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Synergistic Larvicidal and Pupicidal Effects of *Tridax Procumbens* and Marigold Flower Waste with *Bacillus Thuringiensis* on *Culex* Species: A Comprehensive Evaluation

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Abstract:
Mosquito-borne diseases indeed pose significant health threats, particularly in tropical and subtropical regions where favorable conditions for mosquito breeding are prevalent. Conventional insecticides have historically been the primary method of controlling mosquito populations. However, the indiscriminate use of neurotoxic insecticides has led to several adverse consequences including environmental toxicity, harming non-target organisms, disrupting ecosystems, and the development of insecticide resistance in mosquito populations. To address these challenges, there's a growing emphasis on developing alternative mosquito control strategies that are more sustainable and environmentally friendly. In the current study the larvicidal and pupicidal activity of combination of *Tridax procumbens* leaves extract, Marigold flower waste, and *Bacillus thuringiensis (Bt)* on *Culex* species was evaluated. Doses ranging from 10 to 450 ppm were tested for their larval and pupal insecticidal effects on *Culex sp*. mosquitoes in controlled laboratory and outdoor environments. The LC_{50} values were determined for extracts from *Tridax procumbens* leaves and Marigold flowers waste using probit analysis. The combined methanolic plant extracts from *Tridax procumbens* leaves and Marigold flower debris displayed larvicidal effects, showing the greatest mortality rate with LC_{50} values of 99.53, 135.1, 249.4 and 370.8 ppm against II^{nd}, III^{rd}, IV^{th} larvae instars and pupa respectively. *Bacillus thuringiensis (Bt)* demonstrated its best activity with LC_{50} values of 31.62, 51.04, 69.93 and 120.2 ppm against II^{nd}, III^{rd}, IV^{th} larvae instars and pupa respectively. However when a combined therapy was given LC_{50} values were 43.24, 69.61, 204.9 and 274.8 ppm. No mortality was observed in the control. These findings indicate that the methanol extracts derived from *Tridax procumbens* leaves and Marigold flower waste, along with the use of *Bacillus thuringiensis (Bt*), holds promise as an environmentally friendly method for managing the malarial vector, *Culex sp*., a primary target in vector control initiatives. This research represents the initial documentation of the joint larvicidal and pupicidal effectiveness of these plant extracts and bacterial toxin agent against *Culex sp.* mosquitoes.
Key Words: *Tridax procumbens*, Marigold flower waste, *Bacillus thuringiensis*, larvicidal activity, Pupicidal activity, *Culex sp.*

Introduction:

Mosquito-transmitted illnesses, such as malaria, persist as a considerable global public health concern. Among the numerous mosquito types accountable for disseminating these ailments, the *Culex* species, colloquially referred to as the southern house mosquito, serves as a predominant carrier for filariasis and various other diseases[1]. It remains imperative to implement efficient methods for controlling these disease vectors to curtail disease transmission. Prioritizing the creation of ecologically sound and sustainable strategies assumes pivotal significance in this regard[2]. In recent studies, researchers have been examining the use of plant extracts and

microorganisms as potential means to combat mosquito vectors[3]. Within this framework, the current research seeks to explore the larvicidal and pupicidal impacts of combining extracts derived from *Tridax procumbens* leaves, discarded Marigold flowers, and *Bacillus thuringiensis (Bt)* on the larvae and pupae of *Culex* species.

The universal and hardy blooming plant *Tridax procumbens*, commonly referred to as coat buttons or wild daisies, has drawn the interest of researchers and herbal experts due to its exceptional therapeutic qualities[4]. This *Asteraceae*-family herbaceous plant is indigenous to many regions of the earth, including South America, Asia, and Africa[5]. *Tridax procumbens* has many medical benefits, including anti-inflammatory[6], antioxidant[7], antibacterial[8], and analgesic properties[9]. Synthetic pesticides, which frequently pose threats to both human health and the environment, can be replaced by these natural substances as an environmentally conscious option[10].

Marigold flowers waste, a byproduct of the florist business, are frequently thrown away. However, it includes a complex blend of bioactive substances, such as terpenoids, flavonoids, alkaloids, and essential oils, that may have repellent effects[11,12]. Since these substances naturally repel insects, marigold flower debris is a desirable option for eco-friendly and sustainable pest control methods[13].

A Gram-positive, spore-forming bacterium known as *Bacillus thuringiensis (Bt)* has attracted a lot of interest and relevance in the field of pest control, notably for its larvicidal action against a variety of insect pests[14]. *Bt*-based biopesticides are proven as an efficient and sustainable alternative to chemical insecticides considering the bacterium's inherent pesticidal characteristics[15]. Contrary to synthetic pesticides, *Bt*-based products are biodegradable, non-toxic to humans and animals, and do not encourage pests to develop pesticide resistance[16].

Botanical extracts have garnered considerable attention for their potential in insect management owing to their natural origins and relative harmlessness to non-target organisms[3]. *Tridax procumbens*, a widely distributed weed, and Marigold flowers have both exhibited promise in prior investigations for their insecticidal attributes[17]. *Bacillus thuringiensis*, a bacterial insecticide, is also noted for its selectivity towards particular insect species, rendering it a valuable contender for integrated vector control approaches[18].

The current research encompassed an extensive evaluation of various concentrations (ranging from 10 to 450 ppm) of these extracts and *Bacillus thuringiensis* against *Culex sp.* mosquitoes, undertaken in both controlled laboratory settings and outdoor environments. The lethal concentration values, denoted as LC_{50} and LC_{90}, for the extracts sourced from *Tridax procumbens* leaves and discarded Marigold flower components, were ascertained through probit analysis.

Materials and Methods:

Collection of plant materials and authentication:

In the present study, two aromatic plants were used. *Tridax procumbens L.* was obtained from Sanjivani Hill (18°31'59"N, 76°46'54"E), which is located in Latur, Maharashtra. Plant specimen were identified and authenticated by botanist and were deposited in Blatter herbarium, St. Xavier's College, Mumbai, India and Marigold flower waste was obtained from the temples in Latur during Mahashivratri.

Preparation of extracts:

The methanolic extract was prepared by taking 40 grams powder of the plant materials in 400 ml of methanol. For four days, this mixture was shaken at 50 rpm on an orbital shaker. After 4 days the mixture was filtered using Whatman filter paper No. 1. The filtrate was allowed to evaporate using rotary evaporator and extractive values were calculated in terms of percentages. For subsequent use, the extract was preserved in the refrigerator. The stock solution of the extract was prepared by taking 100 mg extract per ml of Dimethyl sulfoxide (DMSO). The stock solution was kept chilled, at 4°C[19]. The percentage of yield (*w*/*w*) was calculated using the below mentioned formula[20].

Percentage of yield = (Weight of dry extract)/(Weight of plant material) × 100

Phytochemical screening:

The extracts obtained from the dried leaves of *Tridax procumbens L.* and marigold flowers were subjected to the analysis for detection of phytochemicals. Preliminary phytochemical screening was carried out for the detection of alkaloids, saponins, tannins, sterols, flavonoids, phenols and anthraquinones [21, 22]. The chemicals and reagents used for the above tests were freshly prepared in laboratory.

Soil sample collection:

Soil samples were collected from the maize field (*Zea Mays L.*) in Zari Bk., Latur district, Maharashtra, India. 20g of samples were retrieved from a 5 to 10 cm depth by scraping soil surface with a sterile spatula. Samples were transferred in sterile plastic bags and were stored at 4°C until further use.

Bacterial isolation and culturing:

In 100 ml of St. Nutrient broth (with 1% starch), 1g of soil sample was added, it was incubated at room temperature for 48 to 72 hours. Following incubation, 1 ml of the enrichment broth was diluted 10-fold and surface plating was done to get isolated colonies. By using the streak plate method, selected colonies were grown on M9 medium agar medium for further purification[23]. The colonies showing Bt like colony morphology: rough, white, and spread out over the plate were sub-cultured on nutrient agar plates and incubated for 24 h at 37°C. They were stored using nutrient agar slants at 4°C until further use.

Morphology identification:

Isolated colonies were subjected to Gram's staining, Negative staining (an acidic dye, Nigrosin), Spore staining (Malachite green, decolorizer and safranin) and CBB staining (0.133% Coomassie Brilliant Blue (CBB) G250 in 50% acetic acid). Characterization of bacteria was performed by conducting biochemical tests that are presented in the form of descriptive data[24].

Cry protein extraction:

The liquid culture medium was centrifuged at 8000 RPM, 4°C for 10 min and the pellet obtained was washed twice by suspending in 1 M NaCl containing 0.01% Triton X-100. The pellet was then suspended in 1ml of saline solution. 100 µl hexane was added to a ratio ≤10% (100 µl/ml of aqueous suspension). The suspension was sonicated at 100W for 10 min and later centrifuged at 6000 RPM for 10 min. The obtained pellet was resuspended in a saline solution, the organic solvent was added again, and the same procedure was repeated 3 times. Finally, the pellet was washed twice with cold distilled water by centrifuge at 6000 RPM for 10 min.

Collection of mosquito larvae:

Mosquito larvae were collected from unused water fountain located in the botanical garden premises. They were kept separate in different plastic trays and fed with a powdered brewer's yeast, until they reached the pupal stage[25]. The larvae were kept free from exposure to pathogens, insecticides, or repellants and maintained at 30–35°C. The transformed pupae were separated manually with a dropper into a glass beaker (300 mL) filled with normal tap water. The beaker was kept in glass cages for the emergence of adult mosquitoes.

Identification of mosquito larvae and pupae:

Mosquito larvae from the collected stagnant water bodies were identified morphologically using a light microscope. Various characteristics of the mosquito larvae were considered for the identification as follows[26],

1) The size and orientation of the siphon of the larvae
2) The number of hair tufts present beyond pectin
3) The orientation of the body of the larva while it breathes at the water surface
4) The type and speed of mobility of the larvae in the water body

Mosquito larvae and their lifecycle were identified by using the Photographic manual of mosquito identification.

Preparation of test and control solutions:

1 gm of the dried methanol extract of *Tridax procumbens* leaves and Marigold flower waste were placed in a standard measuring flask with distilled water to prepare 100 ml of 1% stock solution; From the 1% stock solution, concentrations of 50, 150, 250, 350, 450 ppm were prepared by adding the appropriate volume of dilution. Distilled water was used as the negative control.

Larvicidal and pupicidal activity test:

The bioassay was done as per the standard method of WHO[2]. Combined methanolic extracts of *Tridax procumbens* leaves, Marigold flower waste and *Bacillus thuringiensis* were assessed using the WHO standard method. Five concentrations of cry protein extracts (10, 30, 50, 70 and 90 ppm) were prepared using the McFarland turbidity standard (Standard number 0.5, 1, 2 and 3)[28] . Five different concentrations (50, 150, 250, 350 and 450 ppm) of extracts were tested against freshly moulted second (IInd), third (IIIrd) and fourth (IVth) instar larvae of *Culex sp.* and pupa of *Culex sp*. Twenty numbers of second to fourth instar larvae and pupae were introduced into a 300 ml glass beaker containing 149 ml distilled water and 1 ml of desired concentrations of plant extract (dilution was done by distilled water)[29,30]. At each tested concentration, two trials were made and each trial consisted of two replicates. The control was set up by providing 150 ml of distilled water. Larval food was added to all tested and control beakers. The larval mortality was observed and recorded after 24 h of post treatment. The mortality rates were recorded by using Abbott's formula[31]:

The corrected mortality = ([% mortality in treatment – % mortality in control]/[100 – % mortality in control]) × 100.

Field trail:

Field trial larvicidal activity was conducted in a 500-liter Sintex tank (Sintex Reno) containing 300 liters of drinking water. Based on laboratory LC_{50} and LC_{90} values, the total surface area of the drinking water tank in each habitat was estimated to determine the necessary amount of plant extract residues and *Bacillus thuringiensis (Bt)* for the field trial. The required amounts of *T. procumbens*, marigold flower, and *Bt* extracts were thoroughly combined with water in a bucket, and the mixture was continuously stirred. 0.05 percent of cornstarch was used as an emulsifier[32]. *T. procumbens* and marigold flower extract mixture was evenly spread with *Bt* onto the surface of the drinking water in each habitat using a pressure sprayer bottle. Larval density was previously determined via dipper sampling[33] and counting 24, 48, and 72 hours after the treatment. In order to identify the structure of every larval habitat, a different sample was taken. For combined *T. procumbens*, Marigold flower extracts and *B. thuringiensis* alone and combination of the treatment, two trials were done. The following formula was used to get the percentage of reduction:

*Reduction percentage = C-T/C * 100*;

Where C is the overall mosquito population under control and T is the overall mosquito population under treatment[34].

Determination of LC_{50} and LC_{90} of the crude extracts against larvae and pupa:

Statistical analysis of the experimental data was performed using the computer software MS EXCEL 2021 to find the regression equations (Y = mortality; X = concentrations) and regression coefficient values. The average mortality after 24 h was recorded and used to determine LC50 and LC90 values[35]. For means comparisons to find the significant difference ($p<0.05$ was considered as significant) using IBM Statistical Package for the Social Sciences (SPSS); windows 64-bit. The LC_{50} and LC_{90} (LC required to kill 50% and 90% of larvae) were calculated by Probit analysis using SPSS[36].

Results and Discussion:

Plant material and extraction:

The plant specimen was identified by Dr. Rajendra D. Shinde of St. Xavier's College in Mumbai, India, as *Tridax procumbens L.* and deposited in the Blatter Herbarium, Department of Botany, with specimen number 5524. For drying, a known quantity of (40gm each) was left at room temperature. Both plant materials were then dried and ground into powder using a mixer. Before usage, the powdered components were kept in sealed polythene bags.

The extracts were checked for its percentage yield:

The percentage of extraction yield for methanolic extracts ranged from 10 to 17% (w/w), as indicated in (Table 1). *Tridax procumbens L*. was found to be able to produce a dry weight yield of 10.6% (w/w) and a dry weight yield of 16.2% (w/w) for extract of marigold flower waste.

Table 1: Weight and percentage yield of methanolic extracts from the plant samples.

Plant sample	Part	Percentage yield (w/w %) Dry methanolic extract
Tridax Procumbens	Leaves	10.6
Marigold flower waste	Flowers	16.2

Determination of phytochemical screening of plant extracts:

In the present study, the analysis of qualitative phytochemical screening was done in methanol leaves extract of *Tridax procumbens* and marigold flowers waste. The presence of alkaloids, saponins, tannins, sterols, flavonoids, phenols and anthraquinones was detected in the extracts (Table 2). Alkaloids, saponins, tannins, sterols, flavonoids and phenols were observed in

methanolic extract of *Tridax procumbens L.* except anthraquinones. In case of marigold flowers waste extract all phytochemicals were present.

Table 2: Qualitative estimation of phytochemicals in methanolic extract of *Tridax procumbens L.* and Marigold flowers waste.

Sr. No.	Phytochemicals	*Tridax procumbens L.*	Marigold flowers waste
1.	Alkaloid	+	+
2.	Saponins	+	+
3.	Tannins	+	+
4.	Steroids	+	+
5.	Flavonoids	+	+
6.	Phenol	+	+
7.	Anthraquinones	-	+

(+) Presence; (-) Absence

Isolation of bacterial culture:

The 8 (C1-C8) distinct colonies from 1% starch agar plates, based on appearance were chosen (Table 3).

Table 3: Colony characteristics of 8 different isolates

Isolates	Shape	Margin	Elevation	Texture	Color	Size	Gram Stain	Spore-Forming
C1	Circular	Entire	Raised	Rough	Whitish/Creamy	Medium	Gram-positive	Yes (Forms endospore)
C2	Circular	Irregular	Mucoid	Smooth	Whitish/Creamy	Medium	Gram-positive	Yes
C3	Circular	Entire	Raised	Rough	Whitish/Creamy	Medium	Gram-positiv	Yes

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C4	Circular	Entire	Raised	Rough	Whitish/Creamy	Medium	Gram-positive	Yes
C5	Circular	Entire	Raised	Rough	Whitish/Creamy	Medium	Gram-positive	Yes
C6	Irregular	Entire	Raised	Rough	Whitish/Creamy	Small	Gram-positive	Yes
C7	Circular	Entire	Raised	Rough	Whitish/Creamy	Medium	Gram-positive	Yes
C8	Circular	Entire	Raised	Rough	Whitish/Creamy	Medium	Gram-positive	Yes

Characterization of bacteria:

Characterization of *Bacillus thuringiensis* was performed by conducting catalase test, oxidase test, methyl red test, citrate utilization test, urease test, starch hydrolysis test, indole production test and sugar fermentation tests. The results of the tests are presented in the form of descriptive data in (Table 4).

Table 4: Biochemical tests to understand bacterial metabolic capabilities.

Test	C1	C2	C3	C4	C5	C6	C7	C8
Catalase test	+	+	+	+	+	+	+	+
Oxidase test	-	-	-	-	-	+	-	-
Growth on MacConkey Agar	-	-	-	-	-	-	-	-
Methyl Red test	-	-	-	-	-	+	-	-
Citrate Utilization test	-	-	-	-	-	-	-	-
Urease test	-	-	-	-	-	-	-	-
Starch Hydrolysis test	+	+	+	+	+	+	+	+
Indole production test	-	-	-	-	-	+	-	-

Fermentation of Sugars (Glucose, Lactose)	-	-	-	-	-	+	-	-

(+) Presence; (-) Absence

Identification of mosquito larvae:

Mosquito larvae and their lifecycle were identified by using photographic manuals of mosquito identification. *Culex sp*. larvae usually hang on the water surface. They have a well-developed head with mouth brushes, large thorax and eight segmented abdomen. In the larva phase, they have no legs. Siphones are found on the eighth abdominal segment. The body of *Culex sp.* larvae are stout and wings are unspotted[6].

Larvicidal and pupicidal activity:

After being treated with methanol extracts of *T. procumbens* and Marigold flower waste extract, *Culex* species larval and pupal mortality was noted. The mortality of *Culex sp*. larvae and pupae (II to IV instars and pupa) after exposure to *T. procumbens* + Marigold flower waste extract at several doses (50 to 450 ppm) is shown in (Table 5). At 50 ppm, *T. procumbens* + Marigold flower treatment caused 40% mortality in IInd instar larvae; however, at 450 ppm, 90% mortality resulted from *T. procumbens* + Marigold flower treatment. For all *Culex sp.* instars treated with varying concentrations of plant extracts, a similar tendency has been observed. The values for the LC_{50} and LC_{90} were as follows: for the IInd instar, the LC_{50} value was 99.53 ppm, for the IIIrd instar, 135.1 ppm, and for the IVth instar, 249.4 ppm, respectively. The LC_{90} values for the IInd instar, IIIrd instar, and IVth instar were 787.2 ppm, 1860.0 ppm, and 3715.9 ppm, respectively. Pupae had an LC_{50} value of 370.8 ppm and an LC_{90} value of 3239.7 ppm, respectively.

Table 5: Larval and pupal toxicity effect of *T. procumbens* + Marigold flower waste extract against *Culex sp.*

Mosquito life stage	% of larval and pupal mortality					LD_{50} (LCL-UCL)	LD_{90} (LCL-UCL)	P-value	Chi-square Value	Regression equations Y=a+bx
	50 ppm	150 ppm	250 ppm	350 ppm	450 ppm					
IInd Instar	40	50	65	80	90	99.533 (38.38-153.8)	787.2 (420.4-5199.5)	0.05	2.463	3.01+1.51*x
IIIrd Instar	35	45	60	70	75	135.1 (43.32-229.9)	1860.0 (685.8-221791.6)	0.05	0.668	2.4+1.13*x
IVth Instar	25	35	50	55	65	249.4 (135.6-677.9)	3715.9 (1043.3-4962356.1)	0.05	0.182	2.59+1.08*x

Pupa	15	25	35	50	60	370.8 (238.3-1106.6)	3239.7 (1093.2-290790.1)	0.05	0.932	3.37+1.31*x

Table 6 displays the *Culex sp.* larval mortality (II to IV instars and pupa) following treatment with *B. thuringiensis* at various dosages (10 to 90 ppm). At IInd instar larvae, *B. thuringiensis* treatment at 10 ppm resulted in a 30% mortality rate; however, at 90 ppm, the mortality rate jumped to 80%. All of *Culex sp.'s* instars have shown comparable patterns at various intensities. The values for the LC_{50} and LC_{90} were as follows: for the IInd instar, the LC_{50} value was 31.62 ppm, for the IIIrd instar, it was 51.04 ppm, for the IVth instar, it was 69.93 ppm, and for the pupa, it was 120.2 ppm. The LC_{90} values for the IInd instar, IIIrd instar, IVth instar, and pupa were 271.4 ppm, 530.05 ppm, 760.5 ppm, and 1761.1 ppm, respectively.

Table 6: Larval and pupal toxicity effect of *B. thuringinesis* against *Culex sp.*

Mosquito life stage	**% of larval and pupal mortality**					**LD_{50} (LCL-UCL)**	**LD_{90} (LCL-UCL)**	**P-value**	**Chi-square Value**	**Regression equations Y=a+bx**
	10 ppm	30 ppm	50 ppm	70 ppm	90 ppm					
IInd Instar	30	40	55	77	80	31.62 (16.39-49.55)	271.4 (124.2-3730.2)	0.05	1.684	2.06+1.38*x
IIIrd Instar	25	30	40	55	75	51.04 (30.96-109.9)	530.05 (185.7-41394)	0.05	3.311	2.12+1.25*x
IVth Instar	20	25	35	50	65	69.93 (43.33-237.3)	760.5 (229.0-208.39)	0.05	2.187	2.2+1.19*x
Pupa	15	20	30	40	50	120.2 (64.5-4387.4)	1761.1 (335.5-571.1)	0.05	0.88	2.2+1.05*x

For all larval instars, Table 7 shows the combined larval mortality following treatment with *T. procumbens* + Marigold flower and *B. thuringiensis*. For IVth instar larval mortality, the concentration at 250+50 combined *T. procumbens* + Marigold flower + *B. thuringiensis* treatment was 85%. Following are representations of LC_{50} and LC_{90} values: The LC_{50} values for the second instar, third instar, and fourth instar were 43.24 ppm, 69.61 ppm, and 204.9 ppm, respectively. The LC_{90} values for the IInd instar, IIIrd instar, and IVth instar were 253.6 ppm, 357.0 ppm, and 1657.3 ppm, respectively. $P < 0.05$ is considered significant for the Chi-square values. Additionally, the LC_{50} and LC_{90} (LCL-UCL) values at 95% confidence levels were computed. After 24 hours of exposure, death of larvae and pupae was noted. There was no mortality in the control group.

Table 7: Combined effect of Larval and pupal toxicity effect of *T. procumbens* + Marigold flower waste extract and *B. thuringinesis* against *Culex sp.*

Mosquito life	**% of larval and pupal mortality**	**LD_{50} (LCL-**	**LD_{90} (LCL-UCL)**	**P-value**	**Chi-square**	**Regression equations**

stage	50 +10 ppm	150 +10 ppm	150 +30 ppm	250+30 ppm	250+50 ppm	**UCL)**			**Value**	**Y=a+bx**
IInd Instar	65	70	85	90	100	43.24 (2.838-78.11)	253.6 (165.8-1044.7)	0.05	4.363	1.98+1.28*x
IIIrd Instar	50	65	75	85	95	69.61 (19.31-105.64)	357.0 (231.9-1390.5)	0.05	2.208	3.73+2.01*x
IVth Instar	45	55	70	80	85	204.9 (127.05-476.3)	1657.3 (606.9-1135.2)	0.05	1.131	3.23+1.4*x
Pupa	40	45	55	65	75	274.8 (197.1-650.5)	1403.4 (613.6-5137.1)	0.05	1.34	4.23+1.73*x

In the drinking water sintex tank, 110 *Culex sp.* larvae were discovered. Larval density was decreased by 20.91%, 44.55%, and 79.1% at 24, 48 and 72 hours following treatment with *T. procumbens* + marigold flower extracts. Similar to this, following treatment with *B. thuringiensis,* the reductions in *Culex* sp. larval densities were 16.37%, 34.55%, and 72.7%, respectively. At 24, 48, and 72 hours, respectively, *T. procumbens,* Marigold flower, and *B. thuringiensis* had a combined impact that was 36%, 70%, and 100% (Tables 8, 9).

Table 8: Field study by using plant extracts of *T. procumbens* + Marigold flower waste and *B. thuringiensis* drinking water tank against *Culex sp.*

Trial Sets	**Before treatment**	**After treatment**					
		***T. procumbens* + Marigold flowers waste**			***B. thuringiensis***		
		24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
1	60	49	35	13	52	41	18
2	50	38	26	10	40	31	12
Average	55	43.5	30.5	11.5	46	36	15
Total	110	87	61	23	92	72	30

Table 9: Field study by using combination effect of plant extracts of *T. procumbens* + Marigold flowers waste + *B. thuringiensis* drinking water tank against *Culex sp.*

Trial Sets	Before treatment	After treatment		
		24 hrs	48 hrs	72 hrs
1	60	35	18	0
2	50	29	12	0
Average	55	32	15	0
Total	110	64	30	0

Conclusion:

The current findings unveiled the potent larvicidal and pupicidal effects of *Tridax procumbens* and Marigold flower extracts in tandem with *Bacillus thuringiensis* (Bt) against *Culex* species, addressing the critical need for sustainable mosquito control strategies. Methanolic extraction yielded noteworthy yiedls with *Tridax procumbens* leaves producing 10.6% (w/w) and Marigold flower waste yielding 16.2% (w/w). Phytochemical screenings revealed the presence of alkaloids, saponins, tannins, flavonoids, and phenols in these extracts, emphasizing their potential as natural insecticidal agents.

Controlled laboratory bioassays demonstrated dose-dependent mortality rates in *Culex* larvae and pupae upon exposure to the combined extracts. The calculated LC_{50} and LC_{90} values delineated the concentrations necessary for 50% and 90% mortality, underscoring the efficacy of these extracts at varying doses. Individual extracts also displayed promising insecticidal properties, suggesting their potential as standalone agents for mosquito control.

Field trials corroborated laboratory findings, showcasing reductions in larval densities post-treatment with the combined extracts and Bt. The practical efficacy observed in real-world settings emphasizes the viability of these natural alternatives for mosquito vector control programs. Their demonstrated effectiveness, coupled with their environmentally friendly nature, suggests potential integration into integrated vector management strategies aimed at curbing mosquito-borne diseases.

These findings highlight the significance of exploring eco-friendly alternatives to synthetic pesticides for mosquito control. The study's comprehensive approach, encompassing extraction methods, phytochemical screenings, bioassays, and field trials, lays a robust foundation for considering *Tridax procumbens* and Marigold flower extracts, along with *Bacillus thuringiensis*, as promising components in the arsenal against mosquito-borne illnesses. Further investigations and application-based studies are essential to validate their efficacy, safety, and practicality in real-world scenarios, potentially revolutionizing mosquito vector control with sustainable, nature-based solutions.

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Conflict of interest:

The authors declares that there is no conflict of interest.

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