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Efficacy of certain botanicals against cotton mealybug *Phenacoccus solenopsis* L. (Pseudococcidae: Hemiptera) on Okra

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Abstract
Okra, (*Abelmoschus esculentus*), herbaceous hairy annual plant of the family Malvaceae and its edible fruit. Currently, a mealy bug species, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), has emerged as a major pest of Okra in the recent decades. The present study with methanol and ethanol leaf extracts of botanicals namely *Andrographis panicilata* and *Eucalyptus globulus* and were tested for biocidal and repellence property against *P. Solenopsis* in laboratory conditions against the test insect in different concentrations (1%, 2%, 4%, 8% and 10%). The 10% methanol leaf extracts of *Andrographis panicilata* and *Eucalyptus globulus* has shown the mortality of 100% and 96.08% respectively. In concern with repellency the methanol and ethanol leaf extract of *Andrographis panicilata* has given 99.02% and 97.73% repellence effect respectively.

Keywords: Okra, malvaceae, cotton mealybug, biocidal, repellency

Introduction
Okra *Abelmoschus esculentus* L. (Moench), is an economically important vegetable crop belonging to family Malvaceae and grown in tropical and sub-tropical parts of the world. It has a great nutritional value and is said to be "a perfect villager's vegetable" (Holser and Bost, 2004; Adamou *et al*., 2010) [14, 2]. The total area and production under okra is reported to be 1148.0 thousand ha and 7896.3 thousand tons respectively. Largest area and production is in India (72% of the total world production) followed by Nigeria. In India the land under cultivation of okra is about 501.00 ha and production is about 5783.00 tons (2016-2017). In recent reports, the area under okra cultivation is 11 thousand ha and the production is 75.4 thousand tons (2014-15). However, the productivity of the country is low compared to other countries due to yield losses caused by insect pests, diseases and nematodes. The crop is said to be attacked by more than 72 insect pests and infests the crop from seedling to harvest stage (Srinivasa and Rajendran, 2003) [32].

With the introduction of Bt cotton in 2002, the pest status has changed considerably. Presently, a mealy bug species, *Phenacoccus Solenopsis* Tinsley (Hemiptera: Pseudococcidae), has emerged as a major pest of cotton, vegetables and fruits (Tanwar *et al*., 2008) [35]. In India, *P. Solenopsis* has been reported on many crops (Suresh & Kavitha, 2008) [33] and is found to cause economic losses in various states including Punjab, Haryana, Rajasthan, Gujarat, Maharashtra, Andhra Pradesh and Tamil Nadu (Nagrare *et al*., 2009) [23]. Widespread infestation of this mealybug in India occurred over large areas of different cropping systems across the three cotton growing regions, attaining pest status during 2008.

Both nymphs and adults cause damage by sucking the plant sap from the growing points resulting the infested plants to lose their vitality, remain stunted, leaves turning yellow, dry up and finally death of plants in case of heavy infestation. In addition to the direct losses that the insects can cause by sucking the phloem sap, its feeding secretions (honeydew) cause further losses to the plants by disturbing the photosynthesis activity and inducing fungal contaminations (Arif *et al*., 2003) [7]. Due to the highly polyphagous nature and the ability to oviposit on a wide range of host plants (Arif *et al*., 2009) [6], *P. Solenopsis* has huge potential to invade new areas and to adapt to a wide range of ecological situations. This clearly indicates that *P. Solenopsis* can pose a serious threat to agricultural and horticultural production, especially in tropics and subtropics where the temperatures are expected to increase under projected climate change. There is a need for undertaking adaptive strategies against this pest to lessen the yield losses and safeguard the interest of crop growers (Babasaheb *et al*., 2014) [8].

In an intention to manage the insect pest, farmers mostly rely on insecticides (Saeed *et al*., 2007) [28]. Presently, insecticides from different insecticide classes including organophosphates, carbamates, pyrethroids (Saeed *et al*., 2007; Ahmad *et al*., 2007; Kranthi *et al*., 2012) [28, 3, 19] and new chemicals (David *et al*., 2010; Lysandrou *et al*., 2012) [11, 20] are being used for controlling *P. Solenopsis*. However, due to inadequate public awareness on the dangers posed by usage of pesticides and deficient end-user protection; the use of pesticides is often unsophisticated and obnoxious causing severe human health problems, development of pest genotypes resistant to insecticides (Ahmed *et al*., 2003; Ishtiaq and Saleem, 2011; Khan *et al*., 2011; Basit *et al*., 2011; Shad *et al*., 2012; Khan *et al*., 2013a,b) [4, 15, 16, 9, 30, 17, 18], resurgence, upset of insect pests and environmental pollution (Nas, 2004; Nadeem *et al*., 2014) [24, 22]. Recent reports of control failure of *P. Solenopsis* in cotton growing areas in Punjab, Pakistan (Anonymous, 2011) [5] revealed that insecticide resistance could be the probable reason for such failures in the field.

India has a vibrant repository of medicinal plants and perhaps the world's most sophisticated indigenous medical heritages. The wealth is not only in terms of the number of unique species documented so far for their medicinal use but also the depth of the traditional knowledge base about the uses for human, veterinary health care and crop protection (Ved and Goraya, 2008) [37]. Botanical pesticides are biodegradable and their use in crop protection is a practical sustainable alternative. It maintains biological diversity of predators, and reduces environmental contamination and human health hazards. Botanicals are safe for home gardens and green houses. Therefore the present study attempts to evaluate the efficacies of some native botanicals against cotton mealybugs. They induce fumigant and topical toxicity as well as antifeedant or repellent effects. They are toxic to adults but also inhibit reproduction. Although mechanisms depend on phytochemical patterns and are not yet well known, this widespread range of activities is more and more being considered for both industrial and household uses for controlling insect pests.

Materials and methods

The present study entitled as "Effect of certain botanicals against Cotton Mealybug, *Phenacoccus Solenopsis* L. (Pseudococcidae: Hemiptera) on okra" was carried out during 2016-2018 in the Department of Entomology, Faculty of Agriculture, Annamalai Nagar, Chidambaram, Tamil Nadu. The following are the description about the materials and methods performed to conduct the study.

Collection on plant materials

The plant materials were collected from different places of Tamil Nadu. The leaves of Eucalyptus (*Eucalyptus globulus*) and leaves of Andrographis (*Andrographis paniculata*) were collected from Chidambaram (11.4° N 79.7° E) Tamil Nadu. The collected materials were packed in separate zip-lock pouches and were labeled and stored under cool, dry condition in room temperature 27 ± 2 °C and 75% relative humidity.

Extraction of plant material

The collected plant materials were washed with water and shade dried for duration of about five to seven days. Then the shade dried samples were powdered using the Willey mill. 50g of powdered botanicals from each was weighed and transferred to a cellulose extraction thimble. These powders were loaded in Soxhlet apparatus and refluxed with ethanol and methanol for 8 hours and the extracts were decanted from the flask separately. The extracted solvents were evaporated in a hot plate. The final extracts were elucidated with corresponding solvents and used for the evaluation of experiments. Further dilutions were made for further experiments (Prishanthini, 2013) [26].

Storage and packing of plant extracts

The botanical extracts were stored in tight, light- resistant containers and were avoided from exposure to sunlight and excessive heat. These extracts were placed under refrigerated condition at 4 °C in separate bottles for maximum of three days. Freshly prepared extracts were utilized for each batch of bioassay. Labeling was done to indicate the name of the plant part used and the names of the solvents used in extraction.

Culturing of test insect

The test insect cotton mealy bug (*Phenacoccus Solenopsis)* were collected from infested plants of cotton and okra and were ensured that the plants were not treated with pesticides and had no residues of chemicals. The mealy bugs were then introduced to a pumpkin that was well ripe and had prominent grooves in them. To ensure multiplication of mealy bug the grooves were given slight cuts and honey solution was dripped in the grooves. Before the introduction of the test insect on the pumpkin, the pumpkin was sterilized using 1% bavistine solution and the cage (45 cm x 40 cm x 40 cm) into which the pumpkin was placed was sanitized. The cage was wrapped with sheets of paper to ensure shade for the multiplication of the mealy bugs. In the laboratory, the maximum and the minimum temperature and relative humidity of the study area ranged from 25.6 to 36.5 °C and 23.4 to 25.5 °C, and 40.5 to 92.5% RH respectively.

Transferring the mealy bugs from infested plants to pumpkin was done using a camel hair brush (No.1). After introduction of test insect on the pumpkin, the emergences of ovisacs were witnessed in a couple of weeks. Observations on survival and molt of the crawlers were recorded daily under stereoscopic microscope.

Evaluating the Biocidal properties of plant extracts against *Phenacoccus Solenopsis*; Laboratory bioassay: On-plant assay for Mortality Assessment

The test insects (crawlers and adults) were transferred to fifteen days old seedlings of okra (*Abelmoschus esculentus*) separately at 10-15 insects/plant and allowed to settle. After 24 hours, artificially infested plants were treated with various doses of various plant extracts separately. Three replications were maintained for each treatment. Observations were made on the mortality of crawlers and adults after 12, 24 and 48 hours of treatment. The doses of plant extracts tested under on-plant bioassay were 1, 2, 4, 8, and 10% per plant. The mealybug that lacked mobility was considered dead and taken into account. The mortality records for all treatments were obtained in percentage values.

Percent corrected mortality was calculated by following formula described by Schneider-Orelli's (1947) [29] and Puntener (1981) [27].

$$\% \text{ mortality over control} = \frac{\% \text{ mortality in treatment} - \% \text{ mortality in control}}{100 - \% \text{ mortality in control}} \times 100$$

Laboratory bioassay: for repellency assessment

A promising variety of okra (*Arka anamika*) was selected as a test variety for this study. About 15 days old saplings under greenhouse condition was developed with necessary culture practices. The botanical plant extracts were treated with means of an atomizer on the okra plant. It was allowed to dry for a while in the normal greenhouse temperature. About 20 second instar mealy bug nymphs were released on the treated plant and were examined for its repellent movement.

The plant extracts of different dose levels (1, 2, 4, 8, and 10%) were treated on the okra plants. The repellent effect of the botanical leaf extracts were calculated by repellent movement of these mealy bugs away from corresponding treated okra plant. Appropriate observations were recorded at 24 and 48 hours after insect release on the okra plants. The experimental treatments were replicated thrice and subjected to the statistical analysis for its significance among various botanical leaf extract treatments (V. Sathyaseelan and V. Bhaskaran, 2010) [36].

Percentage repellency (PR) values were computed using the formula suggested by Singh *et al*. (2012) [31].

$$PR = [(NC - NT)/ (NC+NT)] \times 100$$

Where NC = No. of insects present on control

NT = No. of insects on the treated plant

Statistical analysis

The recorded data in the experiments were subjected to analysis of variance (ANOVA) under Completely Randomized block design by adopting the procedures described by Gomez and Gomez (1984) [13]. Necessary data transformation was made before analysis and the computer based on OPSTAT package was used for the calculation.

Results and discussion

The results that were witnessed due to the outcome of the treatments implemented are elucidated in the forthcoming proceedings. Several authors have reported that plant extracts possess similar type of anti-feedant insecticidal and growth inhibition activities against insects (Elumalai *et al*., 2013; Swati *et al*., 2015; Maria *et al*., 2015) [12, 34, 21]. The present study was carried out in laboratory condition with two botanicals namely *Andrographis panicilata* and *Eucalyptus globulus* were tested for biocidal and repellence property against *P. Solenopsis* in laboratory conditions. All the extracts i.e. methanol and ethanol extracts showed both biocidal and repellence effect against the test insect in different concentrations (1%, 2%, 4%, 8% and 10%). Among the methanol leaf extracts of botanicals, the best results regarding the repellency of *P. Solenopsis* was observed with 10 % leaf extract of *A. paniculata* (99.02%) followed by leaf extract of *E. globulus* (95.28%). Midst the ethanol extracts highest repellence effect was exhibited by 10% leaf extract of *A. paniculata* (97.73%) in 48 h. The highest percent repellency recorded was in the treatment with 10 % methanol leaf extract of *A. paniculata.* The percent repellency was 99.02% in 48 h interval after treatment with extract. It was followed by ethanol leaf extract of *A. paniculata* in which the percent repellency were 97.73% in 48 h respectively. In accordance with the results of percent mortality, among the 10 % methanol leaf extract *A. paniculata* unveiled maximum mortality (100%) was witnessed in 48 h. Followed by *E. globulus* that showed 96.08% mortality. *Ethanol extract of A. paniculata* revealed maximum mortality (96.69%) among the ethanol extracts. The least mortality effect was given by *E. globulus.* The percent mortality noted was 85.01%. Overall peak mortality effect was revealed in treatment with methanol extract of *A. paniculata*. The percent mortality as recorded as 100% for the above mentioned botanical leaf extract.

The forthcoming proceedings are concerned to be the insecticidal properties of the selected botanicals in comparison with the former studies and researches. By the results of the present study it was sorted that the selected botanicals had innate insecticidal properties when extracted with different solvents.

In the present study evaluating the repellency of the test insect, *Phenacoccus solenopsis* (Cotton Mealybug), the repellency was recorded the highest when the test plants (okra) were treated with methanol extract of *Andrographis paniculata*. The extract was remarkably noticed to have good repellence effect against the cotton mealybug. The repellency effect perceived during treatment with 10% concentration at 12h and 24 h were 76.16% and 98.12% respectively. This extract showed 99.02% of repellency at the concentration of 10%. Thus this was considered best in regards to repellence effect.

Similar study by Sathyaseelan and Bhaskaran (2010) [36] also viewed nearly the same results as the above study. The percent repellency was marked to be about 98.12% and 99.00% at 10 % concentration of methanol extracts of *A. paniculata* in 24h and 48 h intervals respectively.

In the present study, the percent repellency effect of *P. Solenopsis* in using the ethanol extract of *A. paniculata* was observed to be 65.52% in 12 h, 97.11% in 24 h and 97.73% in 48 h respectively at 10% concentration. The repellency was maximum in methanol leaf extract of *A. paniculata* compared with other two solvent extracts of *A. paniculata*.

In regards to the ethanol and methanol extracts in achieving maximum mortality, the methanol leaf extract of *A. paniculata* had remarkable result of succeeding 100% mortality in all consecutive intervals while the ethanol extract exhibited 96.69% mortality in 48h at 10% concentration.

Oparaeke *et al*. (2005) [25] found that the treatment of mixtures of neem and eucalyptus leaf extract with extracts of lemongrass, African curry, tomato, bitter leaf and African bush tea on *Maruca vitrata* Fab. Pod borers and *Clavigralla tomentosicollis* Stal caused great reduction in pod damage/plant and ensured higher yield as compared to untreated plants. *Eucalyptus globulus* Labill. Consisting of 1.8-cineole, a-pinene and p-cyms (Koul *et al*., 2008) has acted as a good repellence against insects. Similarly in the current study, the repellency of various botanicals were studied with varied concentrations such as 1%, 2%, 4%, 8% and 10% (Singh *et al.,* 2012) [31]. The study results provided are as follows. The methanol extracts of *Eucalyptus globulus* exhibited repellence effect against the test insect (cotton mealybug). At 12 h after treatment the percent repellence observed were 16.67%, 34.33%, 44%, 61.33% and 70.33% at 1%, 2%, 4%, 8% and 10% concentrations correspondingly. Whereas at 24 h after treatment the percent mortality was 44%, 55%, 71%, 77.3% and 93 % at 1%, 2%, 4%, 8% and 10% concentrations correspondingly. These results coincides with the observed experimental results was 19.14%, 27.28%, 40.09%, 54.24% and 64.36% at 12 h interval at 1%, 2%, 4%, 8% and 10% concentrations respectively while the readings at 24 h were observed as 44.16%, 55.15%, 71.26%, 77.27% and 93.30% as per the former concentrations. On the other hand the repellency exhibited by the *Eucalyptus globulus* ethanol leaf extract was 91.43% respectively in 48 h at 10% concentration.

In concern with the repellency effect of the *Eucalyptus globulus*, Bhuwan *et al.* (2012) [10] conferred that the Eucalyptus oil had shown best results against the storage insects, rice weevil *Sitophilus oryzae* L. (Coleoptera: Curculionidae) and red flour beetle *Tribolium castaneum* Herbs (Coleoptera: Tenebrionidae). The repellent activity of *E. globulus* progressively increases with increase in concentration against both stored-grain insect pests.

Abdul Rehman *et al.* (2013) reported that the effect of the solvent extracts reduces with time and comparing the results of the present study and the former study by Abdul *et al.*, there are evidence and matches regarding the statement, as there was no hike or greater increase in between the consecutive readings especially between 24 h and 48 h. This phenomenon was also witnessed in the present study. For instance, the methanol leaf extract of *E. globulus* had resulted to 19.14%, 44.16 % and 54.28% at 12 h, 24 h and 48 h after treatment at the lowest concentration. Whereas at the highest concentration the repellency was noted as 64.36%, 93.30% and 95.28% for 12h, 24h and 48 h intervals respectively. Among all the extracts the repellency effect was maximum in using methanol leaf extract of *A. paniculata* (99.02) > Ethanol leaf extract of *A. paniculata* (97.73). Regarding the percent mortality or biocidal activity the maximum results were achieved by Methanol leaf extract of *A. paniculata* (100%) during the laboratory assay.

Table 1: Efficacy of Different Extracts of *Andrographis paniculata* against *Phenacoccus Solenopsis* on Okra (Mean Percentage Mortality) @ Different Time Intervals

S. No	Treatment Concentration (%)	Methanol			Ethanol		
		12 h	24 h	48 h	12 h	24 h	48 h
1	1%	36.73 (37.27)	40.38 (39.44)	58.97 (50.14)	17.57 (24.77)	45.26 (41.30)	47.99 (43.83)
2	2%	62.39 (52.15)	69.38 (56.38)	73.12 (58.75)	31.18 (33.93)	61.30 (51.57)	68.61 (55.90)
3	4%	84.57 (66.84)	87.35 (69.14)	92.62 (74.31)	43.52 (41.26)	72.84 (58.56)	76.83 (61.20)
4	8%	97.74 (81.32)	100 (90)	100 (90)	57.06 (49.04)	92.20 (73.78)	93.39 (75.00)
5	10%	100 (90)	100 (90)	100 (90)	63.33 (52.71)	95.83 (78.20)	96.69 (79.46)
6	Control	00.00	00.00	00.00	00.00	00.00	00.00
	SE(d)	0.142	0.092	0.038	0.294	0.495	0.235
	CD	0.314	0.202	0.084	0.648	1.090	0.517

*****Mean of three replications
Values in parentheses are arc sine transformed

Table 2: Efficacy of Different Extracts of *Eucalyptus globulus* against *Phenacoccus Solenopsis* on Okra (Mean Percentage Mortality) @ Different Time Intervals

S. No	Treatment Concentration (%)	Methanol			Ethanol		
		12 h	24 h	48 h	12 h	24 h	48 h
1	1%	20.19 (26.69)	46.05 (42.17)	55.94 (48.39)	10.51 (18.92)	35.48 (36.54)	45.57 (42.44)
2	2%	29.58 (32.93)	57.05 (49.03)	64.13 (53.19)	22.40 (28.25)	40.73 (39.64)	50.56 (45.30)
3	4%	42.18 (40.48)	73.31 (58.87)	78.05 (62.03)	34.56 (35.99)	54.90 (47.78)	61.07 (51.37)
4	8%	56.07 (48.47)	79.16 (62.81)	83.47 (65.03)	46.57 (43.01)	70.17 (56.87)	76.1 (60.71)
5	10%	67.18 (65.02)	94.94 (76.67)	96.08 (78.55)	55.03 (47.87)	84.95 (67.15)	85.01 (67.18)
6	Control	00.00	00.00	00.00	00.00	00.00	00.00
	SE(d)	0.069	0.064	0.077	0.191	0.345	0.303
	CD	0.153	0.140	0.169	0.421	0.761	0.668

*****Mean of three replications
Values in parentheses are arc sine transformed

Table 3: Efficacy of Different Extracts of *Andrographis paniculata* against *Phenacoccus Solenopsis* on Okra (Mean Percentage Repellency) @ Different Time Intervals

S. No	Treatment Concentration (%)	Methanol			Ethanol		
		12 h	24 h	48 h	12 h	24 h	48 h
1	1%	16.14 (23.68)	48.28 (43.99)	52.21 (46.24)	20.21 (26.70)	44.76 (41.97)	44.02 (44.42)
2	2%	24.40 (29.50)	66.46 (54.58)	72.05 (58.06)	32.34 (34.64)	63.14 (53.60)	70.27 (56.93)
3	4%	56.32 (49.00)	79.49 (63.05)	84.52 (66.80)	45.88 (42.62)	74.97 (59.95)	78.40 (62.28)
4	8%	67.16 (55.01)	95.54 (77.77)	96.57 (79.29)	57.73 (49.42)	93.91 (75.70)	95.08 (77.15)
5	10%	76.16 (60.75)	98.12 (82.08)	99.02 (84.30)	65.52 (54.02)	97.11 (80.18)	97.73 (80.54)
6	Control	00.00	00.00	00.00	00.00	00.00	00.00
	SE(d)	0.404	0.114	0.157	0.222	0.724	0.194
	CD	0.889	0.250	0.345	0.489	1.595	0.428

*****Mean of three replications
Values in parentheses are arc sine transformed

Table 4: Efficacy of Different Extracts of *Eucalyptus globulus* against *Phenacoccus Solenopsis* on Okra (Mean Percentage Repellency) @ Different Time Intervals

S. No	Treatment Concentration (%)	Methanol			Ethanol		
		12 h	24 h	48 h	12 h	24 h	48 h
1	1%	19.14 (25.93)	44.16 (41.59)	54.28 (47.43)	14.61 (22.46)	39.36 (38.84)	47.60 (43.99)
2	2%	27.28 (31.44)	55.15 (47.93)	62.31 (52.10)	26.19 (30.76)	44.97 (42.09)	57.16 (47.94)
3	4%	40.09 (39.27)	71.26 (57.55)	76.31 (60.85)	38.13 (38.12)	59.26 (50.31)	64.31 (53.23)
4	8%	54.24 (47.40)	77.27 (61.50)	81.27 (64.33)	50.65 (45.35)	74.57 (59.73)	79.22 (62.86)
5	10%	64.36 (53.32)	93.30 (74.99)	95.28 (77.42)	58.10 (49.83)	91.41 (75.59)	91.43 (72.97)
6	Control	00.00	00.00	00.00	00.00	00.00	00.00
	SE(d)	0.076	0.126	0.091	0.265	0.243	0.743
	CD	0.166	0.277	0.201	0.583	0.535	1.638

*****Mean of three replications
Values in parentheses are arc sine transformed

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