among the solvents tested, methanol consistently exhibited the highest larvicidal efficacy across all exposure durations, achieving 96.67%, 100.0%, and 100.0% mortality at 600 ppm after 24, 48, and 72 hours, respectively. Hexane and butanol also showed substantial effectiveness, particularly at higher concentrations and prolonged exposure, whereas the aqueous extract remained markedly less potent throughout. Statistical analysis (Tukey HSD, $p \leq 0.05$) revealed significant differences among treatments, indicating that methanol was the most effective solvent for extracting bioactive compounds responsible for larval toxicity. These findings highlight the strong potential of methanolic *N. tabacum* extracts, especially at 500–600 ppm, as a reliable and eco-friendly larvicidal agent for integrated mosquito management programs. The data presented in Table 4.2.2 reveal a statistically significant, time- and concentration-dependent reduction in egg hatching of *Aedes aegypti* following exposure to *Nicotiana tabacum* extracts across various solvents. Among the tested solvents, methanol consistently exhibited the strongest ovicidal activity**,** reducing egg hatching to 10.00%, 5.00%, and 5.00% at 600 ppm after 24, 48, and 72 hours**,** respectively. This was followed by hexane and butanol, which also showed moderate inhibition. At the same time, aqueous extracts remained the least effective, with egg hatching above 20.00% even at the highest concentration and longest exposure. The statistical analysis (Tukey HSD, $p \leq 0.05$) confirmed that methanol-treated groups differed significantly from others, especially at higher concentrations. These results conclusively identify methanol as the most effective solvent and 600 ppm as the most potent concentration for extracting ovicidal compounds from *N. tabacum*, making it a promising botanical tool for breaking the reproductive cycle of *Aedes aegypti* in integrated vector control programs. The results are presented in Table 4.2. 3 show a clear and statistically significant increase in *Aedes aegypti* pupal mortality with rising concentrations and extended exposure to *Nicotiana tabacum* extracts across different solvents. Methanol once again emerged as the most effective solvent, with pupal mortality escalating from 75.00% at 600 ppm after 24 hours to an impressive 95.00% at 72 hours**,** indicating strong residual efficacy. Hexane and butanol also demonstrated notable effects**,** particularly at higher concentrations and prolonged exposure, with mortality rates of 91.67% and 81.67%, respectively, at 600 ppm after 72 hours of exposure. In contrast, aqueous extracts remained the least effective**,** with a maximum mortality of 76.67% under the same conditions**.** The statistical differences confirmed by Tukey HSD ($p \leq 0.05$) reinforce methanol's superior extraction of bioactive compounds, which consistently produced significantly higher pupal mortality compared to the other solvents. These findings underscore the high larvicidal and pupicidal potential of methanolic *N. tabacum* extract**,** particularly at 600 ppm, as a strong candidate for eco-friendly and time-effective interventions in mosquito vector management strategies.

Table 4.2.1 Percent mortality of *Nicotiana tabacum* extract at different organic solvents against *Aedes aegypti* larvae to various concentrations after different exposure times.

Conc.(ppm)	Solvents	Methanol	Hexane	Butanol	Water
24 hrs					
200	65.00	60.00	55.00	15.00	
300	76.67	70.00	65.00	35.00	
400	85.00	75.00	70.00	40.00	
500	90.00	80.00	75.00	46.67	
600	96.67	85.00	80.00	55.00	
48 hrs					
200	80.00	70.00	65.00	40.00	
300	85.00	75.00	70.00	45.00	
400	90.00	85.00	75.00	50.00	
500	95.00	88.33	80.00	55.00	
600	100.0	96.67	85.00	60.00	
72 hrs					
200	85.00	75.00	70.00	45.00	
300	90.00	85.00	75.00	50.00	
400	95.00	90.00	80.00	55.00	
500	98.33	95.00	85.00	60.00	
600	100.0	100.0	90.00	65.00	

Means followed by the same letter are not significantly different from each other within each solvent for each time interval (Tukey HSD, $p \leq 0.05$).



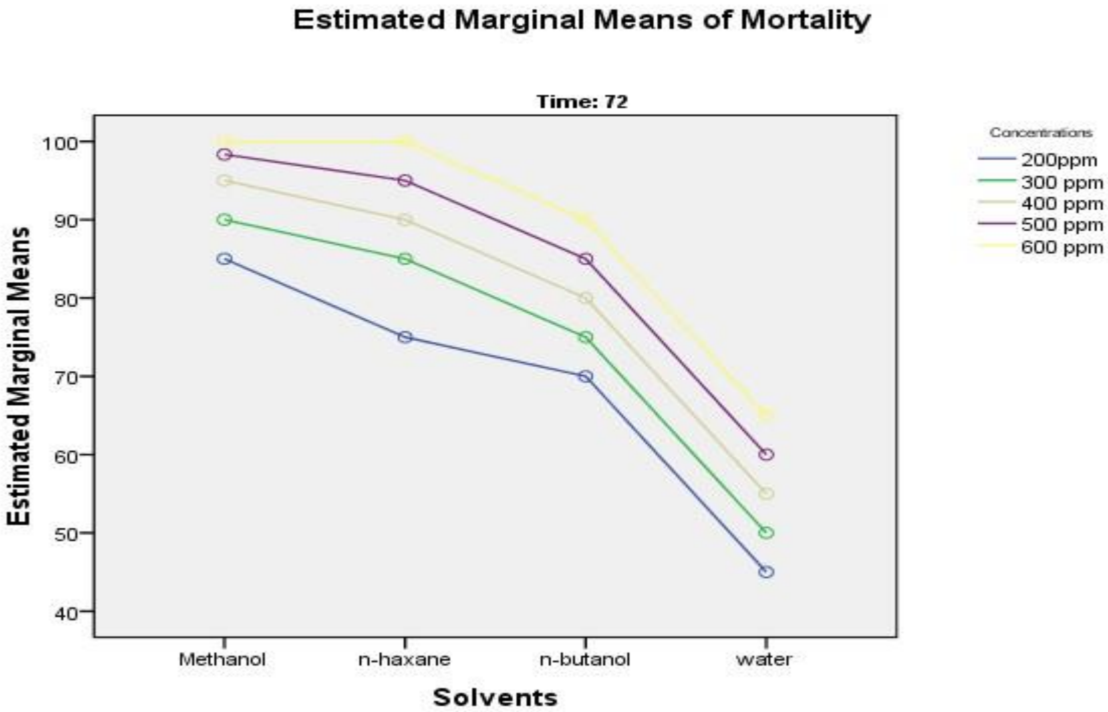


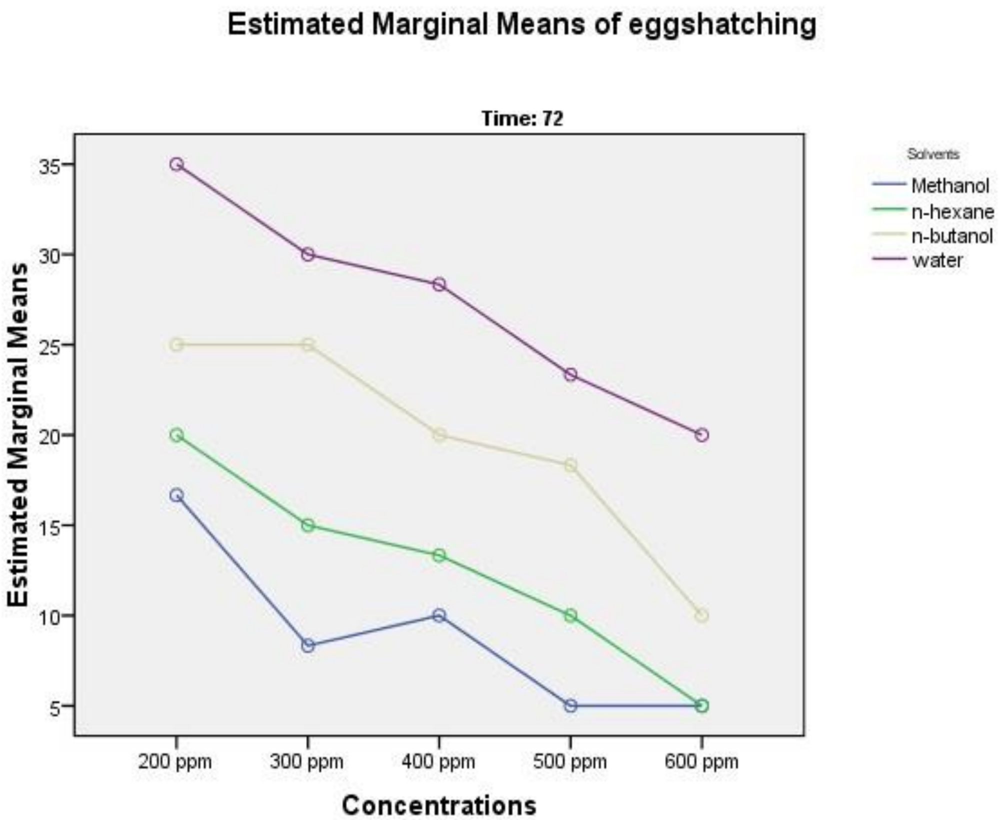
Fig: 11, 12 Percent mortality of *Nicotiana tabacum* extract at different organic solvents against *Aedes aegypti* larvae to various concentrations after 72 hours of exposure time.

Table 4.2.2 Percent egg hatching of *Nicotiana tabacum* extract at different organic solvents against *Aedes aegypti* to various concentrations after different exposure times.

Conc.(ppm)	**Solvents**			
	Methanol	**Hexane**	**Butanol**	**Water**
	24 hrs			
200	23.33	30.00	35.00	41.67
300	18.33	25.00	30.00	40.00
400	16.67	21.67	23.33	40.00
500	15.00	21.67	20.00	40.00
600	10.00	15.00	20.00	31.67
48 hrs				
200 20.00		25.00	30.00	35.00
300 15.00		21.67	23.33	35.00
400 15.00		18.33	26.67	33.33
500 10.00		15.00	20.00	31.67
600 5.00		10.00	15.00	25.00
72 hrs				
200	16.67	20.00	25.00	30.00
300	8.33	15.00	25.00	30.00
400	10.00	13.33	20.00	28.33

500	5.00	10.00	18.33	23.33
600	5.00	5.00	10.00	20.00

Means followed by the same letter are not significantly different from each other within each solvent for each time interval (Tukey HSD, $p \leq 0.05$).



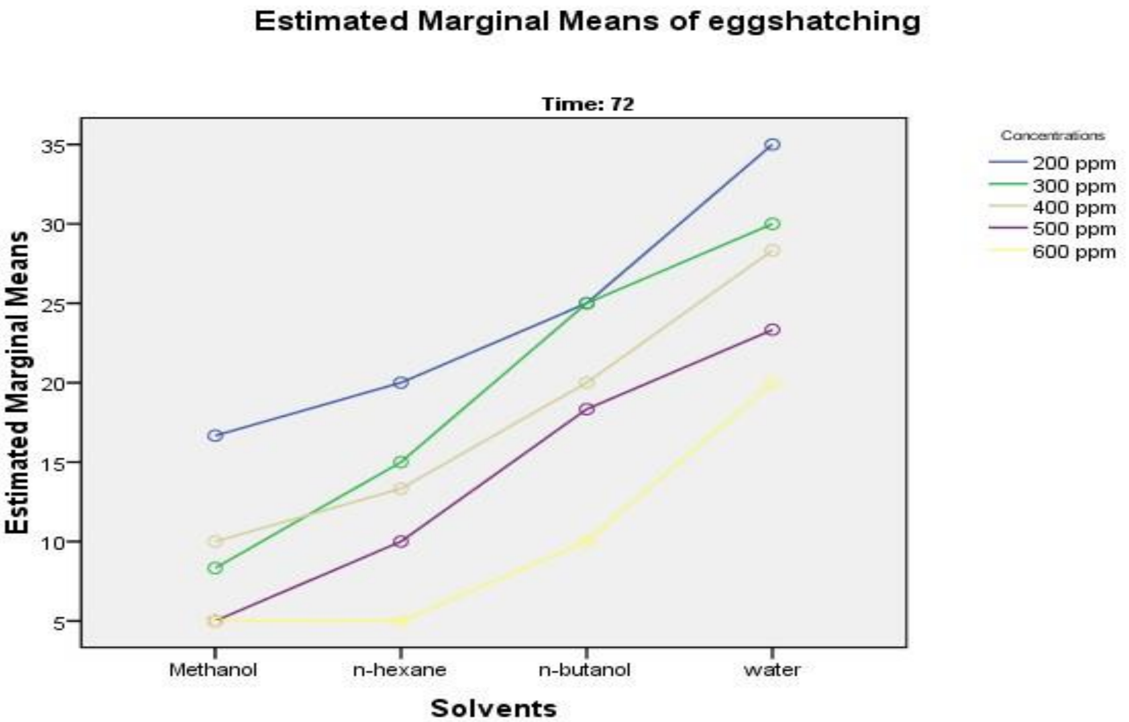
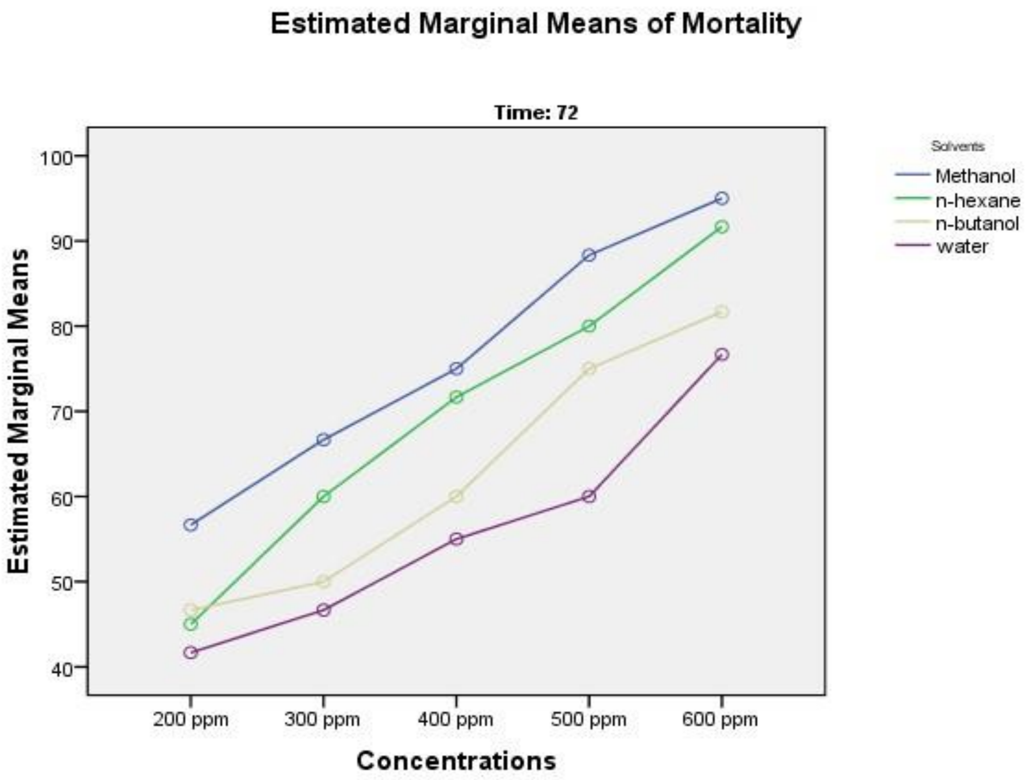


Fig: 1, 2) Percent egg hatching of *Nicotiana tabacum* extract at different organic solvents against *Aedes aegypti* to various concentrations after 72 of exposure time.

Conc.(ppm) **Solvents**	**Methanol**	**Hexane**	**Butanol**	**Water**
	24 hrs			
200	48.33	40.00	31.67	25.00
300	56.67	45.00	33.33	30.00
400	60.00	51.67	41.67	31.67
500	70.00	55.00	51.67	48.33
600	75.00	70.00	65.00	51.67
48 hrs				
200 50.00		46.67	35.00	30.00
300 55.00		51.67	38.33	35.00
400 70.00		60.00	51.67	41.67
500 80.00		75.00	56.67	50.00
600 88.33		80.00	75.00	60.00
72 hrs				
200	56.67	45.00	46.67	41.67
300	66.67	60.00	50.00	46.67
400	75.00	71.67	60.00	55.00
500	88.33	80.00	75.00	60.00
600	95.00	91.67	81.67	76.67

Means followed by the same letter are not significantly different from each other within each solvent for each time interval (Tukey HSD, $p \leq 0.05$).



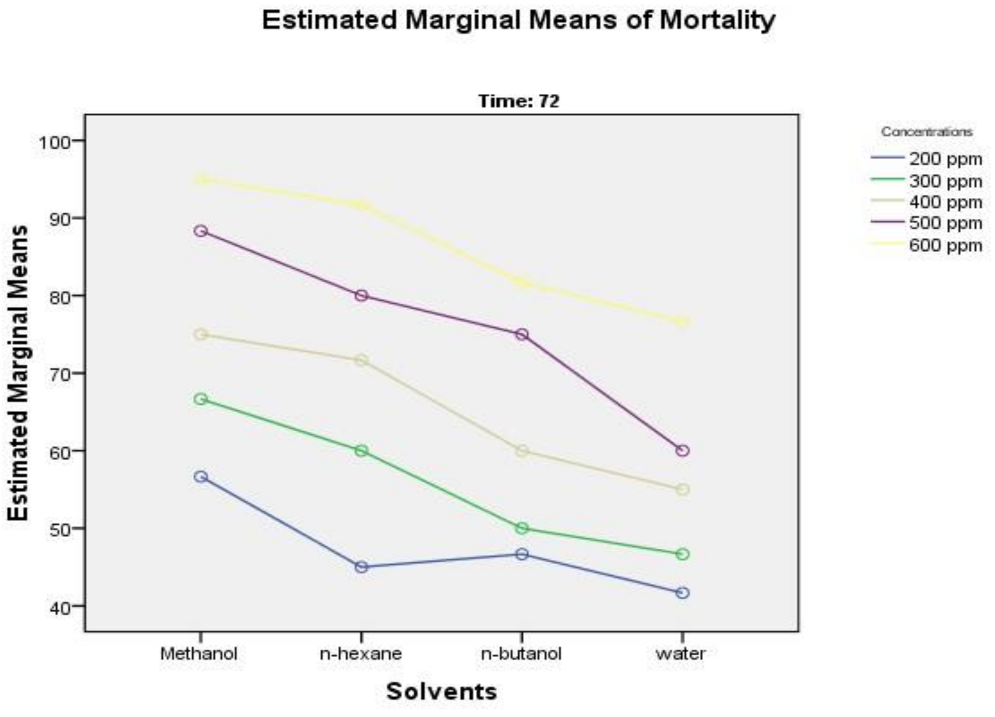


Fig: P2. (5, 6) Percent pupae mortality of *Nicotiana tabacum* extract at different organic solvents against *Aedes aegypti* to various concentrations after 72 hours of exposure time.

Discussion:

According to the results summarized in Tables 4.2.1, 4.2.2, and 4.2.3, *Nicotiana tabacum* extracts demonstrated a highly significant, dose- and time-dependent biocidal effect against all developmental stages of *Aedes aegypti*, with methanol consistently proving to be the most potent solvent. At 600 ppm, methanolic extracts achieved complete larval mortality (100%) by 48 hours, drastically reduced egg hatching to 5%, and induced up to 95% pupal mortality by 72 hours, outperforming hexane, butanol, and aqueous extracts. The pronounced efficacy observed in methanol-based formulations, statistically supported by Tukey HSD analysis ($p \leq 0.05$), highlights its superior ability to extract active phytochemicals with strong ovicidal, larvicidal, and pupicidal properties. These findings position methanolic *N. tabacum* extract at 600 ppm as a highly promising**,** eco-compatible biopesticide for integrated mosquito control strategies targeting multiple life stages of *Aedes aegypti*. Hexane and n-butanol extracts were also effective, whereas water extracts appeared to be less effective. These results suggest that N. tabacum, which is locally available in Pakistan, may play a significant role in eco-friendly mosquito control. If used as a botanical insecticide, it could mean less dependence on harmful chemicals and a local tool for communities to fight dengue. This strategy aligns with the IVMM strategy and promotes sustainable public health practices in Pakistan. The results of the present study are supported by the report of Yahaya et al. (2021), which indicated that the methanol extract of N. tabacum exhibited maximum larvicidal activity (70%) against Aedes after 72 h of treatment. Furthermore, they noted that the methanol extract of N. tabacum yielded the lowest LC50 (2.58±0.1 mg/mL). In contrast, the hexane extract of Datura metel had a lower LC50 value (3.13 ± 0.1 mg/mL) against Anopheles larvae, indicating a higher larvicidal potential. These results are in agreement with Manzano et al. (2020), who reported 93% larval mortality at 50 mg/L ethanol extract after 72 hours of exposure. The present results are also in agreement with Rita and John (2013), who reported a 100% mortality rate against E. Fischer for ethanol and hexane extracts at 750–1000 ppm after 24 hours. Berkov and Zayed (2004) reported that the larvicidal effect of N. tabacum methanol extract on the tested mosquitoes in this work might be due to the presence of alkaloids and saponins in the extract. This finding is similar to the results of Rizvi et al. (2018), who reported that alkaloids and saponins, the major components of various plant parts, vary according to the part and species. They are known to reduce depression in the central nervous system, with a predominant effect on the hypothalamus (Kutama et al., 2010). In particular, the greater mortality in larvae, pupae, and adults treated with leaf extract of N. tabacum relative to seed extract treatment might be related to the components of phytochemicals phytochemicals in the plant. A similar observation was also reported in a study by Mittai et al. (2013). Regarding egg hatching, among the solvents used, methanol had the lowest egg-hatching rate (12.89 eggs), followed by hexane (17.78 eggs) and butanol (22.78 eggs). The maximum number of eggs were hatched in water (32.33 eggs). The number of eggs hatched also decreased from 25.92 (24 hours) to 21.50 (48 hours) and 16.92 (72 hours). The results of the current study are also in agreement with the studies of Elango (2009), who had reported 86% and 100% ovicidal activity at 500 ppm and 1000 ppm, respectively, among An. Subpictus and Aedes aegypti. In a separate study, the methanol extract of A. paniculata demonstrated 100% ovicidal activity at a 150 ppm concentration against An. stephensi eggs. The hexane extract of Aegle marmelos had moderate ovicidal effects of 53.6% and 48.8% at 500 ppm concentration on Aedes aegypti eggs. Shoukat et al. (2020) reported that extracts from Sophora alopecuroides exhibited significant antifeedant and oviposition deterrent activities, suppressing the egg hatchability of Aedes albopictus. Meanwhile, Zhiqing et al. (2013) demonstrated the potential of toosendanin as a botanical insecticide to inhibit the egg-hatching rate of Aedes aegypti. Pupae-wise, among the solvents, ethanol showed the highest mortality (69.00%), followed by hexane (62.56%) and butanol (52.89%), while water was the least effective (45.56%). Mortality increased with time, being at least 24 hours (49.08%), moderate after 48 hours (56.50%), and maximum after 72 hours (66.17%). The current study is in agreement with Shehata et al. (2020) regarding the 100% ovicidal and suicidalidal action of ethanol extracts of Pulicaria jaubertii at a low concentration (150 ppm).

Conclusions: Our results indicate that methanol extracts from Nicotiana tabacum were very effective in reducing the number of larvae and pupae of Aedes mosquitoes. The leaf extract of N. tabacum was, however, the most potent, followed by other solvent extracts in suppressing all stages of dengue mosquitoes, including larvae, eggs, and pupae. Thus, N. tabacum leaf extract can be regarded as a potential insecticide used for dengue vector control. These findings are an important step forward in the development of sustainable VBD interventions that rely on plant-based products.

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