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Phytochemical profiling and larvicidal activity of *Zea mays* peel extracts on mosquito vectors

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Abstract
The primary cereal crop in the world, after rice and wheat, is corn (*Zea mays* L.), a member of the *Poaceae* (Gramineae) family. In the current investigation, *Z. mays* peel was extracted using different polarity of solvents and then analyzed using TLC, GC-MS, mosquito larvicidal activity, and qualitative phytochemical screening. The majority of the total weight of *Zea mays* is still made up of peels, which remain the main byproduct. Nowadays, samples of crude extract from peels are sparking interest in the development and manufacturing of alternative insecticides. Peels are the best source of phytochemical compounds or chemical compounds for treating different diseases. The present investigation employed aqueous, chloroform, hexane, ethanol, and methanol solvent systems to extract phytochemicals from corn peels. The extracts' larvicidal efficacy against *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* larvae in their fourth instar was subsequently evaluated. The current investigation's findings showed that the three mosquito vectors under investigation were effective larvicidal activity. The detection of phytocompounds in the peel extract by qualitative phytochemical profiling, TLC, and GC-MS revealed the presence of chemical constituents such as terpenoids, flavonoids, carbohydrates, and tannins, among others. Extracts from *Z. mays* peels thus provide great promise as a biocontrol agent against mosquito vectors. In the future, the bioactive phytocompounds that were isolated could be used to make powerful pesticides.

Keywords: *Zea mays*, larvicidal activity, phytochemical profiling, GC-MS analysis

Introduction

In more than 100 countries, endemic mosquito-borne diseases pose a threat to around 2100 million people worldwide, resulting in at least one million juvenile fatalities and about two million deaths annually (Muhammad *et al*., 2017) [1]. Because of India's perfect climate, mosquito-borne diseases are widespread there (Sumodan Elumalai *et al*., 2016) [2]. Over 553 million individuals in India are at risk of infection due to the endemic status of 17 states and six union territories (Joshi, 2018) [3]. Nearly 50% of health issues in developing nations are caused by diseases spread by mosquitoes, including malaria, dengue fever, filariasis, and viral encephalitis (Anoopkumar and Aneesh, 2022) [4]. Mosquito populations have resurfaced as a result of the frequent use of synthetic insecticides to reduce them, which has interfered with their natural biological control systems. Furthermore, it caused problems for human health and the environment, evolved resistance, and negatively impacted non-target species (Dahmana *et al*., 2020) [5].

The widespread use of synthetic insecticides to control mosquitoes has disrupted traditional natural pest management methods and resulted in an increase in mosquito populations. The emergence of resistance (Dahmana and Mediannikov, 2020) [5], negative impacts on non-target species, and a rise in environmental and human health problems (Khan and Ahmad, 2019) [6] have prompted a quest for alternate control strategies. Plants are thought to be a rich source of bioactive chemicals and may also provide mosquito control agents. Natural plant chemicals with insecticidal qualities have recently been tested for the management of a variety of insect pests and vectors (CE *et al*., 2019) [7].

Zea mays

The plant known as maize, or corn (*Zea mays* L.), belongs to the *Poaceae* family. It is a monoecious annual plant that is widely grown all over the world. Sweet corn (*Zea mays* var. saccharata), flint corn (*Zea mays* var. indurata), and feed corn (Denata) are among the various types of maize. Each part of the maize plant has a specific function, such as feeding animals or people. *Zea mays* peels have long been used to cure a variety of illnesses, despite the fact that its extracts offer a wide range of pharmacological qualities. Many uses have been found, including antioxidative, antidiabetic, antimutagenic, anticoagulant, antifungal, antiadipogenic, antiobesitic, antihypertensive, antihyperlipidemic, antilithiatic, antibiotic, antibacterial, antiseptic, anti-inflammatory, antidepressant, and anti-fatigue effects.

The current study aimed to evaluate the extracts from *Z. mays* peel against *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* mosquito vectors. TLC and GC- MS also examined the extracts for its secondary metabolites.

Materials and Methods

Selection and procurement of fruit peels

We purchased disease-free and nutritious corn (*Zea mays*) (Fig.1), a member of the Poaceae family, from Koyambedu market in Chennai, Tamil Nadu, India. *Zea mays* (L.) corn peel waste was used for the investigation. The Plant Anatomy Research Centre (PARC), located in Tambaram, Chennai, Tamil Nadu, India, identified and authenticated the corn peel waste.

Fig 1: *Z. mays* peels

Selection of mosquito species

For this study, *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* larvae in their fourth instar were selected (Fig. 2). *Aedes aegypti* is an anthropophile that is widely dispersed and extremely domesticated. The arbovirus that causes dengue and dengue hemorrhagic fever is spread by the Aedes species (Foster and Walker, 2019) [13]. The most severe form of malaria is caused by *Plasmodium falciparum*, and all four malaria-causing parasites can be spread by Anopheles species (Saif, 2017) [14]. Wuchereria species that cause lymphatic filariasis, which is common in trophic zones, are disseminated by *C. quinquefasciatus* (Nchoutpouen *et al*., 2019) [15].

Fig 2: Mosquito species selected for the study

Preliminary phytochemical analysis

Phytochemical screening was performed on the extracts using the protocols developed by Senthil Kumar and Reetha (2009) and Nweze *et al*., (2004) [16]. Proteins, carbohydrates, alkaloids, flavonoids, phytosterols and steroids, anthocyanin and beta cyanin, phenols, tannins, saponins, and glycosides were all present in the peel extract samples.

TLC Plate preparation

Aluminum sheets covered with silica gel 60 F 254 were cut to 1.5 x 5.5 cm, and the produced methanol peel extract was placed on a silica plate to air dry.

Mobile phase preparation

The extracts were standardized in Hexane: ethyl acetate: chloroform (2:1:1) ratio, showed separated bands (Luong *et al*., 2021) [18].

GC-MS analysis

The methanol extract was examined by GC-MS (Agilent 7890A-240 MS with Ion Trap). The GC-MS was equipped with an Agilent J&W, HP-5ms silica capillary column that measured 30 m x 0.250 mm x 0.25 m (Agilent Technologies) and was coupled to MSD. Helium (1 ml/min) served as the carrier gas and nitrogen (35 ml/min) as the mobile phase. Individual components discovered by GC were compared to standard chemicals from the NIST and Willey libraries. The chromatogram displays the relative concentration of each fatty acid component.

Larvicidal Bioassay

Three trials against the vector mosquito, each with five duplicates, were carried out for the larval susceptibility test. *A. ageypti*, *A. stephensi*, and *C. quinquefasciatus* larvae in their fourth instar were used to determine the pathogenicity of the crude extract. After dissolving 100 mg of crude extract in 1 ml of acetone, the volume was increased to 100 ml with distilled water to create the extract stock solution (1000 ppm). Twenty larvae in their fourth instar were released into 200 milliliters of deionized water at varying dilutions of 25 ppm, 50 ppm, 75 ppm, 100 ppm, and 150 ppm. Mortality was then recorded after a 24-hour period. The larvae were exposed to 200 ml of water with 0.1 ml of acetone as a control, and the beakers were kept at 29°C to 2°C in a temperature control room. Tonk *et al*., (2006), replicated each treatment five times.

In glass beakers with 200 ml of the peel extract solution, groups of 20 larvae were introduced. Parallel control tests without extraction were performed. The quantity of dead larvae in each solution was counted after a 24-hour incubation period. Abbott's (1925) [20] method was utilized to account for mortality in the control group, and the percentage mortality was calculated using the average of five replicates.

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

Statistical analysis

Probit analysis was performed on the average larval mortality data to determine the LC_{50} and LC_{90} (Finney, 1971) [21] and other statistics at 95% fiducial limits of upper and lower confidence limit. The Han *et al*. (2013) [23] software was used to determine chi-square values. Results were considered statistically significant at $P < 0.05$, using SPSS (V.24).

Results

Qualitative Phytochemical Analysis of *Z. mays*

According to the phytochemical examination, the extracts of *Z. mays* contained significant amounts of phenol, terpenoids, triterpenoids, alkaloids, carbohydrates, saponins, and glycosides. Tanin, cardiac glycosides, and sterods were present in trace amounts in the methanol extract. However, the methanol extract did not include flavonoids, coumarins, quinone, or anthocyanins. Hexane and chloroform extracts likewise showed a moderate amount of phytocompounds when compared to methanol extract aqueous (Table 1).

Table 1: Phytochemical screening of *Z. mays* plant extracts

S. No	Secondary metabolites	Aqueous	Chloroform	Methanol	Hexane
1	Carbohydrate	+++	++	+++	++
2	Tannins	+	-	++	-
3	Saponins	+++	++	+++	+++
4	Flavonoids	+	-	-	-
5	Alkaloids	+	-	+++	++
6	Anthocyanin	++	++	-	+
7	Quinones	-	-	-	-
8	Glycosides	++	+	+++	+
9	Cardiac glycosides	+	-	++	-
10	Terpenoids	+	++	+++	++
11	Triterpenoids	++	-	+++	-
12	Phenols	+	++	+++	++
13	Coumarins	++	-	-	+
14	Fatty acids	-	-	+	-
15	Protein	+	-	+	-
16	Steroids	++	+	++	+++

+++ Strong presence ++ moderate presence + trace amount- absent

Larvicidal activity *Z. mays* peel extracts

Results of the probit analysis between *Z. mays* peel extract concentrations and fourth instar larvae of *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* following a 24-hour exposure are shown in Tables 2, 3, and 4. With LC_{50} and LC_{90} values of 34.656 ppm 97.692 and 35.983, 70.771 ppm, respectively, the methanol peel extracts of *Z. mays* were shown to be more toxic against *C. quinquefasciatus* and *A. stephensi* than they were against *A. aegypti*, which had LC_{50} values of 25.120 ppm and LC_{90} values of 56.397 ppm.

Table 2: Mosquito larvicidal activity of *Z. mays* peel extracts against the fourth instar larvae of *A. aegypti*

Extracts	Concentration (ppm)	24hr % Mortality	LC_{50} (LCL–UCL) (ppm)	LC_{90} (LCL–UCL) (ppm)	Chi-Square
Aqueous	25	24	45.192 24.525±62.975	101.271 71.240±275.993	16.763
	50	48			
	75	70			
	100	95			
	150	100			
Chloroform	25	14	77.576 69.744±86.708	242.612 195.065±331.379	5.086
	50	27			
	75	42			
	100	63			
	150	81			
Ethanol	25	27	41.877 24.501±56.464	94.736 68.692±207.082	13.213
	50	53			
	75	75			
	100	96			
	150	100			
Hexane	25	12	87.201 77.821± 99.104	296.591 228.985±434.732	2.612
	50	25			
	75	39			
	100	59			
	150	73			
Methanol	25	53	25.120 2.713±39.570	56.397 36.997±346.863	18.991
	50	74			
	75	100			
	100	100			
	150	100			

Control- nil mortality, Significant at $p < 0.05$ level, LC_{50}-Lethal concentration that kills 50% of the exposed larvae LC-Lethal concentration that kills 90% of the exposed larvae UCL-Upper confidence limit; LCL-Lower confidence limit

Table 3: Mosquito larvicidal activity of *Z. mays peel* extracts against the fourth instar larvae of *A. stephensi*

Extracts	Concentration (ppm)	24hr % Mortality	LC_{50} (LCL–UCL) (ppm)	LC_{90} (LCL–UCL) (ppm)	Chi-Square
Aqueous	25	21	50.084 5.149±72.839	110.502 75.382±421.632	21.167
	50	38			
	75	66			
	100	92			
	150	100			
Chloroform	25	19	53.465 28.869±77.237	120.381 82.052±449.823	19.749
	50	36			
	75	61			
	100	87			
	150	100			
Ethanol	25	27	39.69 21.819±54.441	80.489 8.234±187.003	16.736
	50	53			
	75	86			
	100	100			
	150	100			
Hexane	25	17	57.780 37.589±78.776	136.725 95.605±369.382	13.547
	50	33			
	75	59			
	100	79			
	150	98			
Methanol	25	30	35.983 22.986±46.769	70.771 53.879±125.760	11.461
	50	62			
	75	93			
	100	100			
	150	100			

Control- nil mortality, Significant at $p < 0.05$ level, LC_{50}- Lethal concentration that kills 50% of the exposed larvae LC_{90}-Lethal concentration that kills 90% of the exposed larvae UCL- Upper confidence limit; LCL- Lower confidence limit

Table 4: Mosquito larvicidal activity of *Z. mays* peel extracts against the fourth instar larvae of *C. quinquefasciatus*

Extracts	Concentration (ppm)	24hr % Mortality	LC_{50} (LCL–UCL) (ppm)	LC_{90} (LCL–UCL) (ppm)	Chi-Square
Aqueous	25	25	41.165 23.206±56.248	84.331 61.100 ±193.661	16.437
	50	53			
	75	81			
	100	100			
	150	100			
Chloroform	25	27	39.074 23.681±51.898	81.314 60.376±159.902	13.028
	50	59			
	75	82			
	100	100			
	150	100			
Ethanol	25	30	35.617 24.170±45.263	69.849 54.310±113.959	9.535
	50	64			
	75	93			
	100	100			
	150	100			
Hexane	25	21	45.156 26.075±61.909	90.930 65.720±218.067	17.799
	50	44			
	75	79			
	100	98			
	150	100			
Methanol	25	34	34.656 10.899±51.791	67.692 45.944±321.577	24.557
	50	59			
	75	98			
	100	100			
	150	100			

Control- nil mortality, Significant at $p < 0.05$ level, LC_{50}-Lethal concentration that kills 50% of the exposed larvae LC_{90}- Lethal concentration that kills 90% of the exposed larvae UCL- Upper confidence limit; LCL- Lower confidence limit

Z. mays ethanol extract exhibited strong larvicidal action against each of the three tested mosquito larvae. In contrast to *A. stephensi* (39.69 ppm and 80.489 ppm) and *A. aegypti* (41.877 ppm and 94.736 ppm), it demonstrated LC50 and LC90 values of 35.617 ppm and 69.849 ppm against *C. quinquefasciatus*, respectively. Comparing the methanol and ethanol peel extracts of *Z. mays* to the other studied extracts (hexane, aqueous, and chloroform), all of them demonstrated moderate mosquito larvicidal efficacy at a comparatively high concentration. The methanol extract underwent an additional Thin Layer Chromatography assay since, in comparison to the other extracts, it revealed strong larvicidal efficacy.

Thin Layer Chromatography analysis of methanol peel extract of *Zea mays*

TLC of a methanolic extract of *Z. mays* revealed 11 main bands with RF values of 0.75, 0.71, 0.60, 0.52, 0.48, 0.46, 0.37, 0.32, 0.29, 0.24, and 0.19. According to Figure 3, these bands represent important substances including triterpenoids, steroids, phenolic compounds, glycosides, anthocyanin, saponin, alcohols, terpenoids, phenols, and alkaloids.

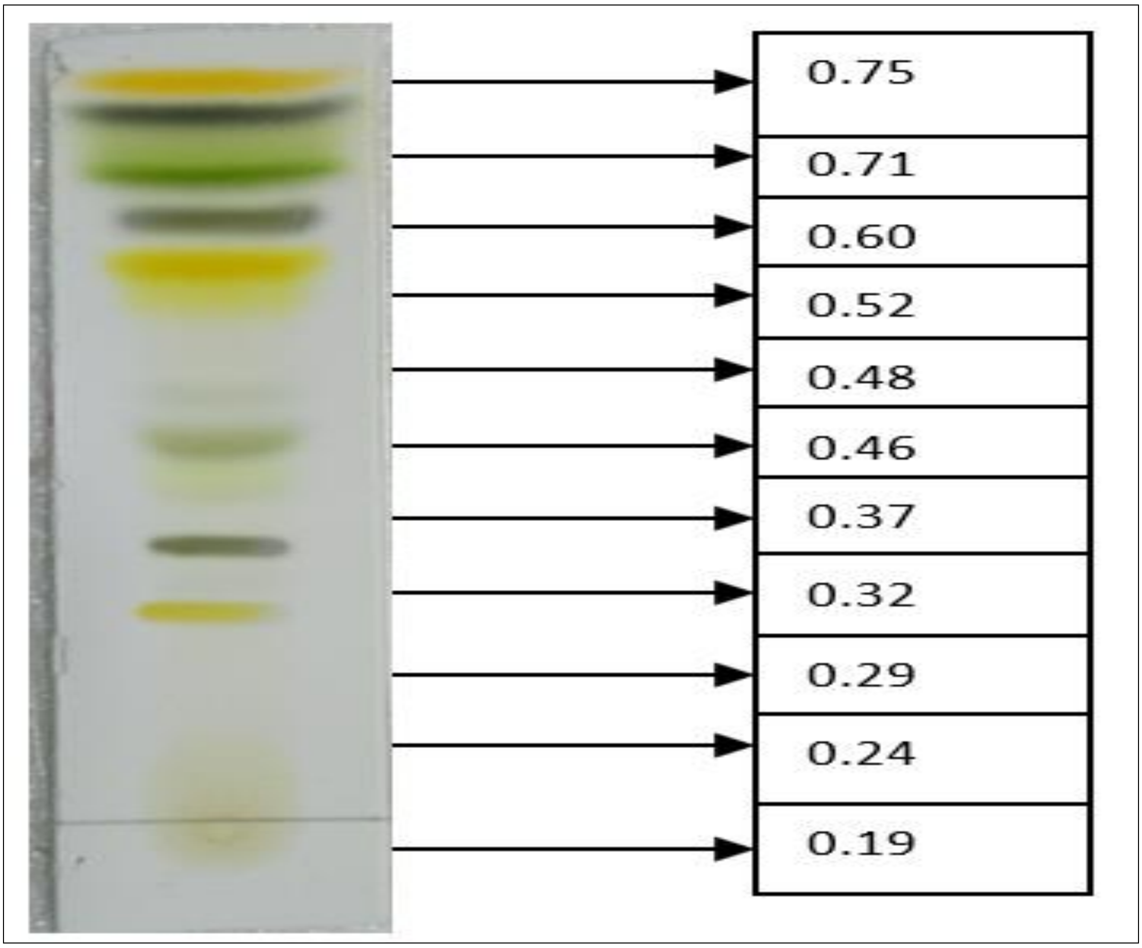

Fig 3: Thin layer chromatography analysis of methanol peel extract of *Z. mays* GC-MS analysis of methanol peel extract of *Z. mays*

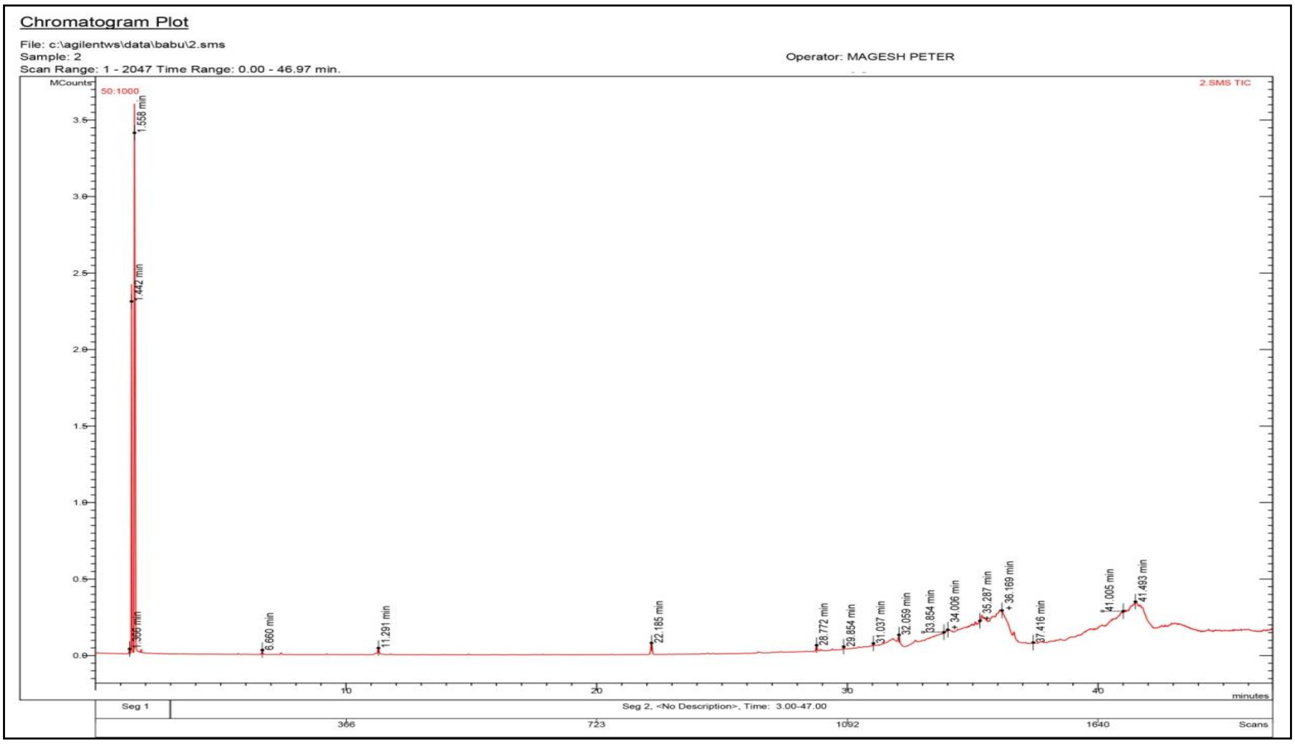

Fig 4: GC-MS analysis of methanol peel extract of *Z. mays*

The chemical composition and identification of the primary compounds found in *Z. mays* methanol peel extracts were shown in Table 5. 17 compounds were identified by GC MS. The main compounds were beta.-Acetoxy-1',1'-dicar , 3,7-Dimethyl-6,7-di(methylth, 7-Octen-2-ol, 2,6-dimethyl-, 5-Hydroxymethylfurfural, Diethyl Phthalate, Neophytadiene, 3,7,11,15-Tetramethyl-2-hexa, Hexadecanoic acid, 14-methyl, Oleic Acid, 18-Pentatriacontanone, Stearic anhydride, 3,9-Epoxypregnane-11,14,18-t, 2-[(1-Hexadecylpyrrolidin-2-, 3,9.beta.:14,15-Diepoxypregn, Octadecanoic acid, 1-[(tetra, n-Hexadecanoic acid, Tetracyclo[11.4.0.0(1,10) (Table 5 and Fig. 4).

Table 5: GC-MS analysis of methanol peel extract of *Z. mays*

S. No	RT	Name of the compound	Peak Area (%)
1.	1.366	17.beta -Acetoxy-1',1'-dicar	67657
2.	1.558	3,7-Dimethyl-6,7-di (methylth	8.79
3.	6.660	7-Octen-2-ol, 2,6-dimethyl-	74448
4.	11.291	5-Hydroxymethylfurfural	125761
5.	22.185	Diethyl Phthalate	292023
6.	28.772	Neophytadiene	142701
7.	29.854	3,7,11,15-Tetramethyl-2-hexa	69349
8.	31.037	Hexadecanoic acid, 14-methyl	45415
9	32.059	Oleic Acid	220268
10	33.854	18-Pentatriacontanone	9264
11	34.006	Stearic anhydride	39151
12	34.394	3,9-Epoxypregnane-11,14,18-t	6152
13	34.990	2- [(1-Hexadecylpyrrolidin-2-	27613
14	35.155	3, 9. beta.:14,15-Diepoxypregn	13304
15	35.287	Octadecanoic acid, 1- [(tetra	52772
16	36.641	n-Hexadecanoic acid	134800
17	37.416	Tetracyclo [11.4.0.0(1,10).0(	4321

Discussion

Multiple investigations on mosquito control have shown that various phytochemicals made from various plant species are effective against various mosquito species (Ali *et al*., 2013) [24]. Our results indicated that *Z. mays* peel extracts in water, methanol, and hexane chloroform demonstrated improved larvicidal activity against all three of the mosquito vectors under investigation. There has never been any prior research on the effectiveness of *A. comosus* peel extracts as larvicidal agents.

The results aligned with the 2020 observation made by Rodrigues. Marin *et al.*, (2020) [25, 26] investigated the efficacy of *C. sinensis* ethanolic peel extract against *A. aegypti* and *C. quinquefasciatus* and found that it was more effective against *A. aegypti*, with an LC_{50} value of 92.27 ppm, whereas the value reported for *C. quinquefasciatus* was 244.70 ppm. These results are in line with their findings.

When evaluated against *A. aegypti*, the methanol peel extract of *Z. mays* shown a high propensity for irritation, with LC_{50} and LC_{90} values of 25.120 ppm and 56.397, respectively. Significant mortality against *A. stephensi* was shown by the extracts' LC_{50} and LC_{90} values for *C. quinquefasciatus*, which were 34.656 ppm and 67.692 ppm, respectively. When adults of *C. limetta* and *C. sinensis* were exposed to leaf extracts of *Parthenium hysterophorus* made in different solvents, Kamaraj *et al.*, (2023) [29] found that *A. aegypti* exhibited a comparable repellency behavior.

The results of this study are similar to those of earlier works by Mahalakshmi *et al.*, (2018) [30]. GC MS analysis of *Z. mays* methanol extracts confirms the results of the phytochemical screening. The chemicals that were most prevalent were beta. 7, Octen-2-ol, 2,6-dimethyl-, 5-Hydroxymethylfurfural, Diethyl Phthalate, Neophytadiene, 3,7,11,15-Tetramethyl-2-hexa, Hexadecanoic acid, 14-methyl Oleic Acid, Acitoxy-1',1'-dicar, and 3,7-Dimethyl-6,7-di(methylth). Terpenoids are typically thought to be responsible for the larvicidal effects on mosquito larvae. Therefore, the high mortality rate seen in *Ocimum sanctum* plant extracts may be due to the presence of terpenoids and triterpenoids, which are hydrocarbons present in the extract and inhibit the developmental stages of insects (Velu *et al.*, 2015 [31]; Baseer and Jain, 2016 [32]; Saravanan, 2022 [33]; Dhama *et al.*, 2023) [34].

The significant presence of tannins, saponins, phenols, quinones, triterpenoids, cardiac glycosides, alkaloids, and terpenoids found in *Z. mays* methanol peel extracts were confirmed by TLC and GC MS analysis. The ethanol extract from the peel of *Zea mays* was qualitatively analyzed to identify the main active ingredients, which included terpenoids, glycosides, cardiac glycosides, carbohydrates, and tannins.

The results of this study showed that the fruit peels of *Zea mays* had a larvicidal effect on the mosquito species that was being studied that was mild, moderate, or extremely substantial. The effectiveness of botanical extracts against mosquito development stages may be influenced by the extraction solvent (Veni *et al.*, 2017) [35], and the solvents' polarity may also have an effect (Raveen *et al.*, 2017) [36].

The methanol peel extracts of *Zea mays* were found to have a very strong larvicidal effect at relatively low concentrations when compared to other plant extracts. This has allowed for additional investigation on their efficacy.

Conclusion

Our research has clearly demonstrated that *Zea mays* waste peels may be used as powerful mosquito-controlling agents. Utilizing wastes as useful products would not only help manage unmanageable waste release and decrease waste load, but it would also lower the pollution load and enhance the environmental profile of the fruit juice manufacturing business.

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