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ANTIFEEDANT ACTIVITY OF NANOFORMULATIONS OF *CLEOME VISCOSA*, AGAINST PEST- *TRIBOLIUM CASTANEUM USING HPLC*

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Conflicts of Interest: Nil

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Abstract:

Background: Now many agricultural engineers are seeking knowledge of nano-materials for engineering bio-pesticides, fertilizer coatings and other purposes. These Nanoparticles can be exploited as they offer a greater surface area and circulate more easily.

Materials & Methods: Insecticidal activity of *C. viscosa* Column fractions against stored product pests. Antifeedant Bioassay was done by fumigation method. Separation and fractionation was done by Column chromatography. TEM morphology of nano-sio2 particle was performed on transmission electron micrograph. Statistical analysis: The average larval mortality data were subjected to probit analysis for calculating lethal concentrations LC_{50} and LC_{95} and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit were calculated.

Results: The extracts of *C. viscose* was moderately toxic and needed at least 2 mg/ ml to obtain 50% mortality. *C. viscosa* showed high toxicity with a LC_{50} of 0.8 mg/ml and LC_{95} of 1.95 mg/ml. Thus it can be concluded that the terpenes adsorbed on the silica through strong hydrogen bonding. All the results above further illustrate that adsorption of terpene has an added advantage that in spite of less agglutination it also exhibit the high dispersion stability.

Conclusion: Among the tested plant materials, *C. viscosa* proved as highly toxic against stored product pests as well as mosquito larvae. This active fraction will be further processed for the pure compound isolation, identification for the principle bioactive compound. The two principle bioactive compounds showed enhanced antifeedant activity against agricultural pests on Nanoformulations.

Keywords: Nanopesticide, *Cleome viscosa*, *Tribolium castaneum, FT-IR analysis,* HLPC

Introduction

Nanotechnology is a broad interdisciplinary area of research, development, and industrial activity that has been growing rapidly worldwide for the past decade. The field of nanoparticle covers many frontline research areas like chemistry, physics, biological and materials science. Now many agricultural engineers are seeking knowledge of nano-materials for engineering bio-pesticides, fertilizer coatings and other purposes.[1] However, little work has been done on pesticide incorporated with Nanoparticles and their control release. These Nanoparticles can be exploited as they offer a greater surface area and circulate more easily and in lepidopteran system.

Agriculture in a tropical country like India, owing to its climatic conditions and its particular environment, suffers severe losses due to pests like *S. litura* and *A. janata*. The Army worm *Spodoptera litura*. Fabricius, is most devastating insect species, which attacks and damages many important crops resulting in great losses.

Stored grains are highly damaged by insect pests like *Tribolium castaneum* H. Almost all the stages of the insect (adult, larvae, except pupa) will attack all the aerial parts of the crop as well as roots in the soil and the grain, which is stored. The yellow fever mosquito, *Aedes aegypti*, is a mosquito that can spread the dengue fever, Chikungunya and yellow fever viruses, and other diseases. The mosquito can be recognized by white markings on legs and a marking of the form of a lyre on the thorax. The mosquito originated from Africa but is now found in the tropics worldwide.[2]

Now the usage of pesticides is inevitable. Our dependence on chemical pesticides has increased to that extent that if we stop using them, our crop production will decrease drastically which will ultimately affect the cost of food grain, which will lead to food shortage severely. According to studies, when pesticide is applied on the field, only 2% of it is taken by plant and 98% of it goes into different environmental components, as pesticide residues have been found to produce biochemical changes in soil,[3] in air and water. Plants are rich source of biological pesticides. The insect damage causes huge losses to crops and food grains. The drastic use of synthetic pesticides pollutes the environment and leads to health hazards. Urgent need is for safe, successful control of the destructive pests that ravage and destroy crops and other resources that sustain a productive agricultural system. Botanical pesticides are good alternatives to chemical pesticides

Today, the major problem in the agricultural pest management is the development of suitable agents which can kill insects physically. Hence, nanosilica mediated gene delivery in plants and pests control have got more emphasis. White fly (Trialeurodes vaporium), an important pest has become possible to control by electrostatically spraying of amorphous silica. Coconut mite (*Dermanyssus gallinae*) is causing havoc throughout India. There are no remedies at present

Immunity in beneficial insects can be developed using nanosilica. This was observed in case of Bombyx mori where grasserie disease caused by nuclear plyhedrosis virus (NPV) could be controlled with the help of lipophilic amorphous silica nanoparticles (LASN)-live BmNPV conjugate as drug. The international Agency for Research on Cancer (IARC) has rated amorphous silica dust as non-carcinogenic.

So here we have terpenes adsorbed on to nano-SiO_2 particles. As Silica surface is generally embedded with hydroxyl groups and ethereal linkages, and hence considered to have a negative charged surface prone to adsorption of electron deficient species. Application of nanoparticles on the leaf and stem surface does not alter either photosynthesis or respiration in several groups of crop plants.

Materials & Methods:

Our present study of the objectives are: Screening the of plant material for insecticidal property. Formulation of the botanicals with Nanoparticles at different concentrations to assess the adsorption compatibility. Evaluating the toxicity of nanoformulation against agricultural pests. Studies on drug loading/control release of bioactive compound from Nanoformulations by using HPLC.

Cleome viscosa, is a medicinal plant, is taken for the present work, plant material used was leaves. The plant materials were collected from the premises of Indian Institute of Chemical Technology (IICT), Hyderabad. *Tribolium castaneum* is an agricultural pest, the larvae were collected from infested castor (*Ricinus communis* (L)) plants growing in the laboratory of Indian Institute of Chemical Technology, Hyderabad, India. The larvae were collected from the fields and were maintained in the laboratory at 28 ± 2º C, 70 – 75 % relative humidity (RH) and a 16:8 hours light/ dark photo period. The larvae were reared on castor leaf.[4]

Red flour beetle, *Tribolium castaneum* were maintained at 28 ± 2°C and 65-75% r. h. in ornamentals, wild plants, weeds and shade trees. Groundnut yield losses up to 71% have been reported in India (Amin 1988). Another agriculture pest is *Achaea janata* which feeds on many different species of plants. Castor bean and croton are preferred hosts. Red flour beetle, *Tribolium castaneum* were maintained at 28 ± 2°C and 65-75% r. h. in Indian Institute of Chemical Technology (IICT)dian Institute of Chemical Technology (IICT) found all over the world. They are strong, accomplished fliers, and will fly from one field to another, infesting grains before the actual harvest.

Silicon dioxide Nanoparticles of average particle diameter 10 nm, specific surface area 115m2 g−1 were procured from sigma-Aldrich. Other reagents were of HPLC grade and used as received. Pure botanical compounds i.e. Eugenol and 1,8-Cineole are the terpenes isolated from two plants were provided by Indian Institute of Chemical Technology (IICT).

Collection and preparation of plant material for extraction was dony by Extraction apparatus (Soxhlet). A rotary evaporator or rotovapor, is a device used in chemical and biochemical laboratories for the efficient and gentle evaporation of solvents. Insecticidal property of the botanical extracts against stored product pests, agricultural pests and yellow fever mosquito was carried out by the following methods. Antifeedant activity against Agricultural pests and Antifeedant Bioassay was carried out.

Thin layer chromatography was used to identify fractions containing similar compounds. Preparation of nanoformulations- Pure botanical compounds Eugenol and 1,8-Cineole were adsorbed on to the nano silica. The native nano-SiO2 particles about 100mg were dispersed in 6ml chloroform, and amount of terpene (25µl, 50µl, 75µl and 100µl) was added into the flask, and subjected to sonication for 10 minutes and then vacuum drying for 24 h. TEM morphology of nano-SiO2 particle- The morphology analysis was performed on transmission electron micrograph (TEM) analyzer (Jeol 100CX–II JAPAN).

Statistical analysis: The average larval mortality data were subjected to probit analysis for calculating lethal concentrations LC50 and LC95 and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit were calculated.

RESULTS:

Insecticidal activity of *Cleome viscosa*, against *Tribolium castaneum*-

Insecticidal activity of crude leaf extract of *Cleome viscosa* was tested on *Tribolium castaneum. C. viscosa* had insecticidal activity. Insecticidal activity of crude extract from *C. viscosa* is as follows:

Table 1: Insecticidal activity of *C. viscosa* Column fractions against *Tribolium castaneum*-

Column fractions	Mean % mortality
100% Hexane	Nil
10% Ethyl Acetate	100
50% Ethyl Acetate	Nil
100% Ethyl Acetate	Nil
100% Acetone	Nil

The crude leaf extract of plant *Cleome viscosa* had insecticidal activity. *Cleome viscosa* plant material was taken for column chromatography for fractionation step. Fractionation was carried out by using organic solvents low polar solvents followed by high polar solvents at a constant flow rate. Fractions containing dissolved phytochemicals were collected at particular time intervals. The collected fractions were assayed for insecticidal activity by both contact and fumigation method. Among the entire fractions Ethyl acetate 10% fraction (25mg/100cc) showed toxic activity against tested insects in fumigation method suggesting the presence of principle bioactive compound (in Table 1).

Table 2: Insecticidal activity of various crude extracts (mg/ml) to the third instars larvae of *Tribolium castaneum*-

Sr. No	Plants	Mortality (in %)		
		0.5	1.0	2.0
1	*Cleome viscosa*	20	50	100

From the (Table-2), it appears that four of the five leaf extracts tested possessed high larvicidal activities in various degrees. The floral extract of *C. viscose* and *was* the most lethal among the five plant extracts tested. At dosage of 2 mg /ml two plant extracts caused 100% mortality in the treated larvae and almost all the extracts were ineffective in producing mortality at lower doses such as 0.5 mg/ ml against *A. aegypti* larvae. A gradient increase in mortality with increase in concentration was observed in all the treatments. The extracts of *C. viscose* was moderately toxic and needed at least 2 mg/ ml to obtain 50% mortality. No significant difference in larval mortality was found in less than 0.5mg/ml concentrations compared to the control treatment.

Table 3: LC_{50} and LC_{95} with fiducial limits (95%) of tested botanical extracts against larvae of *Tribolium castaneum*-

Sr. No	Plants	Activity (mg/ml) (95% FL)	
		LC_{50}(LCL-UCL)	LC_{95}(LCL-UCL)
1	*Cleome viscose*	0.8(0.69-0.99)	1.95(1.63-2.68)

FL: Fiducial limit, UCL: upper confidence limit, LCL: lower confidence limit

More accurate data on the toxicity of the plant extracts were obtained by calculation of their LC_{50} and LC_{95}. In contrast to the results obtained from screening procedure it was observed that *C. viscosa,* showed increased mortality rate (Table 3). *C. viscosa* showed high toxicity with a LC_{50} of 0.8 mg/ml and LC_{95} of 1.95 mg/ml.

Table 4: Toxicity of Nanoformulated botanical compounds (mg/12cm^2) against *Tribolium castaneum*- 3rd instar larva by antifeedancy method.

Compounds	Antifeedant index					
	0.25mg	0.50 mg	0.75 mg	1.00 mg	2.00 mg	3.00 mg
NF Eugenol	30.2	98.1	100	100	100	100
NF 1,8-Cineole	15.3	29.3	49.0	68.0	100	100

*NF: Nanoformulated

The effect of Nanoformulated pure compounds on *Tribolium castaneum*- are presented in Table 4. A gradient increase in Antifeedancy with increase in concentration was observed in both the treatments. At dosage of 0.75mg/12cm^2 caused 100% mortality with Eugenol. were as with 1,8-Cineole dosage of 2mg/12cm^2 caused 100% mortality. Antifeedancy is doubled in NF Eugenol compared to pure eugenol. In the case of NF 1,8-Cineole there was no increase in antifedancy compared to pure 1,8-Cineole. In contrast to the results obtained by the NF 1,8-Cineole, the toxicity of the NF Eugenol showed increased Antifeedancy. It was observed that larvae became slowly inactive within 12 h. They extended the duration of the various larval instars and of pupation at very low concentration and showed toxicity at higher concentrations.

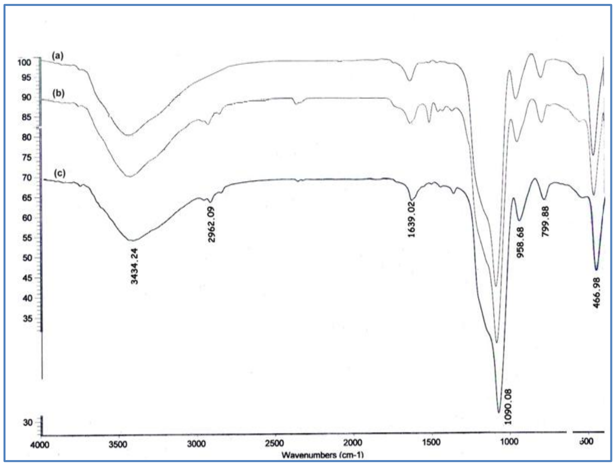
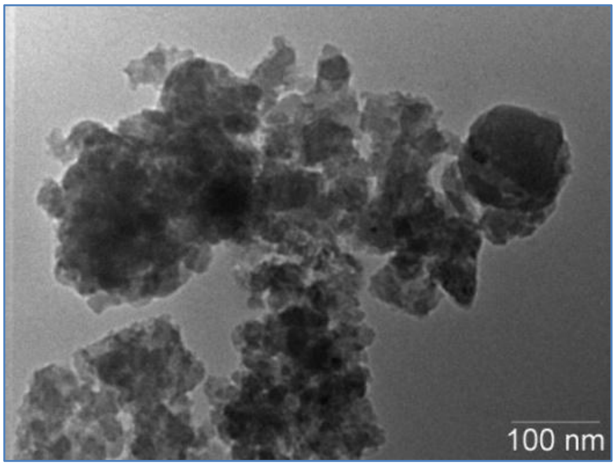


Figure 1: FT-IR spectra of native and Nanoformulated (100 µl/100mg) compounds

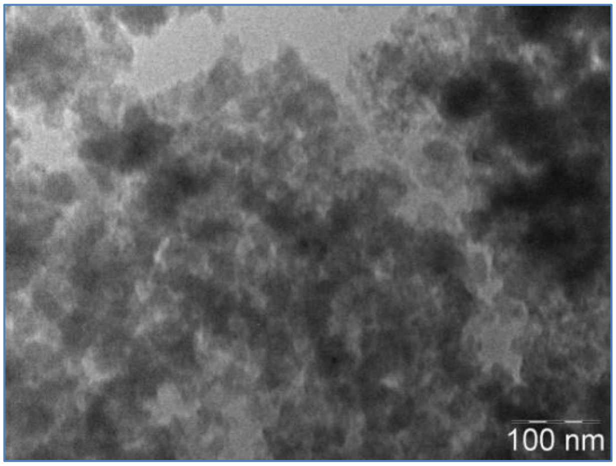
(a) Native nano-SiO$_2$, (b)NF Eugenol 100 µl /100mg and (c) NF 1,8-Cineole 100 µl /100mg

Fig 1. shows FT-IR spectra of native nano-SiO$_2$ particles and the formulated nano-SiO$_2$ particles with Eugenol and 1,8-Cineole, respectively. From fig 4.7.(a) the FT-IR spectra of unmodified nano-SiO$_2$, we can see that the peak at 3422cm−1 is attributed to O–H stretching mode, shear vibration near 1630cm−1, bending mode near 1390cm−1. The peak at 1103cm−1 corresponding to the Si–O–Si adsorption bands, the asymmetric stretching vibration near810 cm−1, bending vibration near 487 cm−1.

In Fig 1. (b) ,(c), fig 4.8 and fig 4.9 The peak at 2926 cm−1 is assigned to the methane symmetrical stretch vibration in terpene, which confirmed the adsorption of the terpene on silica surface. Peak at 1635 cm−1 indicates the presence of moisture in the sample. Slight decrease in OH bending band (800 cm−1) and Si-O bond rocking band (467 cm−1) was observed. A peak of hydroxyl group (between 3200 to 3600 cm−1), The Si-OH band stretch (958 cm−1) and asymmetric Si-O-Si stretching (1090 cm−1) does not alter after adsorption. Thus it can be concluded that the terpenes adsorbed on the silica through strong hydrogen bonding. The morphological information was of native nano-SiO$_2$ and terpene anchored nano-SiO$_2$ suspensions in methanol were obtained by TEM images as shown in fig.2.



(a) Native nano-SiO$_2$



(b)NF Eugenol 100 µl /100mg

Figure 2 (a,b): TEM morphology of nano-SiO$_2$ particles

The agglomeration was observed in both the samples, however less agglutination was observed in terpene adsorbed nanosilica suggests that physical bonding or chemical bonding occurs between the terpene and nanosilica which bring mutual exclusion and steric hindrance effect, thus the surface free energy has been reduced correspondingly and the agglomeration controlled. All the results above further illustrate that adsorption of terpene has an added advantage that in spite of less agglutination it also exhibit the high dispersion stability.

DISCUSSION:

Today, the environmental safety of an insecticide is considered to be of paramount importance. An insecticide does not have to cause high mortality on target organisms in order to be acceptable but it should prevent the breeding. Phytochemicals may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, inexpensive, and are readily available throughout of the world. According to Bowers *et al.*[5]the screening of locally available medicinal plants would generate local employment; reduce dependence on expensive imported products. The biological activity of these plant extracts might be due to the various compounds, including phenolics, terpenoids, and alkaloids, existing in plants. These compounds may jointly or independently contribute to produce insecticidal activity. In our study, strong insecticidal activity against adults of *Tribolium* was obtained with 10% ethyl acetate fraction from *C. viscosa* having potent insecticidal activity on *T. castaneum* was slower (over 90% mortality was obtained at 1-2 days after treatment). This plant material confirm its usefulness as potent insect-control agents. Many plant-derived materials with high volatility act as fumigants. It has been reported that many phytochemicals have broad insecticidal activity against agricultural, stored-product and medical insect pests, and acts as a fumigant. In our study, *C. viscosa* was much more effective against adults of *T. castaneum* in fumigation than in contact method. These results indicate that the insecticidal mode of action of the compounds may be largely attributable to fumigant action: they may be toxic by penetrating the insect body via the respiratory system.

This work demonstrates the potency of *C. viscosa* extract in the control of *A. aegypti* mosquito larvae with LC_{50} value 0.86mg/ml. Our results corroborates with the results obtained by Pushpalatha and Muthukrishnan et al[6] who stated that the fractions of *Vitex negundo* and *Syzygium jambolanum* prolonged the larval and pupal duration of *Culex quinquefasciatus* and *Anopheles stephensi*. However, the mechanism by which molecule kills the larvae is the subject of research currently under way by our team.

Till now, there has been no promising solution for sustainable control of dengue vectors. The trend for dengue vector *A. aegypti* control in this region has shifted from relying solely on insecticides to biological control, source reduction and environmental management through community participation. These findings have re-emphasised the need to explore the possibility of using herbal-based larvicides as supplementary and complimentary measures for malaria control. This will reduce the chemical burden on the environment. It is noteworthy that foliar extract of *C. viscosa* was very promising. Furthermore, these plants grow wild in uncultivated dry zones in the southern India hence the plant materials could be easily collected without any additional cost. Therefore, plant materials could be used as a larvicidal agent in an integrated vector control programme. Research into their mode of action, effect on non-target organisms and field evaluation are presently under investigation. Further investigations are needed to elucidate this activity against a wide range of mosquito species and also the separation of active ingredient(s) of the extract responsible for larvicidal activity in *A. aegypti* should be identified and utilized. The two-plant extracts show promising activity in mosquito control and commercial utilization seems to be very much feasible.

The terpenes and nano adsorbed terpenes tested for their efficacy as insect antifeedants against *A. janta* and *S. litura*. Both the compounds demonstrated the most effective feeding deterrence against *S. litura*. The effect of pure botanical compounds Eugenol and 1,8-Cineole on *A. janata* resulted a gradient increase in Antifeedancy with increase in concentration was observed in both the treatments. At dosage of $1\mu l/12cm^2$ caused 100% mortality with Eugenol. where as with 1,8-Cineole dosage of $3\mu l/12cm^2$ caused 100% mortality. The effect of Nanoformulated pure compounds Eugenol and 1,8-Cineole on *A. janata* showed a gradient increase in Antifeedancy with increase in concentration was observed in both the treatments. At dosage of $0.75mg/12cm^2$ caused 100% mortality with Eugenol. where as with 1,8-Cineole dosage of $2mg/12cm^2$ caused 100% mortality. Antifeedancy is doubled in NF Eugenol compared to pure Eugenol. In the case of NF 1,8-Cineole there was no increase in antifedancy compared to pure 1,8-Cineole. In contrast to the results obtained by the NF 1,8-Cineole, the toxicity of the NF Eugenol showed increased Antifeedancy.

FT-IR spectra of the formulated nano-SiO_2 particles with Eugenol and 1,8-Cineole showed the peak at

2926 is assigned to the methane symmetrical stretch vibration in terpene, which conformed the adsorption of the terpene on silica surface. Peak at 1635 indicates the presence of moisture in the sample. Slight decrease in OH bending band (800 cm-1) and Si-O bond rocking band (467cm) was observed. A peak of hydroxyl group (between 3200 tom3600 cm^1), The Si-OH band stretch (958) and asymmetric Si-O-Si stretching (1090) does not alter after adsorption. Thus it can be concluded that the terpenes adsorbed on the silica through strong hydrogen bonding.

Control release studies of Eugenol with the formulation at 25 µl /100mg nanosilica lead to 20.22% pesticide release when compared to standard, this indicated that rest 79.78% of pesticide is loaded on to the nanosilica. The rest loded pesticide will release subsequently after two to five days, which prevent the subsequent infestation from pests. The control release efficiency of the formulation constant with the increase in compound in formulation up to at 75 µl /100mg and finally reach25.04%, at 100 µl /100mg which has high stability as well as high bioactivity. Control release studies in case of 1,8-Cineole with formulation at 25 µl /100mg nanosilica lead to 15.53% pesticide release when compared to standard, this indicated that rest 84.47% of pesticide is loaded on to the nanosilica. 100% loaded pesticide will release subsequently after two to five days which hinder the further pest infestation. Control release efficiency varies with concentration of the formulations prepared. At 50µl /100mg 75 µl /100mg nanosilica the control release efficiency is around 35 % where as at 100 µl /100mg showed enhanced control release with 28.75% .

The dispersion stability of terpene adsorbed nano-SiO_2 particles in methanol is compared with native nano-SiO_2 particles showed that the native nano-SiO_2 particles have completely precipitated for about 3 days, while the terpene adsorbed SiO_2 nano-particle have a stable colloidal dispersion even after 3 days. The hydroxyl groups (–OH) from nano-SiO_2 particles can interacted with methyl group of terpene which play a vital role in reducing the agglutination of the inorganic nano-particles for a long time.

CONCLUSION:

Among the tested plant materials, *C. viscosa* proved as highly toxic against stored product pests as well as mosquito larvae. This active fraction will be further processed for the pure compound isolation, identification for the principle bioactive compound. The two principle bioactive compounds showed enhanced antifeedant activity against agricultural pests on Nanoformulations. These Nanoformulations are stable and exhibited high compatibility by releasing the compounds slowly from the formulation. These formulations will be further processed for their mode of action against agricultural pests as insect control agents.

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