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ANTIBACTERIAL, TOXICITY & LARVICIDAL POTENTIALITY OF MICROCOS PANICULATA BARKS

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Abstract

The objective of this study was to examine the phytochemical constituents along with the antibacterial, toxicity & 4th instar Culex quinquefasciatus mosquito larvicidal potentiality of BME (Bark Methanol Extract), BCE (Bark Chloroform Extract) & BWE (Bark Water Extract) from Microcos paniculata. Phytochemical constituents were determined through the various qualitative tests including Molisch's test, Fehling's test, Mayer's test, Wagner's test, Dragendorff's test, Frothing test, FeCl3 test, Alkali test, Salkowski's test and Baljet test. The different assay methods were used that were accompanied by agar disc diffusion, brine shrimp lethality bioassay (BSLB) & standard WHO protocol with slight modification for evaluating the antibacterial, toxicity & 4th instar Culex quinquefasciatus mosquito larvicidal potentiality. BME exposed a broad spectrum of antibacterial activity in contrast to other extracts, particularly most significant against the gram negative bacteria, Escherichia coli & Serratia spp. (zone of inhibition 32 mm in both cases). Besides, the BCE was found the most toxic to Brine Shrimp nauplii, with LC50 of 73.3 µg/ml. Both the BME & BCE exhibited considerable larvicidal effects with LC50 of 299.2 µg/ml & 360.2 µg/ml respectively.In total, these results suggest the beneficial role of BME against some gram positive & gram negative bacteria, along with its larvicidal potentiality and substantial toxic effect of BCE.

Key Words: Antibacterial, toxicity, larvicidal, Microcos paniculata, barks.

Introduction

Science has confirmed man's efforts to identify the plants and their medicinal properties through informal discoveries, since the earliest times. About 50% of the drugs used are from the isolated principles of medicinal plants, 25% of the plant origin and 75% drugs are synthetic in nature. Secondary metabolites having a range of molecular structures are synthesized from the primary metabolism, that are found in all parts of the plant. Many researchers seek out for substances with diverse biological actions which have fascinated them [1]. Resistance of the bacteria with a resulting loss of therapeutic effectiveness originates from the past or current misuse of antibiotics [2]. Due to its inexpensiveness, simplicity and requirements of low dose to carry out the brine shrimp assay, it is very appealing in nature [3]. Mankind faced and continuously facing some terrible diseases where mosquitoes act as vectors. Normally mosquitoes are liable dangerously for spreading diseases than all other insects [4]. Human filariasis, which is a disfiguring, disabling and an endemic disease is carried out by *Culex quinquefasciatus* Say (Diptera: Culicidae), a pantropical pest and urban vector of *Wuchereria bancrofti* [5]. Reports show that various plant extracts and their phytoconstituents can harm the mosquitoes [6].

In Bangladesh, *Microcos paniculata* L. of Tiliaceae family is generally known as 'Fattashi or Kathgua'. Being a herbaceous plant, it is commonly dispensed and naturally grown all over the Bangladesh as well as roughly in Vietnam, Sri Lanka, India, Indonesia, Malaysia, Myanmar, China, Andaman and Nicobar (Andaman Islands), Cambodia and Thailand. Traditionally it is used against the insects, fever, diarrhea, dyspepsia, wound healing, hepatitis, colds, heat stroke and helps the digestive system's better activities. Literature study disclosed that *M. paniculata* demonstrated significant larvicidal, good insecticidal, free-radical-scavenging action, antimicrobial, brine shrimp lethality, antidiarrheal, analgesic, preventative effects for coronary heart disease and angina pectoris along with pesticidal activities, α-Glucosidase inhibitory effect, cytotoxic and nicotinic receptor antagonistic activities [7,8,9,10,11,12,13]. From the current information it is clear that the plant may contain some valuable biological properties. Therefore, the present study was aimed to determine the phytochemical constituents along with the antibacterial, toxicity & 4th instar *C. quinquefasciatus* mosquito larvicidal potentiality of different bark extracts of *M. paniculata.*

Materials and Methods

Collection and Identification of Plant Materials

During January, 2013, the fresh barks of *M. paniculata* were collected from Kohua village, Parshuram, Feni, Bangladesh. And identified by the taxonomist at the Bangladesh National Herbarium, where a dried specimen was placed having the accession number 35348.

Preparation of the Plant Extracts

At beginning, the running water was used for washing the barks of *M. paniculata.* Later on, sterile distilled water was utilized for eliminating the dust particles from the barks which were dried under shade for 7 days. A laboratory grinding mill was used for making powder of the dried plant materials. 190g of the powdered materials were extracted through soxhlet apparatus following hot extraction procedure with 900 ml of methanol, chloroform and water separately. After that, Whatman No.1 filter papers were used for filtering the extracts. Hot air oven at 40°C, then dried the extracts that were stored under refrigeration at 4°C for additional experiments.

Preliminary Phytochemical Screening

Freshly prepared *M. paniculata* plant extracts were subjected to different qualitative tests like Molisch's test for carbohydrate; Fehling's test for reducing sugars; alkaloid test by using Mayer's, Dragendorff's and Wagner's reagents; frothing test for saponin; $FeCl_3$ test for tannin; alkali test for flavonoids; Salkowski's test for triterpenoids; Baljet test for finding glycosides

Test for Carbohydrates

Molisch's Test for Carbohydrates

About 500mg crude extracts were dissolved in 5 ml of distilled water separately that were filtered later. Few drops of Molisch's reagent α-naphthol 10% (w/v) in 90% ethanol were added in filtrates. Then 1ml of concentrated H_2SO_4 was poured carefully by side of the test tube. 2minutes later, 5ml of distilled water was added. Positive test was confirmed through the formation of dull violet or red color at the interphase of the two layers [14].

Fehling's Test for Reducing Sugars

2mg crude extracts were dissolved in 1ml of distilled water individually and filtered. Then 1ml mixture of equal parts of Fehling's solution A and B (a ratio of 1:1) was added in the filtrates which were heated by using water bath for few minutes. Formation of brick red precipitate confirmed the presence of reducing sugars [15].

Test for Alkaloids

5 ml of 1 % aqueous HCl was used to stir 50mg of extracts separately following filtration. Then Mayer's, Wagner's and Dragendorff's reagents were used to test the filtrates for the presence of alkaloids [16].

Mayer's Test

1 or 2 drops of Mayer's reagent (Potassion-mercuric iodide solution, 1.36 g mercuric chloride and 5 gm of potassium iodide were dissolved in 100 mL distilled H_2O)) was added to a 2 ml of filtrates by the side of the test tube. Positive test was confirmed by a white creamy precipitate [16].

Wagner's Test

Few drops of Wagner's reagent (Solution of iodine in potassium iodide) were added to the filtrates separately by the side of the test tube and positive test was confirmed through the formation of reddish-brown precipitate [17].

Dragendorff's Test

Addition of 1 or 2ml of Dragendorff's reagent (Bismuth potassium iodide solution, Dragendorff's reagent was made of two solutions. Solution A contained 1.7 g basic bismuth nitrate in 100 mL water/glacial acetic acid (80 mL water and 20 mL glacial acetic acid in a 4:1 ratio), and Solution B contained 40.0 g potassium iodide in 100 mL of water. Both solutions were mixed in following manner to produce 100 mL Dragendorff's reagent. 5 mL Solution A + 5 mL Solution B + 20 mL glacial acetic acid + 70 mL water) to the 2 ml of filtrate solutions was done individually. Formation of orange-red precipitate indicated the positive test [18].

Test for Saponins

Frothing Test

100mg of the crude extracts were dissolved in the 10ml of respective solvents for making stock solutions. Dilution of stock solutions (0.5ml) were made through the addition of 20ml distilled water. After that the diluted test tubes were shaken for 15 minutes. Positive test was confirmed by the formation of foam on the top part of the test tubes [15,19].

Test for Tannins

$FeCl_3$ *Test*

50mg of the crude extracts were dissolved in 5ml distilled water, followed by the addition of few drops of 5% $FeCl_3$. Tannin was confirmed by the presence of bluish black color [20].

Test for Flavonoids

Alkali Test

Alkali test was employed for the confirmation of flavonoids. Few drops of 5% NaOH solution was added to 1ml of stock solutions individually which produced deep yellow color. The color was disappeared in presence of dilute HCl and confirmed flavonoids [20].

Test for Triterpenoids

Salkowski's Test

1ml of $CHCl_3$ was used for shaking the 2mg plant extracts separately. Addition of a few drops of concentrated H_2SO_4 to these solutions by the side of the test tube produced red brown color at the interface confirming positive test [15].

Test for Glycosides

Baljet test

1 ml of the respective plant extract solution was added to the 1ml sodium picrate solution and the transformation of yellow color to orange color confirmed the positive test for glycosides [18].

Test Microorganisms and Preparation of Stock Culture

Department of Microbiology, Jahangirnagar University, Savar, Dhaka, Bangladesh supplied four gram positive bacteria (*Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Bacillus megaterium*) and nine gram negative bacteria (*Shigella boydii, Escherichia coli, Salmonella typhi, Erwinia spp., Vibrio cholerae, Proteus mirabilis, Serratia spp., Salmonella spp., Pseudomonas spp.*) that were verified by gram staining and sub culturing in appropriate selective media on which the action of the plant extracts were tested.

Preparation of Standard Culture Inoculum of Test Microorganisms

2 ml nutrient broth was used for inoculating three or four isolated colonies and the inoculated colonies were incubated by WHO's recommendation as long as the growth in the broth was equivalent with 0.5% Mac-Farland standard.

Antibacterial Assay

For the initial screening of test bacteria, the agar disc diffusion is utilized as an in vitro method [21]. Necessary Petri plates are prepared, following autoclave technique for 15 minutes at 121°C and the Laminar air flow were used to cool them. 20 ml

of media was transferred into each sterile Petri plates aseptically and solidification was happened. By using sterile glass rod, 1 ml inoculum suspension was spread equivalently over the agar medium to get uniform bacterial distribution. Sterile discs that were prepared instantly were loaded by required doses of plant extracts or standard antibiotic and were put gently over the media. After that, for proper diffusion, 1 hour incubation period at 5°C was completed. Again, the incubation was run for 24 hours at 37°C. Through the measurement of apparent zone around the disc, the antibacterial activity was documented.

Brine Shrimp Lethality Bioassay

Toxicity of the plant extracts can be determined through brine shrimp lethality bioassay by the method of Meyer et al [22]. Seawater was used for performing the hatching of *Artemia salina* Leach (brine shrimp eggs) into matured nauplii (Larvae) within 48 hrs at 25^0C. The seawater contained 10 napulii, where the test solutions were added that were diluted serially. Then the number of alive larvae was counted after 24 hrs incubation period that was carried out at 25^0C. In this bioassay, the positive control was vincristine sulfate.

Larvicidal Bioassay

WHO protocol was modified to some extent for carrying out the larvicidal assay [23]. The 4th instar larvae of *Culex quinquefasciatus* was utilized for determining the larvicidal activities of different extract of *M. paniculata.* In this experiment, a series of glass beakers, each having 200 ml capacity were used and 100ml of tap water was kept in these beakers separately. Then for obtaining exact concentration of the extracts, sufficient quantity of the stock solution was added into each beaker. Only 100 ml of tap water was used as control medium. The larval mortalities of 4th instar larvae of *C. quinquefasciatus* were observed separately in control, 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 ppm concentrations. Insertion of ten 4th instar larvae of *C. quinquefasciatus* were done individually into the control and different concentrations of bark extract. At the end of 24 hrs, the number of alive and dead larvae, response %, corrected response %, linear response %, linear probit, LC_{50}, LC_{90}, lower limit & upper limit and chi-square values were documented.

Statistical Analysis

LdP Line^R software was used for calculating the $LC_{50,}$ LC_{90}, chi-square values and other statistics at 95 percent fiducial limits of upper confidence limit and lower confidence limit from duplicate experiments [24].

Results

Phytochemical Constituents Study

A number of primary and secondary metabolites like carbohydrates, alkaloids, saponins, tannins, flavonoids, triterpenoids and glycosides were confirmed qualitatively, when the crude extracts were tested with various chemical reagents that are presented in Table 1.

Antibacterial Assay

In Table 2, specific concentrations of different extracts of *M. paniculata* showed their antibacterial activity. Dose dependent inhibition was observed by the all extracts against both Gram positive & Gram negative bacteria. In addition, BME showed highest efficiency against most of the bacteria and remarkable inhibition against the Gram negative bacteria *E. coli* & *Serratia spp.* being the most. The plant extracts demonstrated antibacterial activities by the following order.

BME > BWE > BCE

Maximum test organisms were moderately inhibited by the standard antibiotic cefradine. It inhibited the Gram positive bacteria more than the Gram negative bacteria. Although *Shigella boydii* is a Gram negative bacteria, but it was inhibited most.

Brine Shrimp Lethality Bioassay

Having LC_{50} of 73.3 µg/ml, the BCE was found to be the most toxic to Brine Shrimp nauplii than other extracts but, VS (Vincristine sulphate) demonstrated the LC_{50} value 1.7 µg/ml. The toxicity potential of the test samples was decreased by the following order.

VS > BCE > BWE > BME

For the data (Table 3), lower limit & upper limit of LC_{50} & LC_{90} couldn't be calculated by LdP Line^R software as 'g' value was greater than 0.4. 'g' is a factor used in fiducial limit calculations [24]. "With almost all good sets of data, 'g' will be substantially smaller than 0.1, and seldom greater than 0.4" [25].

Larvicidal Bioassay

At the end of 24 hrs, the various extracts of *M. paniculata* were monitored for their larvicidal activity against the 4th instar larvae of *Culex*

quinquefasciatus and the results are represented in (Table 7-9). BME showed the most promising larvicidal effects. The 24 hrs LC_{50} value of BME for *C. quinquefasciatus* was found to 299.2 ppm (Table 7).

Discussion

The variation in the antibacterial properties of the *M. paniculata* extracts might be due to the presence of various secondary metabolites that were found during phytochemical analysis. There is a possibility that due to insufficient antibacterial constituents, some extracts were less effective against certain bacteria. From the existing study, it is clear that the Gram negative bacteria were inhibited more than the gram positive bacteria by the BME of *M. paniculata.* Plant's secondary metabolites include phytochemical components such as phenols, alkaloids, saponins, flavonoids, tannins and a number of aromatic compounds which provide a defense mechanism against insects, herbivores and many microorganisms through anticipation [26]. Different mechanisms are engaged for exerting the antibacterial action of bioactive compounds. When microbes infect plants, then flavonoids are synthesized by plants which are hydroxylated phenolic substances. And these flavonoids acts as an effective antibacterial agent covering a variety of bacteria that was established by in vitro method. Possibly they perform their actions through complex formation with soluble and extracellular proteins along with bacterial cell walls also [27]. Saponins show their antibacterial actions by causing leakage of certain enzymes and proteins from the cell [28]. Triterpenoids exhibit antibacterial activity against Gram positive bacteria, block cell division by inhibiting DNA synthesis and macromolecular synthesis in *Bacillus subtilis*[29]. In case of Gram negative bacteria, the lipopolysaccharide layer creates a barrier for the entry of most of the compounds [30]. Gram negative bacteria's external membrane creates a permeable barrier for stopping access of bulky polar substances into the cell. However, protein channels which are aqueous in nature regarded as porins, can facilitate the entry of small polar substances as well as many small hydrophilic antibiotics into the periplasmic space of Gram negative bacteria. Different Gram negative bacteria varies the number and size of porins. Pseudomonas aeruginosa lacks the classical high permeability porin channels and shows resistant to a wide range of antibiotics. In case of Gram negative bacteria, some antibiotics can pass through the porins by passive diffusion and other can pass across the cytoplasmic membrane via an energy dependent active transport system. For example, the aminoglycosides are transferred across the cytoplasmic membrane, which depend on electron transport because of necessity for a membrane electrical potential. Metabolic energy is needed for some antibiotics to enter into the Gram positive bacteria, but the mechanism is not clear [31]. The observed plant extracts showed remarkable antibacterial activities against most of the Gram negative bacteria which may be due to the presence of porin channels and the usage of either active transport or passive diffusion.

Though BSLB is used for evaluating the bioactivity of the plant extracts, but it is insufficient concerning the explanation of the mechanism of action of these extracts. LC_{50} values lower than 1000 µg/ml are considered bioactive during the toxicity estimation of the plant extracts by BSLB [22]. The crude extracts of *M. paniculata* showed different kinds (e.g. tannins, alkaloids, triterpenoids) of toxic substances which may be responsible for their diverse toxicities (table 3-5).

Filariasis, Japanese encephalitis, malaria, dengue etc. are spread by mosquitoes causing millions of deaths every year and have an immense impact on public health. Disturbances in natural biological control systems, undesirable effects on non-target organisms and development of resistance are occurring by the frequent usage of synthetic insecticides for controlling mosquitoes. Consequently, the environment and human health are affected mostly. Considering these problems plants may be an alternative source of mosquito control agent [32].

The outcomes of the existing phytochemical analysis revealed that the BME part of *M. paniculata* contained alkaloids, flavonoids, triterpenoids, tannins, saponins and BCE showed the presence of flavonoids and tannins that may be responsible for their (except BWE, LC_{50}=1223.4 µg/ml) potential larvicidal activities against 4th instar larvae of *C. quinquefasciatus.*

Conclusion

The results were encouraging and may suggest that among the all extracts, the bark methanolic extract of *M. paniculata* possesses compounds with antibacterial, toxic and larvicidal properties that are in agreement to a certain degree with the traditional uses of the plant. Further research is necessary to determine the quantitative identity of the antibacterial, toxic and larvicidal compounds within the plant and also to determine their full spectrum of efficacy. Moreover, partition method would be advantageous procedure to eliminate a large amount

of inactive fractions which, in turn, may reduce cost and time to find out the active compounds.

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Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

List of abbreviations

LC_{50}= Lethal Concentration, 50%
LC_{90}= Lethal Concentration, 90%
PPM= Parts Per Million

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Table 1. Phytochemical screening of different extracts of barks of *M. paniculata.*

Phytoconstituents	Test name	Observation of various extracts		
		BME	BCE	BWE
Carbohydrates	Molisch's test	+	+	+
	Fehling's test	+	+	-
Alkaloids	Mayer's test	+	-	-
	Wagner's test	+	-	-
	Dragendorff's test	+	-	-
Saponins	Frothing test	+	-	+
Tannins	$FeCl_3$ test	+	+	-
Flavonoids	Alkali test	+	+	+
Triterpenoids	Salkowski's test	+	-	+
Glycosides	Baljet test	-	+	+

Where, **'+' =** Present and '-' = Absent

Table 2. Antibacterial activitiy of *M. paniculata* at 2, 4 and 6 mg/disc including standard cefradine at 10µg/disc.

Organisms	BME			BCE			BWE			Cefradine
	2mg	4mg	6mg	2mg	4mg	6mg	2mg	4mg	6mg	10µg/disc
	Diameter of Zone of Inhibition (mm)									
B. subtilis	8.5	17	23	-	12	18	7.5	13	23	27
B. cereus	6.5	10	21	6.5	13	20	7	15	24	12
B. megaterium	-	12	16	7	15	19	-	6.5	10	22
S. typhi	7	15	22	-	9	16	6.5	12	21	15
V. cholerae	7.5	14	23	-	9	17	8	15	25	15.5
P. mirabilis	7	14	20	-	9.5	18	9.5	18	27	12
E. coli	10.5	20	32	6.5	15.5	22	7	14	23	8
S. aureus	7	16	21	-	13	20	-	11	21	19
Serratia spp.	10	21	32	-	12	18	-	10	20	-
Erwinia spp.	11	16	23	-	10	16	7.5	13	22	25
Pseudomonas spp.	12	19	31	-	12	19	7	14	24	15
Salmonella spp.	7	15	20	-	10	18	5	11	25	20.5
Shigella boydii	7	10	19	7.5	17	22	9	14	16	31

Table 3. Probit analysis, $LC_{50,}$ LC_{90} & calculated chi square (χ^2) values for BME.

Conc.(µg/ml)	Treated	Response %	Corrected response %	Linear response %	Linear probit	LC_{50} (µg/ml)	LC_{90} (µg/ml)	χ^2
1	20	30	26.3	22.0	4.2			
5	20	30	26.3	27.9	4.4			
10	20	30	26.3	30.7	4.5			
20	20	40	30.8	33.6	4.6	LC_{50}=756.3	LC_{90}=45320890	1.8
50	20	40	30.8	37.6	4.7			
100	20	40	30.8	40.7	4.8			
200	20	40	30.8	43.8	4.8			
500	20	60	57.9	48.1	5.0			

Where Conc. = Concentration.

Table 4. Probit analysis, $LC_{50,}$ LC_{90} & χ^2 values for BCE.

Conc.(µg/ml)	Treated	Response %	Corrected response %	Linear response%	Linear probit	LC_{50} (µg/ml)	LC_{90} (µg/ml)	χ^2
1	20	30	26.3	20.7	4.2			
5	20	30	26.3	30.5	4.5	LC_{50}=73.3 Lower limit=25.5 Upper limit=457.3	LC_{90}=61207.6 Lower limit=3524.5 Upper limit=1282783602.6	1.9
10	20	40	36.8	35.2	4.6			
20	20	40	36.8	40.2	4.8			
50	20	40	36.8	47.1	4.9			
100	20	60	57.9	52.4	5.1			
200	20	60	57.9	57.6	5.2			
500	20	70	68.4	64.3	5.4			

Table 5. Probit analysis, $LC_{50,}$ LC_{90} & χ^2 values for BWE.

Conc.(µg/ml)	Treated	Response %	Corrected response %	Linear response %	Linear probit	LC_{50} (µg/ml)	LC_{90} (µg/ml)	χ^2
1	20	20	15.8	19.0	4.1	LC_{50}= 145.6 Lower limit=45 .8 Upper limit=27 58.8	LC_{90}=21 1532.7 Lower limit=6 705.2 Upper limit=2 766694 03548. 7	0.8
5	20	30	26.3	27.6	4.4			
10	20	40	36.8	31.9	4.5			
20	20	40	36.8	36.3	4.7			
50	20	50	47.4	42.5	4.8			
100	20	50	47.4	47.4	4.9			
200	20	50	52.2	52.2	5.1			
500	20	60	58.6	58.6	5.2			

Table 6. Probit analysis, $LC_{50,}$ LC_{90} & χ^2 values for VS.

Conc.(µg/ml)	Treated	Response %	Corrected response %	Linear response %	Linear probit	LC_{50} (µg/ml)	LC_{90} (µg/ml)	χ^2
0.06	20	10	5.3	3.5	3.2	LC_{50}= 1.7 Lower limit=1. 1 Upper limit=2. 9	LC_{90}=18 .7 Lower limit=9. 4 Upper limit=5 4.7	3.3
0.125	20	15	10.5	7.8	3.6			
0.25	20	15	10.5	14.8	4.0			
0.5	20	25	21.1	25.2	4.3			
1	20	45	42.1	38.3	4.7			
5	20	65	63.2	71.6	5.6			
12.5	20	95	94.7	85.6	6.1			
25	20	95	94.7	92.5	6.4			

Table 7. Response %, corrected response %, linear response % and linear probit of 4th instar larvae of *C. quinquefasciatus* exposed for 24 hrs to different concentrations for BME of *M. paniculata.*

Conc.(PPM)	Treated	Response%	Corrected response %	Linear r Esponse %	Linear probit	LC_{50} (µg/ml)	LC_{90} (µg/ml)	χ^2
50	20	15	10.5	5.9	3.4			
100	20	20	15.8	16.8	4.0			
150	20	30	26.3	27.3	4.4	LC_{50}=299.2 Lower limit=242.1 Upper limit=386.4	LC_{90}=1291.3 Lower limit=812.4 Upper limit=3316.1	4.2
200	20	35	31.6	36.2	4.6			
250	20	45	42.1	43.7	4.8			
300	20	45	42.1	50.1	5.0			
350	20	54	52.6	55.5	5.1			
400	20	55	52.2	60.0	5.3			
450	20	75	73.7	64.0	5.4			
500	20	80	79.0	67.4	5.5			

Table 8. Response %, corrected response %, linear response % and linear probit of 4th instar larvae of *C. quinquefasciatus* exposed for 24 hrs to different concentrations for BCE of *M. paniculata.*

Conc.(PPM)	Treated	Response %	Corrected response %	Linear response %	Linear probit	LC_{50} (µg/ml)	LC_{90} (µg/ml)	χ^2
50	20	10	5.3	0.8	2.6			
100	20	10	5.3	6.0	3.4			
150	20	10	5.3	14.4	3.9	LC_{50}= 360.2 Lower limit=306.0 Upper limit=448.4	LC_{90}=1035.4 Lower limit=728.4 Upper limit=2049.9	6.8
200	20	25	21.1	23.8	4.3			
250	20	35	31.6	32.9	4.6			
300	20	40	36.8	41.2	4.8			
350	20	55	52.6	48.6	5.0			
400	20	60	57.9	55.1	5.1			
450	20	65	63.2	60.6	5.3			
500	20	70	68.4	65.5	5.4			

Table 9. Response %, corrected response %, linear response % and linear probit of 4th instar larvae of *C. quinquefasciatus* exposed for 24 hrs to different concentrations for BWE of *M. paniculata.*

Conc.(PPM)	Treated	Response %	Corrected Response %	Linear response %	Linear probit	LC_{50} (µg/ml)	LC_{90} (µg/ml)	χ^2
50	20	10	5.3	1.5	2.8			
100	20	10	5.3	4.3	3.3			
150	20	10	5.3	7.5	3.6	LC_{50}=1223.4 Lower limit=666.3 Upper limit=14256.8	LC_{90}=7923.2 Lower limit=3014.9 Upper limit=8487824.8	7.4
200	20	10	5.3	10.7	3.8			
250	20	10	5.3	13.8	3.9			
300	20	25	21.1	16.7	4.0			
350	20	20	15.8	19.5	4.1			
400	20	20	15.8	22.2	4.2			
450	20	30	26.3	24.6	4.3			
500	20	45	42.1	27.0	4.4			

Figure 1. Identification of 4th instar larvae of *Culex quinquefasciatus.*

Figure 2. Nutrients (yeast powder & glucose) of *Culex quinquefasciatus* soaked in cotton.

Figure 3. Mosquitoes are sucking pigeon's blood for hatching.

Figure 4. Spreader.

Figure 5. Spreader is pulling mosquitoes for hatching purpose.

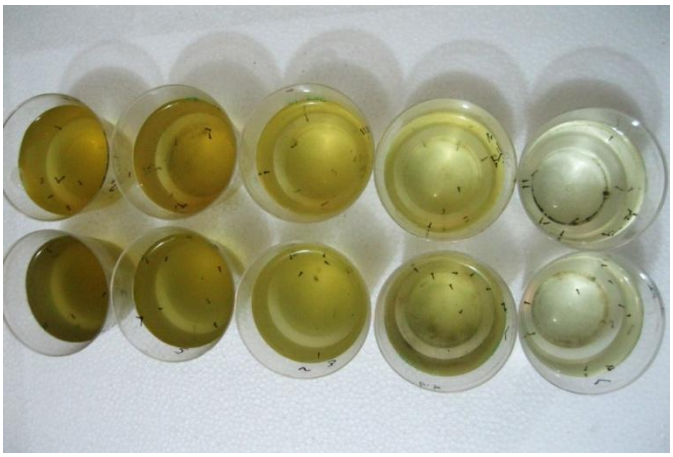

Figure 6. 4th instar *Culex quinquefasciatus* larvae in different concentrations of test sample.