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Screening of botanicals for insecticidal property against pink mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae)

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ABSTRACT: The insecticidal activity of 10 per cent aqueous extract of 46 plant species was evaluated against the pink, grape or hibiscus mealybug, *Maconellicoccus hirsutus* using atomization method. The per cent mortality ranged between 0.0 to 96.7 at 24 hours after exposure (HAE) and 0.0 to 98.3 at 48 and 72 HAE. *Calotropis gigantea* showed the highest mortality *i.e.* 96.7, 98.3 and 98.3% at 24, 48 and 72 HAE, respectively which was statistically superior to rest of the plants. It was followed by *Ricinus communis, Helicteres isora*, *Centella asiatica*, *Spathodea campanulata*, *Colocasia esculenta*, *Ocimum tenuiflorum*, *Curcuma longa*, and *Piper nigrum* which also showed potential in managing pink mealybugs.

Keywords: Botanical insecticides, *Maconellicoccus hirsutus,* Pink mealybug

INTRODUCTION

Pink mealybug, *Maconellicoccus hirsutus* also known as grape or hibiscus mealybug is a polyphagous sucking pest that feeds on a wide range of horticultural and agricultural crops globally distributed over 330 species, (Chong *et al*., 2015). In India, losses due to *M. hirsutus* have been reported in cotton (Muralidharan and Badaya, 2000); grapevine (Manjunath,1985); pigeon pea (Patel *et al*.,1990), coffee, guava, citrus, beans, maize, mulberry (Manjunath *et al.,* 2006; Mala *et al.,* 2007) and sugar cane (Reddy *et al.*, 2009).

The waxy covering and concealing nature makes it difficult to control mealybugs which forces farmers to increase the dosage and frequency of chemical insecticide applications (Williams, 1996).The use of synthetic insecticides has resulted in irreparable harm and damage to our fragile environment in addition with pest resurgence, pesticide residue and elimination of natural enemies. Therefore non-chemical methods provide a better option in mealybug management in a long run. Plant derived insecticides are biodegradable and environmental friendly, hence it serves as an excellent alternative to synthetic one (Koul *et al*., 2008). Botanicals possessing insecticidal and repellent action have been reported against pink mealybug *viz. Azadirachta indica* (Verghese, 1997; Kulkarni and Patil, 2013), *Abrus precatorius (*Anitha *et al*., 1999), *Clerodendron inerme* (Katke and Balikai, 2008), *Balanites aegyptiaca, Quillaja saponaria* (Patil *et al*., 2010), *Pongamia pinnata*, *Madhuca longifolia*, *Lantana camara*, *Adathoda vasica* (Thinnaluri *et al*., 2014) etc. Apart from these, botanicals like tobacco, castor oil, neem oil, pongam oil, mahua oil, sweet flag, *Annona squamosa*, *Calotropis gigantea*, *Allium sativum, Ocimum sanctum* have been tested against different species of mealy bugs (Ahmed *et al*., 2011; Gowda *et al*., 2013; Prashanthini and Vinobaba, 2014; Biswas *et al*., 2015; Manzoor and Haseeb, 2015; and Khan, 2016). In recent few years, mealybugs have succeded to grab attention of researchers with its outbreaks being one of the most difficult pest to control. However, there is no extensive study of botanicals against mealybugs yet inspite of all the reports. In view of above, this research aims to explore more potential botanicals for management of pink mealybug, *Maconellicoccus hirsutus* (Green).

MATERIALS AND METHODS

Maintenance of mealybug culture

All the laboratory experiments were conducted at Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore. First instar crawlers of *M. hirsutus* were obtained from Biocontrol Laboratory, Tamil Nadu Agricultural University, Coimbatore and reared on pumpkins (*Cucurbita maxima*). Fresh green pumpkins with no injuries was purchased from the market, washed with tap water to remove dusts and pesticide residue, treated with 0.1% Bavistin to avoid fungal infection and then dried. After drying, a thread is tied along the grooves to support crawlers movement and spreading over the surface. The culture was maintained by releasing few crawlers over the pumpkins kept in rearing cages; protected from predators with mesh and antwell; at temperature 30±2ºC and relative humidity 70±5% (Chacko *et al*., 1978 and Singh, 1978).

Collection of plants and extract preparation

Cold extraction method with water was followed for the screening since it will give an insight of potential plants against mealybug. Also aqueous extract is much more easier and safe to prepare and apply in small scale for home gardeners and local farmers in short period of time. The plants reported to possess insecticidal activity were collected from TNAU campus. They were washed with clean water and shade dried at room temperature 28±1ºC. After drying, they were ground using mechanical grinder, sieved to fine powder and then pulverized. The plant powders were kept separately in air tight bottles for future use. 10gm of plant powder was mixed in 100ml distilled water *i.e.* 10% aqueous extract and kept overnight. Next day, the solution is filtered using a layer of filter paper over muslin cloth to get a clear extract so that there is no suspended particles which might block the spray nozzle (Maheswari and Govindaiah, 2017). Aqueous extract of 10% is quite a high concentration and going beyond that is uneconomical and impractical sometimes.

Bioefficacy testing by atomization method

An experiment was conducted to study the contact toxicity of various plant extracts against adult female *M. hirsutus* by atomization method under laboratory conditions following Khan *et al*. (2012) with some modifications. Only adult females were tested since they have the highest wax content as compared to crawlers and male adults. In order to test the toxicity of the plant extracts, the mealybugs were sprayed with 10% aqueous extract using a hand atomizer and given exposure for 10 minutes. In control mealybugs were atomized with water alone. Thereafter mealybugs were transferred to glass Petri dishes (9 cm diameter) containing fresh mulberry leaves lined with moistened filter paper to keep the leaves fresh. Each treatment was replicated thrice with 20 adult female mealybugs per replication.

Data collection and Statistical Analysis

The experimental design followed was Completely Randomized Design (CRD). The observations on mortality were recorded at 24, 48 and 72 hours after exposure (HAE). Mealybugs which showed no movement when disturbed with brush were counted as dead. Data obtained were analysed by analysis of variance (ANOVA) and the significance among the treatments were determined according to Tukey's HSD mean separation test at $P = 0.05$. The statistical analysis was performed by using SPSS16.0 software.

RESULTS

The per cent mortality data of *M. hirsutus* adult female at 24, 48 and 72 HAE is presented in Table1 along with the plant parts used in the experiment and families they belong to. The percent mortality in this experiment ranged between 0.0 to 96.7 at 24HAE and 0.0 to 98.3 at both 48 and 72HAE. Out of all the treatments, 7 plants showed more than 80% mortality after 72 hours, 13 of them ranged between 50-80% while remaining 30 treatments showed less than 50%. Among all the treatments highest mortality was shown by *Calotropis gigantea* at all hours after exposure (96.7, 98.3 and 98.3% at 24, 48 and 72HAE, respectively) which was statistically superior to all other treatments. At 24HAE, *C. gigantea* was followed by *Helicteres isora* (75.0%), *Ricinus communis* (73.3%), *Centella asiatica* (66.7%) *Piper nigrum* (63.3%) and *Curcuma longa* (61.7%) statistically on par with each other. Similar trend was observed at 48 and 72HAE with *C. gigantea* followed by *Ricinus communis, Helicteres isora*, *Centella asiatica*, *Spathodea campanulata*, *Colocasia esculenta*, *Ocimum tenuiflorum*, *Curcuma longa*, and *Piper nigrum* while no mortality was observed for *Acorus calamus*, *Anacardium occidentale*, *Chrysopogan zizanioides*, *Cymbopogon schoenanthus*, *Gliricidia sepium, Lantana camara, Solanum virginianum* and *Leucas aspera*.

DISCUSSION

In the present study certain plants have been found to be effective however only few superior ones are discussed here. The reason for ineffectiveness of many botanicals might be due to the mealy wax coating all over the body. This wax prevent the penetration of toxic chemicals through the integument (Arunkumar *et al*., 2017). The experimental material was aqueous extract and hence the bioactive compounds might have failed to penetrate the integument to result effective mortality. However the superior plants like *Calotropis gigantea* was able to dissolve the wax to an extent while ineffective plants could not do so and the mealybugs started producing white mealy matter again after few hours of spraying. Due to lack of extensive studies of botanicals on *M. hirsutus*, the results are being supported by reports on other mealybug species or other insects. *Spathodea campanulata* flower was very effective against *M. hirsutus* in this experiment. Similar effect was observed in other insects as well. 100% mortality of coffee berry borer, *Hypothenemus hampei* was observed by Alarcón-Noguera and Penieres-Carrillo (2013) while Franco *et al*. (2015) found it had control efficiency of 89% of *Sitophilus zeamais* test population. It also has larvicidal and repellent properties against mosquitoes (Aarthi and

Table 1. Efficacy of 10% aqueous extracts of different plants against adult female pink mealybug, *Maconellicoccus hirsutus* (Green) by atomization method

Plant species	Family	Mortality (%)		
		24 HAE	**48 HAE**	**72 HAE**
Justicia adhatoda (leaf)	Acanthaceae	26.7 (26.4)^{ijklmn}	38.3 (37.6)^{ijklmno}	40.0 (39.3)^{ijklmn}
Acorus calamus (rhizome)	Acoraceae	0.0 (0.0)^{p}	1.7 (1.7)^{rs}	1.7 (1.7)^{rs}
Achyranthes aspera (leaf)	Amaranthaceae	55.0 (53.0)^{cdef}	65.0 (61.9)^{efgh}	66.7 (63.4)^{defgh}
Anacardium occidentale (leaf)	Anacardiaceae	0.0 (0.0)^{p}	0.0 (0.0)^{s}	5.0 (5.0)^{qrs}
Annona squamosa (seed)	Annonaceae	1.7 (1.7)^{op}	6.7 (6.7)^{qrs}	10.0 (10.0)^{pqrs}
Centella asiatica (leaf)	Apiaceae	66.7 (63.7)^{bc}	83.3 (78.3)^{bcd}	85.0 (80.0)^{bcd}
Allamanda catharitica (leaf)	Apocynaceae	36.7 (36.4)^{fghijk}	40.0 (39.6)^{hijklmno}	51.7 (49.9)^{ghijklm}
Calotropis gigantea (leaf)	Apocynaceae	96.7 (95.7)^{a}	98.3 (97.2)^{a}	98.3 (97.2)^{a}
Catharanthus roseus (leaf)	Apocynaceae	10.0 (10.0)^{lmnop}	20.0 (20.0)^{nopqrs}	25.0 (24.9)^{mnopqrs}
Colocasia esculenta (leaf)	Araceae	43.3 (42.3)^{defghi}	68.3 (67.1)^{defg}	83.3 (83.8)^{bcd}
Artemisia pallens (leaf)	Asteraceae	28.3 (28.1)^{hijklm}	36.7 (36.3)^{jklmno}	38.3 (38.0)^{ijklmno}
Tagetes tenuifolia (leaf)	Asteraceae	35.0 (34.5)^{fghijk}	38.3 (37.6)^{ijklmno}	46.7 (45.5)^{hijklm}
Tagetes tenuifolia (flower)	Asteraceae	41.7 (40.7)^{efghij}	51.7 (50.0)^{fghijkl}	60.0 (57.6)^{efghij}
Spathodea campanulata (flower)	Bignoniaceae	50.0 (48.3)^{cdefgh}	73.3 (68.9)^{bcde}	75.0 (70.3)^{bcdef}
Cyperus rotundus (leaf)	Cyperaceae	38.3 (37.5)^{fghij}	41.7 (40.6)^{hijklmn}	41.7 (40.6)^{ijklmn}
Jatropha curcas (leaf)	Euphorbiaceae	38.3 (37.7)^{fghij}	48.3 (47.6)^{ghijkl}	51.7 (50.9)^{ghijklm}
Ricinus communis (leaf)	Euphorbiaceae	73.3 (69.1)^{b}	86.7 (81.7)^{b}	88.3 (83.3)^{b}
Cassia alata (leaf)	Fabaceae	10.0 (10.0)^{lmnop}	20.0 (19.9)^{nopqrs}	30.0 (29.8)^{klmnopqr}
Cassia fistula (leaf)	Fabaceae	36.7 (36.1)^{fghijk}	41.7 (40.9)^{hijklmn}	48.3 (46.9)^{hijklm}
Delonix regia (leaf)	Fabaceae	5.0 (5.0)^{mnop}	10.0 (10.0)^{pqrs}	10.0 (10.0)^{pqrs}
Gliricidia sepium (leaf)	Fabaceae	0.0 (0.0)^{p}	6.7 (6.7)^{qrs}	11.7 (11.6)^{opqrs}
Sesbania grandiflora (leaf)	Fabaceae	13.3 (13.3)^{klmnop}	23.3 (23.2)^{mnopqrs}	26.7 (26.5)^{lmnopqrs}
Tephrosia purpurea (leaf)	Fabaceae	20.0 (19.9)^{jklmnop}	35.0 (34.4)^{klmno}	38.3 (37.6)^{ijklmno}
Prosopis juliflora (leaf)	Fabaceae	20.0 (19.9)^{jklmnop}	21.7 (21.5)^{nopqrs}	30.0 (29.6)^{klmnopqr}
Leucas aspera (leaf)	Lamiaceae	0.0 (0.0)^{p}	0.0 (0.0)^{s}	0.0 (0.0)^{s}
Mentha piperita (leaf)	Lamiaceae	50.0 (48.4)^{cdefgh}	53.3 (51.4)^{efghijk}	58.3 (55.9)^{fghij}

Ocimum tenuiflorum (leaf)	Lamiaceae	50.0 (48.4)^{cdefgh}	58.3 (56.0)^{efghij}	80.0 (80.6)^{bcde}
Vitex negundo (leaf)	Lamiaceae	53.3 (52.1)^{cdefg}	63.3 (60.5)^{efgh}	63.3 (60.5)^{efghi}
Strychonos nux-vomica (leaf)	Loganiaceae	5.0 (5.0)^{mnop}	16.7 (16.6)^{opqrs}	18.3 (18.2)^{nopqrs}
Lawsonia inermis(leaf)	Lythraceae	28.3 (28.0)^{hijklm}	30.0 (29.6)^{lmnopq}	35.0 (34.4)^{jklmnop}
Abutilon indicum (leaf)	Malvaceae	20.0 (19.9)^{jklmnop}	25.0 (24.8)^{mnopqr}	31.7 (31.2)^{klmnopq}
Helicteres isora (fruit)	Malvaceae	75.0 (71.6)^{b}	85.0 (84.0)^{bc}	86.7 (85.2)^{bc}
Azadirachta indica (leaf)	Meliaceae	26.7 (26.4)^{ijklmn}	33.3 (32.8)^{klmnop}	35.0 (34.3)^{jklmnop}
Azadirachta indica (seed)	Meliaceae	1.7 (1.7)^{op}	1.7 (1.7)^{rs}	11.7 (11.7)^{opqrs}
Azadirachta indica (seed kernal)	Meliaceae	25.0 (24.8)^{ijklmno}	31.7 (31.3)^{klmnop}	38.3 (37.8)^{ijklmno}
Callistemon viminalis (leaf)	Myrtaceae	36.7 (35.9)^{fghijk}	45.0 (43.7)^{ghijklm}	53.3 (51.3)^{fghijk}
Eucalyptus deglupta (leaf)	Myrtaceae	3.3 (3.3)^{nop}	3.3 (3.3)^{rs}	3.3 (3.3)^{qrs}
Pedalium murex (leaf)	Pedaliaceae	40.0 (39.7)^{fghij}	60.0 (59.4)^{efghi}	70.0 (69.3)^{cdefgh}
Piper nigrum (leaf)	Piperaceae	63.3 (60.6)^{bcd}	71.7 (68.4)^{cdef}	73.3 (70.0)^{bcdefg}
Chrysopogon zizanioides (leaf)	Poaceae	0.0 (0.0)^{p}	0.0 (0.0)^{s}	0.0 (0.0)^{s}
Cymbopogon schoenanthus (leaf)	Poaceae	0.0 (0.0)^{p}	3.3 (3.3)^{rs}	3.3 (3.3)^{qrs}
Cynodon dactylon (leaf)	Poaceae	13.3 (13.3)^{klmnop}	21.7 (21.5)^{nopqrs}	31.7 (31.3)^{klmnopq}
Antigonon leptopus (leaf)	Polygonaceae	30.0 (29.7)^{hijkl}	38.3 (37.8)^{ijklmno}	41.7 (41.1)^{ijklmn}
Murraya koenigii (leaf)	Rutaceae	31.7 (31.2)^{ghijkl}	41.7 (40.7)^{hijklmn}	55.0 (53.2)^{fghijk}
Manilkara zapota (seed)	Sapotaceae	10.0 (10.0)^{lmnop}	21.7 (21.5)^{nopqrs}	31.7 (31.3)^{klmnopq}
Datura metel (leaf)	Solanaceae	41.7 (41.2)^{efghij}	56.7 (54.8)^{efghij}	61.7 (59.6)^{efghi}
Solanum virginianum (leaf)	Solanaceae	21.7 (21.5)^{ijklmnop}	45.0 (43.7)^{ghijklm}	51.7 (50.1)^{ghijkl}
Solanum virginianum (fruit)	Solanaceae	0.0 (0.0)^{p}	0.0 (0.0)^{s}	3.3 (3.3)^{qrs}
Lantana camara (leaf)	Verbenaceae	0.0 (0.0)^{p}	3.3 (3.3)^{rs}	10.0 (10.0)^{pqrs}
Curcuma longa (leaf)	Zingiberaceae	61.7 (59.1)^{bcde}	71.7 (67.7)^{cdef}	80.0 (74.8)^{bcde}
Control (Water)	-	0.0 (0.0)^{p}	0.0 (0.0)^{s}	0.0 (0.0)^{s}
S.E m ±	-	0.023	0.026	0.028
CD (p=0.05)	-	5.88	5.88	7.44

HAE=Hours After Exposure; Values are mean of three replications; Figures in the parentheses are arcsine transformed values; Values followed by different letter in the column differ significantly at 5% level of probability by Tukey's HSD test

Murugan, 2010; Saranya *et al.*, 2013).The compounds responsible for insecticidal activity are thought to be protein (glycosyl transferase family proteins and serine-threonine-protein phosphatase) present in the mucilage of flowers and young shoots that would be dissolved in the nectar (Queiroz *et al*., 2014; Santos *et al*., 2017). Portugal-Araújo (1963) attributed the death of insects in the flowers to floral bud secretion toxicity. This contributes the death of pink mealybugs on *S. campanulata* flower extract. Another effective plant was *Ricinus communis* whose leaves contains compound ricinine toxic to the leaf-cutting ant *Atta sexdens rubropilosa* (Bigi *et al*., 2004) and adults of *Callosobruchus chinensis* and *Tribolium castaneum* (Goyal *et al*., 2005). *R. communis* also contains fatty acids like linoleic acid, linolenic acid, palmitic acid, stearic acid, pentadecanoic acid, etc. (Bigi *et al*., 2004; Ramos-López *et al.,* 2012) and were found to have the insectistatic and insecticidal activities against *Spodoptera frugiperda*. Seed extracts gave a good level of protection of wheat grains for up to 12 weeks according to Mahgoub and Ahmed (1996). Kodjo *et al.* (2011) reported that *R. communis* leaf, root, seed kernel crude extracts and oil emulsion resulted very high mortality rate of *Plutella xylostella* both in laboratory and semi-field condition. Babarinde *et al.* (2011) reported 100% mortality and 80% repellency of *Tribolium castaneum*. The higher percentage mortality was recorded in larva than in adults. They suggested it might be due to more sclerotized adult as compared to larvae which affected the permeability of the toxic compounds of seed extracts. This might be the reason of its effectiveness in this study also because body of pink mealybugs is also less sclerotized. Abida *et al* (2010) found *Curcuma longa* rhizome extract effective against *T. castaneum* adults while its leaf oil showed insecticidal in both contact and fumigant toxicity according to Tripathi *et al. (*2002). Ajaiyeoba *et al.* (2008) found the essential oils from the leaf and rhizome showed toxicity against mosquitoes. The compound ar-turmerone, extracted from rhizomes of *C. longa*, showed high toxicity on *Nilaparvata lugens, Plutella xylostella, Myzus persicae, Spodoptera litura*, *S. zeamais*, *S. frugiperda* at low doses (Lee *et al*., 2001; Tavares *et al*., 2013). Park *et al*. (2002) reported most potent insecticidal alkaloids, pipernonaline and piperoctadecalidine against *Spodoptera litura* and *Myzus persicae*. Amides compounds pipgulzarine and pipzorine exhibited toxicity against fourth instar larvae of *Aedes aegypti* (Siddiqui *et al*., 2003). The hexane extract of *Piper nigrum* fruit showed the highest toxicity at 48 h after treatment against *Spodoptera litura* (Fan *et al*., 2011).The best plant in this study is *Calotropis gigantea* causing 98.3% mortality in aqueous extract. Its efficacy might increase after solvent extraction. Every parts of *C. gigantea* has been studied extensive mainly for its pharmaceutical properties such as analgesic, anticonvulsant, anxiolytic, sedative effect (Argal and Pathak, 2006), antimicrobial, anti-inflammatory, antipyretic, etc. (Palejkar *et al*., 2012; Kumar *et al*., 2013). Apart from these, it had been reported to possess insecticidal, antifeedant and repellent activity by various scientists against stored grain pests, termites, mosquitoes, lepidopteran pests, housefly, whitefly, forest insect pests, flesh fly, locust, etc. This experiment explored the potential utility of *C. gigantea* against *M. hirsutus* causing upto 98.3% mortality in 10% aqueous extract which may increase after solvent extraction. Prashanthini and Vinobaba (2014) found 88.33% mortality at 1.5% ethanol extract against cotton mealy bug *Phenacoccus solenopsis*. Manzoor and Haseeb (2015) reported that it caused mortality of 58% for *Phenacoccus solenopsis* and 32.40% for *Dysdercus cingulatus* at 5% concentration in 24hr. Sumathi *et al*. (2017) reported that 0.2% methanol extract of *C. gigantea* caused 90-95% mortality of *Paracoccus marginatus*. The GCMS analysis of different parts shows presence of more than 100 compounds. Already reported insecticidal compounds like linoleic acid, linolenic acid, palmitic acid, oleic acid, sitosterol, azulene, etc. are among them. Therefore, these compounds altogether or individually, might be causing such a high mortality of pink mealybug as well. It may be confirmed by evaluating the pure form of these compounds against *M. hirsutus*. This experiment can be concluded that botanicals, as it is safe to environment alongside its efficacy, have huge potential to replace chemical insecticides to manage *M. hirsutus* and further studies are required to understand the responsible compounds, extraction and its mode of action.

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