Screening of *Cleome viscosa* (L.) for Dose Mortality, Insect Repellency, Cytotoxicity and Larvicidal Activities in the Laboratory Condition

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Abstract-The Petroleum Ether (Pet.E), chloroform ($CHCl_3$) and Methanol (MeOH) extracts of *Cleome viscosa* (root, aerial part and fruit) have been thoroughly screened through residual film assay and repellent activity test against *Tribolium castaneum* adults; brine shrimp lethality test against *A. salina* nauplii; and larvicidal activity test against mosquito larvae were carried out as a supporting experiment to find bioactive potentials of the test plant. The residual film assay offered promising results with remarkable activity against the adult beetles of *T. castaneum*. A perusal of the data achieved in this experiment clearly showed the presence of insecticidal properties in *C. viscosa* (root, aerial part and fruit) extracts as well as traces of repellent potential. $CHCl_3$ and MeOH aerial extracts showed the highest and the second highest mortality (LD_{50} values were 0.170 and 0.248 mg/cm^2 respectively) against the adult beetles of *T. castaneum.* But $CHCl_3$ root extract was found to show no mortality against the adult beetles of *T. castaneum*. For the repellency test, the MeOH extract of *C. viscosa* aerial part showed the highest repellency between dose interval at 1% level of significance ($P<0.01$) against the adult beetles of *T. castaneum.* The Pet.E. extract of the aerial part and the $CHCl_3$ extract of the root showed repellency at 5% level of significance ($P<0.05$) while the other parts didn't show significant repellent activity against the adult beetles of *T. castaneum.* The cytotoxic effect of *C. viscosa* extracts against the brine shrimp (*A. salina*) nauplii were found promising. The Pet.E. extract of the root and $CHCl_3$ extract of the fruit showed the highest and the second highest toxicity (LC_{50} values were 21.905 and 26.675 ppm after 30h of exposures respectively) against the nauplii. The larvicidal effect of *C. viscosa* extracts against the mosquito larvae of *Culex* sp. were found promising. The $CHCl_3$ extract of the fruit and the $CHCl_3$ extract of the root showed the highest and the second highest toxicity (LC_{50} values were 185.390 and 272.910 ppm after 30h of exposures respectively) against the larvae. A comprehensive phytochemical analyses of the test plants for its insecticidal, repellent, cytotoxic and larvicidal as well as the physiological studies of the active ingredients are very much to be solicited for their effective use in the future pest control and pharmaceutical endeavors.

Index Terms-*Cleome viscose*, *Tribolium castaneum*, mortality, repellency, toxicity, pharmaceutical

1 Introduction

The utilization of plant materials to protect field crops and stored commodities against insect attack has a long history. Many of the plant species concerned have also been used in traditional medicine by local communities and have been collected from the field or specifically cultivated for these purposes. The plant derived materials and phytochemicals which once formed the basis of pest control technology, are again being scrutinized for potentially useful products or as models new lead compounds for introduction into therapeutical screening programs [1].

In fact, plant species is a vast repository of chemical substances that protect plants from attacks by phytophagous insects. Some of these chemicals may expel or kill the insects or deter their feeding, oviposition and reproduction. These properties of the plants are of a great value in protecting stored commodities from insect infestation [2]. To trace the lead components for their further use to enhance the quality of human life it is necessary to go through several steps showed in the figure.

Use of pesticides is often considered to be the most potent control technology for pests. But, continuous heavy use of some pesticides has created serious problems arising from factors, such as, direct toxicity to parasites, predators, pollinators, fish and man [3], pesticide resistance [4, 5], susceptibility of crop plants to insect

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pests. Resistance to one or more pesticides has been reported in at least 477 species of insects and mites (Georghiou and Mellon, 1983), cross and multi-resistant strains in many important insect species have also been reported [6-9].

Plants are like natural laboratories where a great number of chemicals are biosynthesized and in fact they may be considered the most important source of chemical compounds there are. Modern science has discovered one of the rare opportunities in the naturally occurring compounds in plants act as toxic or repellent to the pest. These plant derived materials and phytochemicals, which once formed the basis of pest control technology, are again being scrutinized for potentially useful products or as models for new classes of insecticides [10-11]. *Tribolium* species are major pests of stored grains and grain products in the tropics. Control of these insects relies heavily on the use of synthetic insecticides and fumigants, which has led to problems such as disturbances of the environment, increasing costs of application, pest resurgence, pest resistance to pesticides and lethal effects on non-target organisms in addition to direct toxicity to users [12]. Thus, repellents, fumigants, feeding deterrents and insecticides of natural origin are rational alternatives to synthetic insecticides.

Quite a good number of plants have been identified and utilized for insecticidal and medicinal purpose till to date. But it is true that a large number of plants have still been untouched or less investigated from which significant results can be obtained to control the pest of crops and disease problems of human beings. *Cleome viscosa* L. is such plant that has been studied a lot phytochemically and only a few studies have been done only on its medicinal properties, but in details a very few works have been done till to date on its use for the control of crop pests. The aim of this research is to trace the presence of bioactive potentials (pesticidal, repellent, larvicidal, etc.) in the study plant and to standardize the essences of the test materials through searching literature and web information in comparison with the results of the investigation to be carried out on their possible use in the contemporary pest control technology.

2 Materials and Methods

2.1. Selection and collection of plant materials

In order to arrive at useful compounds in the shortest possible time, careful selection of plant material is obviously very important. Random collection is one method but it is more judicious to base the selection on certain criteria. By the way of illustration, plants used in traditional medicine are more likely to provide pharmacologically active compounds. Similarly, folk use of toxic plants could be taken with desirable output. In this investigation different parts of *C. viscosa* (fruit, aerial part and root) have been collected for the presence of toxic, as well as, bio-active constituents since the plants are well known as medicinal plants and also considered to contain toxic constituents. In case of very small plants, such as herbs, shrubs, grass, etc. normally the whole plant is subjected for extraction, because the distribution of constituents generally not vary too much.

2.2. Preparation of plant materials for extraction

The fresh roots, aerial parts (leaves, stems etc.) and fruit of *C. viscosa* were collected from collector field beside the bank of Padma river of Rajshahi by following way:

Root: Roots were collected by digging up without damaging them and shake and brush away excess soil without washing them with water. Root was cutting into small pieces as thin as possible.

Aerial part: Aerial part belongs to the parts all above the soil including stem, leaves etc. After collection of aerial parts were chopped and spread out to dry without heaping the material together

Fruit: After collection of fruits were cut into small pieces as thin as possible. After collection roots were dried thoroughly in a well-ventilated place. After drying well the plant materials were powdered in a grinder machine avoiding excess heat during grinding.

2.3. Chemical extraction of the collected materials

There are basically two methods for extracting compounds from plant materials. Which one to choose, depends on whether the aim is to extract the more polar compounds (especially glycosides) which are present in the cell vacuole, or to obtain the less polar aglycones present on the surface of the plant, in aerial parts heartwood or roots. In the present study three solvents were selected to extract different parts of *C. viscosa* separately. The three solvents were Petroleum ether (Pet.E.), Chloroform ($CHCl_3$) and Methanol (MeOH). The dried grind materials, viz. aerial part, root and fruit (in case of *C. viscosa*) were extracted with sufficient amount of petroleum ether, chloroform and methanol (150g × 300ml × 3 times) for each of the items. Separate extracts were collected by the cool method after 72 hours of plunging for each of the material. Extracts, thus, obtained are filtered and concentrated on a rotary evaporator at 40ºC and only as residue is left and kept in a refrigerator after labeling.

2.4. Extraction procedures

2.4.1. Extraction procedure for Pet. Ether, $CHCl_3$ and CH_3OH solvents

2.5. Selection of test organisms

To carry on tests for insecticidal properties of the extractives of the selected plants (*C. viscosa*) the red flour beetle *T. castaneum* (Hbst.) (Coleoptera: Tenebrionidae) was selected as the test organism, because it is an easy cultivable and noble laboratory animal. Except *T. castaneum*, brine shrimp nauplii, and mosquito larvae were also selected to carry out further efficiency tests of the extractives.

2.6. Collection of test organisms

Source of test insects- *T. castaneum*, used in the present experiment were taken from the stock cultures of the Crop Protection and Toxicology Laboratory, University of Rajshahi, Rajshahi-6205, Bangladesh; and reared as subcultures to be used in the experimentation. Mosquito eggs (raft) were collected from different drains of RU campus. Brine shrimp cysts were collected from Kataban (different Aquarium shops), New Market, Dhaka, Bangladesh.

2.6.1. *T. castaneum* Hbst.

2.6.1.1. Culture of test insect *T. castaneum*

Mass cultures were maintained in plastic containers (1200ml) and sub-cultures in beakers (1000 ml) with the food medium. The beakers were kept in an incubator at $30^0C \pm 0.5^0C$ without light and humidity control. Each container and beaker contained 250g and 150g of food respectively. About 200 adults in each container and 100 adults in each beaker were introduced. The cultures were checked in regular intervals and eggs and larvae were separated to increase properly. A crumpled filter paper was placed inside each container and beaker for easy movement of the beetles. The containers and beakers were covered with pieces of muslin cloth tightly fixed with the help of rubber bands to avoid possible escape of the beetles.

2.6.2. *A. salina*

2.6.2.1. Culture of *A. salina*

As the *A. salina* is marine crustacean, this is not easy to culture like *T. castaneum* in lab conditions. But, they can be reared in a short edition. To carry on toxicity tests of certain materials these nauplii are very easy to grow from its marketed cysts and to set experiments thereby.

2.6.3. Mosquito larvae

2.6.3.1. Preparation of environment and culture of mosquito larvae

Mosquito eggs are hatched in stagnant water. They are collected from damp drains with special collecting spoon. Collected mosquito eggs (rafts) are placed into a new beaker containing pond water and kept it in a dark place of the lab to hatch.

2.7. Bioassays for activity of the collected extracts

The test systems should ideally be simple, rapid, reproducible, and inexpensive. If active principles are only present at low concentration in the crude extract then bioassay is to be high enough sensitive for their detection. Another factor of special relevance to plant extracts is the solubility of the sample. Finding a suitable system can pose problems. For the selection of bioassays to employ in research on plant constituents, the first step is to choose suitable target organisms. The complexity of the bioassay has to be designed as a function of the facilities and resources available. A list of bioassays taken in this investigation is shown in Table-2.1

2.7.1. Bioassay with residual film/surface film experiments

2.7.1.1. Experiments for surface film test by *T. castaneum* adults

All extracts were diluted with the solvents in which they were extracted and the actual amount of extracted matter in a dose was recorded. The application of dose was carried out by residual film method (Busvine, 1971). For each dose 1 ml of mixture was dropped on a Petri dish (50 mm) in such a way that it made a uniform film over the Petri dish. Then the Petri dishes were air-dried leaving the extraction on it. The actual extract present in one ml mixture was calculated and dividing the value by the area of the Petri dish the dose per centimeter square (mg/cm^2) was calculated. After drying 10 red flour beetles of same age were released in each petridish with 3 replications. A control batch was also maintained with the same number of insects after preparing the Petri dish by applying and evaporating the solvent only. The treated beetles were placed in the incubator at the same temperature as reared in stock cultures and the mortality of the beetles was counted after 30 minutes, 12, 24, 36 and 48 hours of treatment.

This is also one basic application method for doses of toxic substances to any insect population. The test material has been dissolved in an organic solvent with a certain concentration to apply to a petridish of known surface area. After application being volatile the solvent evaporates out immediately simply with the atmospheric temperature. Thus the ingredient goes to make film on the surface of the petridish. Released insects within this captivity might have contact with the substance distributed evenly on the floor. However, being covered with the upper lid of the petridish there could have a

captive environment with the extract distributed even in the air inside and may cause mortality by suffocation. Mortality suffocation may cause promptly if there is any volatile bioactive principles in the test material.

2.7.1.2. Preparation of doses with the crude extracts for the surface film test

In this investigation of dose-mortality efficiency was evaluated through surface film experiment with series of doses applied on *T. castaneum* adults. For *A. precatori* (fruit, aerial and root) extract, a general concentration for the extracts was selected as 50 mg/ml as the stock dose (Pilot dose). The doses for surface film application was obtained as- For root in Pet.E.: 2.547, 2.038, 1.528, 1.010 and 0.509 mg/cm^2, for aerial part in Pet.E.: 3.566, 2.547 and 2.038 mg/cm^2, for aerial part in $CHCl_3$: 4.076, 3.566, 3.057, 2.548 and 2.038 mg/cm^2, for aerial part in MeOH: 2.547, 2.038, 1.528, 1.010 and 0.509 mg/cm^2, for fruit in Pet.E.: 2.038, 1.528,1.010, 0.509 and 0.254 mg/cm^2, for fruit in $CHCl_3$: 3.566, 3.057, 2.548, 2.038 and 1.528 mg/cm^2, for fruit in MeOH: 3.057, 2.547, 2.038, 1.528 and 1.010 mg/cm^2

2.7.1.3. Observation of mortality in surface film tests

After completing the all the arrangements treated Petri dishes were placed in a secured place at room temperature. The whole experiment was observed from time to time and mortality was observed after 30 minutes, 12, 24, 36 and 48 hours of treatment and the data was recorded. A simple microscope was used to check each and every beetle by tracing natural movement of its organs. In some cases hot needle was taken closer to the bodies (without movement) to confirm death. Attention was also paid to recovery of the insects if occurred.

2.7.1.4. Statistical analysis

The mortality records of the residual film experiments done by *T. castaneum* adults were corrected by the Abbott's (1925) formula:

$$P_r = \frac{P_o - P_c}{100 - P_c} \times 100$$

Where,

P_r = Corrected mortality (%)

P_o = Observed mortality (%)

P_c = Control mortality (%) {Sometimes called natural mortality (%)}

Then mortality percentages were subjected to statistical analysis according to Finney (1947) and Busvine (1971) by using a computer program. The dose-mortality relationship was expressed as a median lethal dose (LD_{50}).

2.7.2. Lethality test against the brine shrimp nauplii.

2.7.2.1. Experimental design for lethality test

Brine shrimp cysts are hatched in simulated seawater to get nauplii. Test samples are prepared by the addition of calculated amount of DMSO (Dimethyl sulfoxide) for obtaining desired concentration of test sample. The nauplii are counted by visual inspection and are taken in test-tubes containing 5 ml of simulated seawater. Then samples of different concentrations are added to the premarked test-tubes through pipette. The test-tubes are left for 30 hours and then the nauplii are counted again to find out the cytotoxicity of the test agents and compared to the results with positive control.

2.7.2.2. Preparation of simulated seawater (brine water) and hatching of brine shrimp nauplii

Since the lethality test involves the culture of brine shrimp nauplii that is, the nauplii should be grown in the seawater. Seawater contains 3.8% of NaCl. Accordingly 3.8% sodium chloride solution was made by dissolving sodium chloride (38 g) in normal pond water (1000 ml) and was filtered off.

Brine water was taken in a small tank and *A. salina* cysts (1.5 g/L) were added. Constant temperature (37°C) and sufficient light were maintained to give the sufficient aeration. After 24 hours, matured shrimp as nauplii was collected and used for the experiment.

2.7.2.3. Experimentation of lethality test

All the extracts of *C. viscosa* were applied against brine shrimp nauplii. For each samples, a 'pilot' test was done before final experimentation. 2 mg extract sample was weighted and taken in a small glass vial, and then 1-2 drops of pure Dimethyl sulfoxide (DMSO) added to dissolve initially. 1 ml of pond water was taken into the vial to mix up the sample extract with water to prepare 200 ppm dose. When it mixed up completely added to the test-tube (10ml marked) for conducting tests. This process was also maintained during final experiment. Separate vials were taken for each dose. For each dose three replications were made. For root in Pet.E.: 400, 200, 100, 50, 25 and 12.5 ppm, for root in $CHCl_3$: 400, 200, 100, 50, 25 and 12.5 ppm, for root in MeOH: 400, 200, 100, 50, 25 and 12.5 ppm, for aerial part in Pet.E.: 400, 200, 100, 50, 25 and 12.5 ppm, for aerial part in $CHCl_3$: 400, 200, 100, 50, 25 and 12.5 ppm, for aerial part in MeOH: 400, 200, 100, 50, 25 and 12.5 ppm, for fruit in Pet.E.: 400, 200, 100, 50, 25 and 12.5 ppm, for fruit in $CHCl_3$: 400, 200, 100, 50, 25 and 12.5 ppm, for fruit in MeOH: 400, 200, 100, 50, 25 and 12.5 ppm.

2.7.2.4. Analysis of data

The mortality records of the residual film experiments done *T. castaneum* adults on adults were corrected by the Abbott's (1925) formula:

$$P_r = \frac{P_o - P_c}{100 - P_c} \times 100$$

Where,

P_r = Corrected mortality (%)

P_o = Observed mortality (%)

P_c = Control mortality (%), sometimes called natural mortality (%).

Then mortality percentages were subjected to statistical analysis according to Finney (1947) and Busvine (1971) by using 'computer software'. The dose-mortality relationship was expressed as a median lethal concentration (LC_{50}).

2.7.3. Larvicidal test against mosquito larvae

2.7.3.1. Experimental design for larvicidal test

Mosquito eggs are hatched in stagnant water. Test samples are prepared by the addition of calculated amount of DMSO (Dimethyl sulfoxide) for obtaining desired concentration of test samples. The larvae are counted by visual inspection and are taken in test-tubes containing 5 ml of pond water. Then samples of different concentrations are added to the premarked test-tubes through pipette. The test-tubes are left for 30 hours and then the larvae are counted again after 6h intervals to find out the lethality of the test agents and compared to the results with positive control.

2.7.3.2. Preparation of environment for the hatching of eggs

Collected mosquito eggs are placed into a new beaker containing pond water and kept it in a dark place of the lab to hatch. After 24 hours, hatched larvae are collected and used for the experiment.

2.7.3.3 Experimentation of larvicidal test

$CHCl_3$ (root and fruit) and MeOH extracts of *C. viscosa* were applied against mosquito larvae. 'Pilot' test was done before final experimentation. For each sample, 2 mg extract sample was weighted and taken in a small glass vial, and then 1-2 drops of pure Dimethyl sulfoxide (DMSO) added to dissolve initially. 1 ml of pond water was taken into the vial to mix up the sample extract with water to prepare 200 ppm dose. When it mixed up completely added to the test-tube (10ml marked) for conducting tests.

This process was also maintained during final experiment. Separate vials were taken for each dose. For each dose three replications were made. For root in $CHCl_3$: 400, 300, 200, 100 and 50ppm, for aerial part in MeOH: 500, 400, 300, 200 and 100 ppm and for fruit in $CHCl_3$: 400, 300, 200, 100 and 50 ppm.

2.7.3.4. Analysis of data

The mortality records of the residual film experiments done *T. castaneum* adults on adults were corrected by the Abbott's (1925) formula:

$$P_r = \frac{P_o - P_c}{100 - P_c} \times 100$$

Where,

P_r = Corrected mortality (%)

P_o = Observed mortality (%)

P_c = Control mortality (%), sometimes called natural mortality (%).

Then mortality percentages were subjected to statistical analysis according to Finney (1947) and Busvine (1971) by using 'computer software'. The dose-mortality relationship was expressed as a median lethal concentration (LC_{50}).

2.7.4. Experiments for repellent activity of the extracts

The repellency test used was adopted from the method (No. 3) of McDonald *et al*. (1970) with some modifications by Talukder and Howse (1993, 1994). No significant difference was detected between the repellency of only solvent impregnated and untreated filter papers in tests designed to check for any possible influence of Pet.E. $CHCl_3$ and MeOH.
The average of the counts was converted to percentage repellency (PR) using the formula of Talukder and Howse (1993, 1995):

PR = (Nc – 5) X 20

Where, 'c' is the percentage of insects on the untreated half of the disc. Positive values expressed repellency and negative values for attractant activity.

2.7.4.1. Preparation of doses with the crude extracts for the repellency test

A general concentration for each of the plant extracts was selected as stock dose for repellency application to make other successive doses by serial dilution to give 0.629, 0.315, 0.157, 0.079 and 0.039 mg/cm^2 concentrations.

2.7.4.2. Application of doses in the repellency test

Half filter paper discs (Whatman No. 40, diameter 9 cm) were prepared and selected doses of all the Pet.E. $CHCl_3$ and MeOH extract separately applied onto each of the half-disc and allowed to dry out as exposed in the air for 10 minutes. Each treated half-disc was then attached lengthwise, edge-to-edge, to a control half-disc with adhesive tape and placed in a petridish (diameter 9 cm), the inner surface of which was smeared with fluon to

prevent insects escaping. Three replications were maintained same as the surface film test.

The orientation of the same was changed in the replica to avoid the effects of any external directional stimulus affecting the distribution of the test insects. Each concentration was tested five times. Insects that settled on each half of the filter paper disc were counted after 1h and then at hourly intervals for 5h.

2.7.4.3. Observation and analyses of repellency data

Repellency was observed for one-hour interval and up to five successive hours of exposure, just by counting the number if insects in the treated and non-treated part of the filter paper spread on the floor of the 90 mm petridish. The values in the recorded data were then calculated for percent repellency, which was again developed by arcsine transformation for the calculation of ANOVA.

3 Result

3.1. Bioassay on *T. casteneum* adults

3.1.1. Effects of *C. viscosa* (fruit, aerial part and root) Pet.E. extract against *T. castaneum* by residual film Assay

All the *C. viscosa* Pet.E. extracts (fruit, aerial part and root) were tested against the adult beetles of *T. castaneum* through residual film assay and results found promising. The data was then subjected to probit analysis and the result has been presented in the Table 3.1. To trace acute toxicity an observation of lethality was made after 1/2h of application of the doses.

The LD_{50} values for the Pet.E. fruit extracts of *C. viscosa* were 2.170, 0.888, 0.651 and 0.561 mg/cm^2; while the regression equations were Y = 4.176 + 2.447X, Y = 1.603 + 3.581X, Y = 1.733 + 4.013X and Y = 2.089 + 3.883X; with the χ^2 values (along with their df) 2.876(1), 1.150(3), 1.338(3) 0.897(3) for all of all 12, 24, 36 and 48h of exposures respectively. This was just followed by the aerial Pet.E. extracts of *C. viscosa* for 24, 36 and 48h of exposures with the LD_{50} values 2.881, 1.119 and 1.077 mg/cm^2; while the regression equations were Y = 3.608 + 3.028X, Y = 4.902 + 1.981X, and Y = 4.9138 + 2.655X; with the χ^2 values (along with their df were 9.679(1), 1.806(1) and 0.741(1) respectively.

Table 3.1: LD_{50} values 95% confidence limits and regression equations of fruit, aerial and root extracts (Pet.E.) of *C. viscosa* against *T. castaneum* adults.

Plant organs	Exposure (h)	LD_{50} (mg/cm^2)	95% confidence limits		Regression equations	χ^2 value (df)
			Lower	Upper		
Fruit	12	2.170	1.427	3.300	Y = 4.176 + 2.447X	2.876(1)
	24	0.888	0.755	1.044	Y = 1.603 + 3.581X	1.150(3)
	36	0.651	0.556	0.762	Y = 1.733 + 4.013X	1.338(3)
	48	0.561	0.474	0.665	Y = 2.089 + 3.883X	0.897(3)
Aerial part	12	-	-	-	-	-
	24	2.881	2.330	3.564	Y = 3.608 + 3.028X	9.679(1)
	36	1.119	0.315	3.973	Y = 4.906 + 1.981X	1.806(1)
	48	1.077	0.357	3.250	Y = 4.913 + 2.655X	0.741(1)
Root	12	-	-	-	-	-
	24	2.647	2.123	3.300	Y = 3.018 + 4.686X	4.126(1)
	36	0.548	0.370	0.809	Y = 3.296 + 2.305X	0.266(2)
	48	0.371	0.221	0.620	Y = 3.529 + 2.582X	0.437(2)

This was just followed by the root Pet.E. extracts for 24, 36 and 48h of exposures with the LD_{50} values 2.647 0.548 and 0.371 mg/cm^2; while the regression equations were Y = 3.018 + 4.686X, Y = 3.296 + 2.305X and Y = 3.529 + 2.582X, Y = 3.018 + 4.686X, Y = 3.296 + 2.305X and Y = 3.529 + 2.582X with the χ^2 values along with their df were 4.126(1), 0.266(2) and 0.437(2) respectively.

3.1.2. Effects of *C. viscosa* (fruit, aerial part) $CHCl_3$ extracts against *T. castaneum* adults

All the *C. viscosa* $CHCl_3$ extracts (fruit, aerial part) were tested against the adult beetles of *T. castaneum* through residual film assay and results found promising. The data was then subjected to probit analysis and the result has been presented in the Table 3.2. To trace acute toxicity an observation of lethality was made after 1/2h of application of the doses.

The LD_{50} values for the $CHCl_3$ fruit extracts of *C. viscosa* were 3.734, 2.493, 1.730 and 1.615 mg/cm^2; while the regression equations were Y = 1.381 + 6.323X, Y = 1.903 + 7.804X, Y = 4.025 + 4.092X and Y = 4.126 + 4.189X; with the χ^2 values (along with their df) 3.815(3), 3.906(3), 1.578(3) and 3.832(3) for all of all 12, 24, 36 and 48h of exposures respectively. This was just followed by the aerial $CHCl_3$ aerial extracts of *C. viscosa* for 12, 24, 36 and 48h of exposures with the LD_{50} values 7.650, 2.769, 0.894 and 0.170 mg/cm^2; while the regression equations were Y = 1.954 + 3.447X, Y = 4.547 + 1.023X, Y = 5.085 + 1.770X and Y = 5.956 + 1.244X; with the χ^2 values (along with their df) were 0.422(3), 3.194(3), 4.382(3)and 1.357(3) respectively.

Table 3.2: LD_{50} values 95% confidence limits and regression equations of fruit and aerial extracts ($CHCl_3$) of *C. viscosa* against *T. castaneum* adults.

Plant parts	Exposure (h)	LD_{50}	95% confidence limits	Regression equation	χ^2 value (df)

		(mg/ cm²)	Lower	Upper		
Fruit ($CHCl_3$)	12	3.734	3.215	4.338	Y = 1.381 + 6.323X	3.815(3)
	24	2.493	2.325	2.673	Y = 1.903 + 7.804X	3.906(3)
	36	1.730	1.451	2.062	Y = 4.025 + 4.092X	1.578(3)
	48	1.615	1.333	1.958	Y = 4.126 + 4.189X	3.832(3)
Aeria($CHCl_3$)	12	7.650	3.405	17.184	Y = 1.954 + 3.447X	0.422(3)
	24	2.769	1.729	4.433	Y = 4.547 + 1.023X	3.194(3)
	36	0.894	0.202	3.950	Y = 5.085 + 1.770X	4.382(3)
	48	0.170	2.355	123.42	Y = 5.955 + 1.244X	1.357(3)

3.1.3. Effects of (fruit and aerial part) extracts (MeOH) of *C. viscosa* against *T. castaneum* adults.

All the *C. viscosa* MeOH extracts (**f**ruit, aerial part) were tested against the adult beetles of *T. castaneum* through residual film assay and results found promising. The data was then subjected to probit analysis and the result has been presented in the Table 3.3. To trace acute toxicity an observation of lethality was made after 1/2h of application of the doses.

Table 3.3: LD_{50} values 95% confidence limits and regression equations of fruit and aerial extracts (MeOH) *C. viscosa* against *T. castaneum* adults.

Plant organs	Exposure (h)	LD_{50} (mg/cm²)	95% confidence limits		Regression equation	χ^2 value (df)
			Lower	Upper		
Fruit (MeOH)	12	41.434	0.106	1.6086	Y = 3.580 + 0.877X	1.080(2)
	24	2.794	2.171	3.595	Y = 3.819 + 2.646X	5.105(2)
	36	0.837	0.586	1.196	Y = 5.252 + 3.285X	0.353(3)
	48	0.701	0.432	1.137	Y = 5.472 + 3.064X	1.854(3)
Aerial (MeOH)	24	1.770	1.325	2.365	Y=-0.124 + 4.105X	14.12(3)
	36	0.520	0.378	0.716	Y = 2.810 + 3.056X	2.679(1)
	48	0.248	8.184	0.756	Y = 4.276 + 1.826X	2.733(1)

The LD_{50} values for the MeOH fruit extracts of *C. viscosa* were 41.434, 2.794, 0.837 and 0.701 mg/cm²; while the regression equations were Y = 3.580 + 0.877X, Y = 3.819 + 2.646X, Y = 5.252 + 3.285X and Y = 5.472 + 3.064X; with the χ^2 values (along with their df) 1.080(2), 5.105(2) 0.353(3) and 1.854(3) for all of all 12, 24, 36 and 48h of exposures respectively. This was just followed by the aerial MeOH extracts of *C. viscosa* for 24, 36 and 48h of exposures with the LD_{50} values 1.770, 0.520, and 0.248 mg/cm²; while the regression equations were Y = -0.124 + 4.105X, Y = 2.810 + 3.056X, and Y = 4.276 + 1.826X; with the χ^2 values (along with their df) were 14.12(3), 2.679(1) and 2.733(1) respectively.

3.2. Bioassay on *A. salina* nauplii

3.2.1. Effect of *C. viscosa* fruit extracts (Pet.E, $CHCl_3$, MeOH) against *A. salina* nauplii by brine shrimp lethality test

All the *C. viscosa* **f**ruit extracts (Pet. Ether, $CHCl_3$ and MeOH) were tested against the one day aged brine shrimp nauplii through lethality test and results found promising. The data was then subjected to probit analysis and the result has been presented in the Table 3.4. To trace acute toxicity an observation of lethality was made after 6h of application of the doses.

The LC_{50} values for the Pet.E. fruit extracts of *C. viscosa* were 19714.780, 2752.110, 172.020, 95.421 and 43.279 ppm; while the regression equations were Y = 1.935 + 0.713X, Y = 3.088 + 0.556X, Y = 3.411 + 0.711X, Y = 3.209 + 0.904X and Y = 3.332 + 1.019X; with the χ^2 values (along with their df 0.608(2), 2.092(4), 1.772(4), 2.088(4) and 1.282(4) for all of all 6, 12, 18, 24 and 30h of exposures respectively. This was just followed by the fruit $CHCl_3$ extracts of *C. viscosa* for 6, 12, 18, 24 and 30h of exposures with the LC_{50} values 57610.99, 2492.75, 692.39, 71.418 and 26.675 ppm; while the regression equations were Y = 2.363 + 0.554X, Y = 2.749 + 0.663X, Y = 3.766 + 0.435X, Y = 4.106 + 0.482X and Y = 3.838 + 0.815X; with the χ^2 values (along with their df were 0.510(4), 1.325(4), 0.505(4), 0.153(4) and 0.218(4) respectively. This was just followed by the fruit MeOH extracts for 6h, 12h, 18h, 24h and 30h of exposures with the LC_{50} values 9549.26, 493.53, 112.86, 51.174 and 35.385 ppm; while the regression equations were Y = 2.404 + 0.652X, Y = 2.364 + 0.979X, Y = 3.179 + 0.887X, Y = 3.444 + 0.910X and Y = 3.492 + 0.974X with the χ^2 values along with their df were 1.379(4), 1.713(4), 0.568(4), 2.293(4)and 2.285(4) respectively.

Table 3.4: LC_{50} values, 95% confidence limits, regression equations and χ^2 values of fruit (Pet.E, $CHCl_3$ and MeOH) extracts *C. viscosa* against *A. salina* nauplii.

Extract types	Exposures	LC_{50} values (ppm)	95% confidences limits (ppm)		Regression equations	χ^2 values (df)
			Lower	Upper		
Pet.E.	6h	19714.7	27.220	1.427	Y=1.935+ 0.713X	0.608(2)
	12h	2752.11	167.88	45114	Y=3.088 + 0.555X	2.092(4)
	18h	172.020	81.472	363.23	Y=3.411 + 0.711X	1.772(4)
	24h	95.421	58.060	156.82	Y=3.209 + 0.904X	2.088(4)

	30h	43.279	27.204	68.851	Y=3.332 + 1.019X	1.282(4)
$CHCl_3$	6h	57610.9	35.821	9.265	Y=2.363 + 0.553X	0.510(4)
	12h	2492.75	221.17	28095	Y=2.749 + 0.662X	1.325(4)
	18h	692.390	79.589	6023.4	Y=3.765 + 0.434X	0.505(4)
	24h	71.418	29.492	172.94	Y=4.105 + 0.482X	0.153(4)
	30h	26.675	13.530	52.593	Y=3.838 + 0.814X	0.218(4)
MeOH	6h	9549.26	163.46	55785	Y=2.403 + 0.652X	1.379(4)
	12h	493.530	203.47	1197.1	Y=2.363 + 0.978X	1.713(4)
	18h	112.860	66.870	190.48	Y=3.179 + 0.887X	0.568(4)
	24h	51.174	31.113	84.172	Y=3.444 + 0.910X	2.293(4)
	30h	35.385	21.135	59.245	Y=3.491 + 0.973X	2.285(4)

3.2.2. Effect of *C. viscosa* aerial extract (Pet.E. and $CHCl_3$) against *A. salina* nauplii by brine shrimp lethality test

The *C. viscosa* aerial extract (Pet.E. and $CHCl_3$) were tested against the one day aged brine shrimp nauplii through lethality test and results found promising. The data was then subjected to probit analysis and the results have been presented in the Table 3.5.

To trace acute toxicity an observation of lethality was made after 6h of application of the doses. The LC_{50} values for the Pet.E. aerial extracts of *C. viscosa* were 351.230, 188.360, 80.135, and 41.470 ppm; while the regression equations were Y = 0.807 + 1.647X, Y = 2.545 + 1.079X, Y = 3.462 + 0.807X and Y = 3.919 + 0.668X; with the χ^2 values (along with their df) 29.729(3), 16.311(4) , 7.637(4), and 5.108(4) for all of all 12, 18, 24 and 30h of exposures respectively. This was just followed by the aerial $CHCl_3$ extracts of *C. viscosa* for 12, 18, 24 and 3 0h of exposures with the LC_{50} values 6291.390, 1012.460, 692.390 and 245.050 ppm; while the regression equations were Y = 2.154 + 0.749X, Y = 2.445 + 0.850X, Y = 1.915 + 1.205X, and Y = 2.198 + 1.173X; with the χ^2 values (along with their df) were 5.173(5), 7.794(5), 11.914(5) and 10.156(5) respectively.

Table 3.5: LC_{50} values, 95% confidence limits, regression equations, and χ^2 values of *C. viscosa* aerial (Pet.E and $CHCl_3$) extracts against *A. salina* nauplii.

Extract types	Exposures	LC_{50} values	95% confidences limits (ppm)		Regression equations	χ^2 values (df)
			(ppm)	Lower	Upper		
Pet.E.	12h	351.23	76.858	1605.10	Y=0.806 + 1.647X	29.72(3)
	18h	188.36	66.119	536.600	Y=2.544 + 1.079X	16.31(4)
	24h	80.135	46.519	138.040	Y=3.462 + 0.807X	7.63(4)
	30h	41.470	20.506	83.867	Y=3.919 + 0.668X	5.10(4)
$CHCl_3$	12h	621.39	999.23	39612.0	Y=2.154 + 0.749X	5.17(5)
	18h	112.46	475.27	2156.80	Y=2.444 + 0.850X	7.79(5)
	24h	363.00	202.79	649.790	Y=1.915 + 1.204X	11.91(5)
	30h	245.05	170.79	351.600	Y=2.197 + 1.172X	10.15(5)

3.2.3. Effect of *C. viscosa* root extracts (Pet.E. and MeOH) against *A. salina* nauplii by brine shrimp lethality test

The *C. viscosa* root extract (Pet.E. and MeOH) were tested against the one day aged brine shrimp nauplii through lethality test and results found promising. The data was then subjected to probit analysis and the results have been presented in the Table 3.6. To trace acute toxicity an observation of lethality was made after 6h of application of the doses.

The LC_{50} values for the Pet.E. root extracts of *C. viscosa* were 268883.8, 21441.78, 361.08, 72.008 and 21.905 ppm; while the regression equations were Y = 2.406 + 0.478X, Y = 3.325 + 0.387X, Y = 3.358 + 0.642X, Y = 3.639 + 0.733X and Y = 3.809 + 0.889X; with the χ^2 values (along with their df 0.619(3), 1.508(4), 1.018(4), 1.618(4) and 5.425(4) for all of all 12, 18, 24 and 30h of exposures respectively. This was just followed by the root MeOH extracts of *C. viscosa* for 12, 18, 24 and 30h of exposures with the LC_{50} values 430.30, 115.09, 67.128, and 64.395 ppm; while the regression equations were Y = 1.793 + 1.218X, Y = 0.948 + 1.966X, Y = 1.305 + 2.023X, and Y = 0.588 + 2.439X; with the χ^2 values (along with their df) were 1.554 (2), 3.09 (4), 4.346 (4), and 2.870 (4) respectively.

Table 3.6: LC_{50} values, 95% confidence limits, regression equations and χ^2 values of *C. viscosa* root extracts (Pet.E. and MeOH) against *A. salina* nauplii.

Extract types	Exposures	LC_{50} values	95% confidences limits (ppm)		Regression equations	χ^2 values (df)
		(ppm)	Lower	Upper		
	6h	26888.8	1.027	7.034	Y=2.406+0.47X	0.619(3)

Pet. Ether	12h	2144.78	36.384	1.263	Y=3.325+0.38X	1.508(4)
	18h	361.080	115.75	1126.38	Y=3.358+0.64X	1.018(4)
	24h	72.008	39.860	130.080	Y=3.639+0.73X	1.618(4)
	30h	21.905	11.114	43.174	Y=3.808+0.88X	5.425(4)
MeOH	12h	430.300	200.18	924.920	Y=1.792+1.21X	1.554(2)
	18h	115.090	88.561	149.580	Y=0.947+1.96X	3.09 (4)
	24h	67.128	52.400	85.996	Y=1.304+2.02X	4.346(4)
	30h	64.395	51.700	80.208	Y=0.588+2.43X	2.87 (4)

3.3. Bioassay on mosquito larvae

3.3.1. Effect of $CHCl_3$ (fruit and root) extracts of *C. viscosa* against mosquito larvae by larvicidal assay

$CHCl_3$ (Fruit and Root) extracts of the *C. viscosa* were tested against the one day aged mosquito larvae through larvicidal activity test and found promising. The data was then subjected to probit analysis and the result has been presented in the Table 3.7. To trace acute toxicity an observation of lethality was made after 6h of application of the doses.

The LC_{50} values for the $CHCl_3$ fruit extracts of *C. viscosa* were 812.28, 366.09 and 272.91 ppm; while the regression equations were Y = -1.176 + 2.122X, Y = -2.214 + 2.814X and Y = -2.026 + 2.8846X; with the χ^2 values (along with their df) 0.945 (2), 0.366 (2) and 0.109 (2) for all of all 18h, 24h and 30h of exposures respectively. This was just followed by the $CHCl_3$ root extracts of *C. viscosa* for 12h, 18h, 24h and 30h of exposures the LC_{50} values were 1442.7, 750.28, 361.46 and 185.39 ppm; while the regression equations were Y = 0.152 + 1.534X, Y = -1.239 + 2.170X, Y = 0.576 + 1.729X and Y = 0.511 + 1.979X with the χ^2 values along with their df were 0.326(2), 3.190(2), 0.963(2) and 1.123(2) respectively.

Table 3.7: LC_{50} values, 95% confidence limits, regression equations and χ^2 values of *C. viscosa* $CHCl_3$ (fruit and root) extracts against mosquito larvae.

Plant parts	Exposures	LC_{50} (ppm)	95% confidences limits (ppm)		Regression equations	χ^2 values (df)
			Lower	Upper		
C. viscosa (fruit)	18h	812.28	327.09	2017.1	Y =1.176 + 122X	0.945 (2)
	24h	366.09	281.42	476.23	Y =2.214+2.81X	0.366 (2)
	30h	272.91	223.34	333.48	Y =2.025 +2.88X	0.109 (2)
C. viscosa (root)	12h	1442.7	226.83	9176.8	Y =0.152 +1.53X	0.326 (2)
	18h	750.28	334.69	1681.8	Y =1.239 +2.17X	3.190 (2)
	24h	361.46	238.76	547.21	Y =0.575 +1.72X	0.963 (2)
	30h	185.39	139.0	247.1	Y =0.510 +1.97X	1.123 (2)

3.3.2. Effect of MeOH extracts of *C. viscosa* aerial part against mosquito larvae by larvicidal assay

MeOH extracts of the *C. viscosa* aerial part were tested against the one day aged mosquito larvae through larvicidal activity test and found promising. The data was then subjected to probit analysis and the result has been presented in the Table 3.8. To trace acute toxicity an observation of lethality was made after 6h of application of the doses.

Table 3.8: LC_{50} values, 95% confidence limits, regression equations and χ^2 values of *C. viscosa* MeOH (aerial part) extracts against mosquito larvae.

Solvent	Plant parts	Exposures	LC_{50} (ppm)	95% confidences limits (ppm)		Regression equations	χ^2 values (df)
				Lower	Upper		
MeOH	*C. viscosa* (aerial)	12h	18898.00	7.702	4.6E+07	Y= 1.244+ 0.878X	0.680 (2)
		18h	1131.400	415.54	3080.40	Y= -0.819 + 1.905X	0.471 (2)
		24h	578.490	375.15	892.060	Y= -0.491 + 1.988X	1.252 (2)
		30h	309.490	237.90	402.540	Y= -0.154 + 2.069X	0.889 (2)

The LC_{50} values for the MeOH extracts of *C. viscosa* (aerial part) were 18898.0, 1131.4, 578.49 and 309.49 ppm; while the regression equations were Y = 1.244 + 0.878X, Y = -0.819 + 1.906X, Y = -0.492 + 1.988X and Y = -0.154 + 2.069X; with the χ^2 values (along with their df) 0.680 (2), 0.4711 (2), 1.252 (2) and 0.889 (2) ppm for all of all 12, 18, 24 and 30h of exposures respectively.

3.4. Repellent effect of the test plant against *T. castaneum* adults

All the extracts of the selected plant *C. viscosa* (fruit, aerial part and root) were tested against *T. castaneum* adults for their repellent activity. The extracts of *C. viscosa* (Pet.E., $CHCl_3$ and MeOH) were found to show repellent activity against the adults beetles of *T. castaneum* even for concentration from 0.079 to as less as 0.005 $mgcm^{-2}$ (0.079, 0.039, 0.020, 0.010 and 0.005 $mgcm^{-2}$ for ½ of 90 mm filter paper for all the plant extracts). The data was read with 1h interval for up to 5 hours of exposure and was subjected to

ANOVA after transforming them into arcsine percentage value and the results are given in Table 3.9. The MeOH extract of *C. viscosa* aerial part showed the highest repellency between dose interval at 1% level of significance (P<0.01) against the adult beetles of *T. castaneum*.

Table 3.9: ANOVA results and repellency effect of *C. viscosa* extracts against *T. castaneum* adults.

Types of extract		Source of variation (df)			F-ratio with level of significance		P-value	
		Between doses	Between Time interval	Error	Between doses	Between Time interval	Between doses	Between Time interval
Fruit	Pet.E.	4	4	16	6.041	0.201	0.003	0.933
	$CHCl_3$	4	4	16	2.656	2.178	0.071	0.117
	MeOH	4	4	16	2.744	10.95	0.065	0.001
Arial part	Pet.E.	4	4	16	18.500*	0.223	7.540	0.921
	$CHCl_3$	4	4	16	2.438	1.360	0.089	0.291
	MeOH	4	4	16	54.650**	14.98	3.950	2.850
Root	Pet.E.	4	4	16	1.347	2.837	0.295	0.059
	$CHCl_3$	4	4	16	12.640*	4.634	7.850	0.011

(* = P<0.05; ** = P<0.01)

The aerial Pet.E. and root $CHCl_3$ extract showed repellency at 5% level of significance (P<0.05) while the other parts didn't show the significant repellent activity against the adult beetles of *T. castaneum.*

3.5. Summary of the results

For the detection of bioactive potentials in Pet.E., $CHCl_3$, MeOH extracts of *C. viscosa* (fruit, aerial part and root) insecticidal, insect repellency, brine shrimp lethality and larvicidal activity tests have been carried out. A total outcome of the bioassays carried out is represented in the Table 3.10.

Table 3.10: Summary of the biological activity of the selected plant extracts.

Test samples		*T. castaneum*		*A. Salina* nauplii	Mosquito larvae
		Dose mortality	Repellency	Brine shrimp lethality	Larvicidal activity
C. viscosa (fruit)	Pet.E.	+	-	+	NA
	$CHCl_3$	+	-	+	+
	MeOH	+	-	+	NA
C. viscosa (aerial)	Pet.E.	+	+	+	NA
	$CHCl_3$	+	-	+	NA
	MeOH	+	+	-	+
C. viscosa (root)	Pet.E.	+	-	+	NA
	$CHCl_3$	-	+	-	+
	MeOH	NA	NA	+	NA

(+ = active; - = not active; NA = not attempted)

4 Discussions

The Pet.E., $CHCl_3$ and MeOH extracts of *C. viscosa* (root, aerial part and fruit) have been thoroughly screened through residual film assay and repellent activity test against *T. castaneum* adults; brine shrimp lethality test against *A. salina* nauplii; and larvicidal activity test against mosquito larvae were carried out as a supporting experiment to find bioactive potentials of the test plant. The residual film assay offered promising results with remarkable activity against the adult beetles of *T. castaneum*.

For the control of stored product pests use of plant extract in laboratory trials is no more something new now-a-days. Some researchers [13, 14] showed insecticidal activities of *Thevetia peruviana* seed and leaf extracts respectively. Insecticidal and repellent potentials of spice extracts against *Tribolium castaneum* adults were shown by a reporter [15] for *Cinnamomum zeylanicum, Sygygium aromaticum* & *Myristica fragrans*; and by a worker [16] for *Coriandrum sativum, Trachyspermum ammi* & *Trigonella foenum-graecum*. A researcher [16] revealed nut shell, root bark and stem bark of *Anacardium occidentale* L. as sources of insecticidal properties against *T. castaneum*. Some authors reported the repellent effects of some well known plant such as neem, turmeric etc. and also reported that Begonia leaves at a rate of 1.5 to 2.5kg for 100 kg of rice

grain repelled insects against infestations in the stores. Leaf of *Withania somnifera* repelled *R. dominica* [18]. Leaf powder of Bael (*Aegle marmelos*) also repelled *R. domonica*, *S. cerealella* and *S. oryzae* in paddy.

Now-a-days medicinal plant is gaining popularity in our country because; medicinal plant contains several biological activities for humans. The present study deals with the plant *Cleome viscosa* is a common weed, used in Ayurveda for therapeutic purposes. All parts of the plants are reported to be medicinally important for the treatment of various diseases in Ayurveda. Leaves are used as an external application to wounds and ulcers. It also acts as rubefacient and vesicant [19].

The pharmacological studies have shown that *C. viscosa* possesses various notable biological activities such as antihelmentic, antimicrobial, analgesic, antiinflammatory, immunimodulatory, antipyretic, psychopharmacological, antidiarrheal and hepatoprotective activities [20]. A wide variety of phytoprinciples have been isolated from the plant. The advantage of the traditional use of *C. viscosa* has also been supported by the isolation and identification of several possible flavonoids, saponins, tannins, terpenoids and sterol glycoside. The paste of the root is applied externally in the treatment of earaches. Leaves and young shoots are cooked as a vegetable [21]. *Cleome viscosa* possessed effective wound healing activity [22]. It has sharp mustard like flavor. The pungent seed can be pickled or used as a mustard substitute in curries and the juice of the plant is used as a condiment. Oil obtained from the seeds is used for cooking. All parts of the plant are used in liver diseases, chronic painful joints and mental disorders. The presence of amino acids from seeds of *C. viscosa* has been reported [23].

A perusal of the data achieved in this experiment clearly showed the presence of insecticidal properties in *C. viscosa* (root, aerial part and fruit) extracts and as well as traces of repellent potential. $CHCl_3$ and MeOH aerial extracts showed the highest and the second highest mortality (LD_{50} values were 0.170 and 0.248 mg/cm^2 respectively) against the adult beetles of *T. castaneum.* But $CHCl_3$ root extract was found to show no mortality against the adult beetles of *T. castaneum*.

For the repellency test, the MeOH extract of *C. viscosa* aerial part showed the highest repellency between dose interval at 1% level of significance ($P<0.01$) against the adult beetles of *T. castaneum.* The Pet.E. extract of the aerial part and the $CHCl_3$ extract of the root showed repellency at 5% level of significance ($P<0.05$) while the other parts didn't show significant repellent activity against the adult beetles of *T. castaneum.* The cytotoxic effect of *C. viscosa* extracts against the brine shrimp (*A. salina*) nauplii were found promising. ThePet.E. extract of the root and $CHCl_3$ extract of the fruit showed the highest and the second highest toxicity (LC_{50} values were 21.905 and 26.675 ppm after 30h of exposures respectively) against the nauplii.

The larvicidal effect of *C. viscosa* extracts against the mosquito larvae of *Culex* sp. were found promising. The $CHCl_3$ extract of the fruit and the $CHCl_3$ extract of the root showed the highest and the second highest toxicity (LC_{50} values were 185.390 and 272.910 ppm after 30h of exposures respectively) against the larvae.

5 Conclusions

Thus, a comprehensive phytochemical analyses of the test plants for its insecticidal, repellent, cytotoxic and larvicidal as well as the physiological studies of the active ingredients are very much to be solicited for their effective use in the future pest control and pharmaceutical endeavors. But no information was available from any sources regarding previous investigation on *C. viscosa* for biological activity or so, including cytotoxicity, larvicidal activity, etc. Considering all these activities further investigation is necessary to take the test plants towards a utility stage.

6. References

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