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TOXICITY OF GREEN SILVER NANOPARTICLES OF PLANT EXTRACTS AGAINST CITRUS MEALYBUG *PLANOCOCCUS CITRI*

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ABSTRACT

Planococcus citri is an economically important polyphagous insect pest and is a serious threat to horticultural crops especially mango and citrus. It is mainly controlled by synthetic insecticides which have deleterious effects on humans, livestock and environment. To dispense with the detrimental consequences of insecticides, plant extracts and their silver nanoparticles can be potential alternatives. The present study reports the effectiveness of four plant extracts and their silver based nanoparticles against *P. citri*. All the plant extracts and their silver nanoparticles caused mortalities of 2nd and 3rd instars of *P. citri* at all concentrations and exposure durations. The ginger extracts and their silver nanoparticles caused maximum mortality of 2nd and 3rd instars of *P. citri* (94 and 90% respectively) at the highest concentration after 72 hours followed by neem extracts and their silver nanoparticles. Similarly, the mortalities caused by garlic silver nanoparticles followed the similar pattern. Eucalyptus plant extracts and their silver nanoparticles were found to be the least effective. It was also observed that with an increase in concentration and exposure period, the mortality also increased showing a direct relationship. Ginger plant extract and their silver nanoparticles gave the best results and lowest LC50 values by a large margin. Neem extracts ranked second followed by garlic and eucalyptus and gave significantly poor results as compared to the first two plants. The required concentration for killing 50% of the nymphal population was observed to decrease with the passage of time.

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INTRODUCTION

Citrus mealybug, *Planococcus citri* (Hemiptera: Pseudococcidae) is an economically important polyphagous insect pest causing serious damage to citrus orchards and nurseries (Satar et al., 2013; Uygun and Satar, 2008). The damage is caused by mealybug

through sap uptake, honeydew secretion and associated sooty mold development, toxin injection and virus transmission (Cid et al., 2010). It is very difficult to manage this pest due to its protected habitat and waxy coating on the body. Many control measures have been used to control this pest but the chemical

insecticides are being used extensively (Erdemir and Erler, 2017). The chemical insecticides provide a quick knockdown to the insect pests but the typical use of these synthetic chemical based insecticides have caused many problems such as insecticide resistance, pest resurgence, negative impact on beneficial organisms, disrupting the natural ecosystem, acute adverse health effects and harmful environmental effects (Prishanthini and Vinobaba, 2012).

There is need to replace these synthetic chemicals with other alternatives such as biopesticides that have low toxicity to the environment and non-target organisms (Rasheed et al., 2014). Plant extracts have been used to manage insect pests of different crops around the globe (Ahmadi et al., 2012; Mansour et al., 2018). Natural pesticides extracted from plants such as plant extracts and silver nanoparticles are most reliable products against insect pests (Almadiy et al., 2018). These plant based insecticides like steroids, phenols and terpenoids have been found as promising agents for insect pest control and can cause harmful effects being anti-feedants, repellents, fecundity suppressants and respiration inhibitor (Sarwar, 2015).

Nanotechnology has widely been used in different fields due to its particular properties including chemical stability, correct conductivity, catalytic and most vital antibacterial, insecticidal, antiviral and antifungal activities (Ahmed et al., 2016; Nazir et al., 2019). Green synthesized nanoparticles especially green synthesized silver nanoparticles (AgNPs) have been proved as fast-acting chemicals against many insect pests and have unique characteristics e.g. size and shape depending optical, magnetic properties, and electrical, widely antimicrobial and insecticidal properties (Benelli, 2016). Very little research has been carried out on the use of these natural plant based biopesticides such as plant extracts and green synthesized silver nanoparticles against citrus mealybug. Therefore, the current study was planned to evaluate the toxicity of four plant extracts and their green synthesized silver nanoparticles against 2nd and 3rd nymphal instars of *P. citri*. The study will provide alternative control measures to manage the insect pest, which will have no or low toxicity to the environment, non-target organisms and lessen the chance of resistance in citrus mealybug.

MATERIALS AND METHODS

Plant materials: Four plants i.e. Eucalyptus (*Eucalyptus camaldulensis*), neem (*Azadirachta indica*), ginger

(*Zingiber officinale*) and garlic (*Allium sativum*) were used for the preparation of plant extracts and their green synthesized silver nanoparticles to evaluate their toxicity against 2nd and 3rd nymphal instars of citrus mealybug.

Plant extraction: The above-mentioned plant materials were washed and dried under shade for about one week. The dried plant materials were crushed into fine powders with the help of electric grinder and sieved through 20 mesh size sieves to obtain a favorable size range. The 100 g powder was mixed with 500 ml ethanol in a conical flask. The suspension was left for 5 days and mixed twice a day manually. After 5 days, the suspension was filtered through Whatman No. 1 filter paper and the crude extract was obtained through rotary evaporator. The dry powder of crude extract was stored under room temperature.

Green synthesis of silver nanoparticles: For the preparation of green synthesized nanoparticles, 10 g extract powder of plant material and 1 Mm of silver nitrate (AgNO₃) were added in 250 ml sterile distilled water. The solution was boiled for 5 minutes until change of color of solution to brown which indicated the formation of green synthesized AgNPs.

Collection and mass rearing of *Planococcus citri*: The nymphs of citrus mealybug were collected from different host plants. The nymphs were released on well matured and cleaned pumpkins placed in specially designed plastic cages. The culture was maintained throughout the research period without contamination.

Experimental bioassay

Toxicity of plant extracts and green synthesized silver nanoparticles against nymphs of *Planococcus citri*: The experiment was carried out in the Insect Molecular Laboratory of Department of Entomology, University of Arid Agriculture, Rawalpindi under controlled conditions. The four plant materials with six concentrations and five replications were used for insecticidal toxicity of plant extract against 2nd and 3rd nymphal instars of *P. citri*. Forty two nymphal instars were used for each concentration of each treatment. The mortality data were recorded after 24, 48, and 72 hours. The same procedure was followed for the evaluation of green synthesized silver nanoparticles against the insect pest.

Statistical analysis: Data were analyzed by using probit analysis. Mortality data were corrected using Abbot's formula (Abbott, 1925). Statistical software SPSS 16 was

used for the statistical analysis of all the data.

RESULTS

Nymphicidal responses of plant extracts and their silver nanoparticles against *Planococcus citri*:

All the plant extracts and their silver nanoparticles caused mortalities of 2nd and 3rd instars of *P. citri* at all

concentrations and exposure durations. The ginger extracts caused the maximum mortality of 2nd and 3rd instars of *P. citri* (94 and 90% respectively) at the highest concentration after 72 hours (Table 1 and 2). Similar trend of mortality was observed with ginger silver nanoparticles (Table 3 and 4).

Table 1: Effect of ginger plant extract on mortality and corrected mortality of 2nd nymphal instar of *Planococcus citri*.

Concentration (mg/100 ml)	24 h		48 h		72 h	
	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality
Control	0	0	2	0	6	0
0.50	24	24	28	26.53	32	27.66
1	38	38	44	42.86	50	46.81
3	48	48	62	61.22	70	68.09
7	58	58	74	73.47	80	78.72
15	84	84	88	87.76	94	93.62

Table 2: Effect of ginger plant extract on mortality and corrected mortality of 3rd nymphal instar of *Planococcus citri*.

Concentration (mg/100 ml)	24 h		48 h		72 h	
	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality
Control	0	0	2	0	4	0
9	10	10	14	12.24	22	18.75
19	26	26	32	30.61	44	41.67
38	42	42	50	48.98	62	60.42
76	60	60	68	67.35	74	72.92
153	76	76	80	79.59	90	89.58

Table 3: Effect of ginger Ag-nanoparticles on mortality and corrected mortality of 2nd nymphal instar of *Planococcus citri*.

Concentration (mg/100 ml)	24 h		48 h		72 h	
	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality
Control	0	0	0	0	6	0
9	6	6	10	10	16	10.64
19	20	20	26	26	36	31.91
38	36	36	42	42	56	53.19
76	54	54	60	60	68	65.96
153	76	76	76	76	82	80.85

Table 4: Effect of ginger Ag-nanoparticles on mortality and corrected mortality of 3rd nymphal instar of *Planococcus citri*.

Concentration (mg/100 ml)	24 h		48 h		72 h	
	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality
Control	0	0	0	0	0	0
0.50	24	24	28	28	32	32
1	36	36	38	38	42	42
3	46	46	48	48	52	52
7	56	56	58	58	62	62
15	90	90	92	92	96	96

In case of garlic extract, the maximum mortality of 2nd instars of the insect pest was caused at the highest concentration after 72 hours (Table 5). Similarly, the maximum mortality of 3rd instar was recorded with garlic extract at the same concentration and exposure duration (Table 6). The mortalities caused by garlic silver

nanoparticles followed the similar pattern (Table 7 and 8). As regards neem extracts, all the concentrations at three exposure periods caused mortalities of 2nd and 3rd instars of *P. citri*. The highest concentration of neem extract after 72 hours resulted in the highest mortalities of both the instars (Table 9 and 10).

Table 6: Effect of garlic plant extract on mortality and corrected mortality of 3rd nymphal instar of *Planococcus citri*.

Concentration (mg/100 ml)	24 h		48 h		72 h	
	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality
Control	0	0	2	0	4	0
152	6	6	10	8.16	16	12.5
304	14	14	20	18.37	30	27.08
608	34	34	40	38.78	52	50
1216	52	52	62	61.22	68	66.67
2432	66	66	70	69.39	78	77.08

Table 7: Effect of garlic Ag-nanoparticles on mortality and corrected mortality of 2nd nymphal instar of *Planococcus citri*.

Concentration (mg/100 ml)	24 h		48 h		72 h	
	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality
Control	0	0	0	0	4	0
15	10	10	14	14	22	18.75
30	20	20	36	36	40	37.50
60	42	42	52	52	60	58.33
121	60	60	68	68	76	75
243	72	72	78	78	82	81.25

Table 8: Effect of garlic Ag-nanoparticles on mortality and corrected mortality of 3rd nymphal instar of *Planococcus citri*.

Concentration (mg/100 ml)	24 h		48 h		72 h	
	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality
Control	0	0	0	0	6	0
15	8	8	10	10	18	12.77
30	14	14	28	28	38	34.04
60	36	36	44	44	50	46.81
121	52	52	60	60	68	65.96
243	66	66	72	72	80	78.72

Table 9: Effect of neem plant extract on mortality and corrected mortality of 2nd nymphal instar of *Planococcus citri*.

Concentration (mg/100 ml)	24 h		48 h		72 h	
	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality
Control	0	0	4	0	6	0
75	14	14	20	16.67	28	30.40
150	30	30	44	41.67	58	55.32
300	44	44	62	60.42	70	68.09
600	64	64	70	68.75	84	82.98
1200	80	80	86	85.42	92	91.49

Table 10: Effect of neem plant extract on mortality and corrected mortality of 3rd nymphal instar of *Planococcus citri*.

Concentration (mg/100 ml)	24 h		48 h		72 h	
	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality
Control	0	0	0	0	4	0
75	12	12	16	16	20	16.67
150	24	24	36	36	44	41.67
300	38	38	54	54	62	60.42
600	60	60	66	66	78	77.08
1200	76	76	82	82	88	87.5

The silver nanoparticles of neem behaved in the same way causing maximum mortalities at higher concentrations (Table 11 and 12). In case of eucalyptus plant extracts, the maximum mortality of 2nd and 3rd instars of *P. citri* was obtained with the highest dose and the maximum time interval (Table 13 and 14). In the same way, silver nanoparticles of eucalyptus gave maximum mortalities of both the instars at the same dose and exposure period (Table 15 and 16). It was also observed that with an increase in concentration and exposure period, the mortality also increased showing a direct relationship.

LC50 values for plant based extracts against 2nd and 3rd instars of *P. citri*: LC50 values along with the fiducial limits at the 95% for the plant extracts of ginger, garlic, neem, and eucalyptus against 2nd and 3rd instars of *P. citri* are given in Table 17 and 18. Ginger plant extract gave the best results and lowest LC50 values by a large margin. Neem extracts ranked second followed by garlic and eucalyptus and gave significantly poor results as compared to the first two plants. The required concentration for killing 50% of the nymphal population was observed to decrease with the passage of time.

Table 11: Effect of neem Ag-nanoparticles on mortality and corrected mortality of 2nd nymphal instar of *Planococcus citri*.

Concentration (mg/100 ml)	24 h		48 h		72 h	
	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality
Control	0	0	0	0	4	0
7	16	16	24	24	38	35.42
15	30	30	46	46	60	58.33
30	42	42	62	62	70	68.75
60	64	64	70	70	82	81.25
120	82	82	80	80	94	93.75

Table 12: Effect of neem Ag-nanoparticles on mortality and corrected mortality of 3rd nymphal instar of *Planococcus citri*.

Concentration (mg/100 ml)	24 h		48 h		72 h	
	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality
Control	0	0	0	0	4	0
7	20	20	28	28	46	43.75
15	34	34	52	52	64	62.5
30	48	48	70	70	76	75
60	70	70	82	82	88	87.5
120	88	88	94	94	100	100

Table 13: Effect of eucalyptus plant extract on mortality and corrected mortality of 2nd nymphal instar of *Planococcus citri*.

Concentration (mg/100 ml)	24 h		48 h		72 h	
	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality
Control	0	0	0	0	4	0
208	8	8	12	12	20	16.67
416	20	20	26	26	36	33.33
832	38	38	44	44	58	56.25
1664	54	54	62	62	70	68.75
3328	72	72	78	78	84	83.33

Table 14: Effect of eucalyptus plant extract on mortality and corrected mortality of 3rd nymphal instar of *Planococcus citri*.

Concentration (mg/100 ml)	24 h		48 h		72 h	
	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality
Control	0	0	0	0	4	0
208	6	6	10	10	14	10.42
416	16	16	20	20	30	27.08
832	30	30	40	40	46	43.75
1664	42	42	50	50	62	60.42
3328	68	68	74	74	80	79.17

Table 15: Effect of eucalyptus Ag-nanoparticles on mortality and corrected mortality of 2nd nymphal instar of *Planococcus citri*.

Concentration (mg/100 ml)	24 h		48 h		72 h	
	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality
Control	0	0	0	0	2	0
20	14	14	20	20	28	26.53
41	28	28	34	34	42	40.82
83	48	48	56	56	64	63.27
166	60	60	72	72	78	77.55
332	78	78	86	86	90	89.80

Table 16: Effect of eucalyptus Ag-nanoparticles on mortality and corrected mortality of 3rd nymphal instar of *Planococcus citri*.

Concentration (mg/100 ml)	24 h		48 h		72 h	
	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality	% Mean Mortality	Corrected Mean Mortality
Control	0	0	0	0	4	0
20	8	8	14	14	22	18.75
41	20	20	26	26	36	33.33
8	40	40	50	50	58	56.25
166	52	52	60	60	70	68.75
332	70	70	76	76	84	83.33

Table 17: Comparison of LC50 values of plant extracts at 3 time intervals against 2nd instar of *Planococcus citri*.

Plant extracts (mg/100 ml)	LC50 with fiducial limit		
	24 hours	48 hours	72 hours
Ginger	53.192 41.658-70.269	40.679 31.620-52.602	26.399 19.890-33.669
Neem	353.136 274.978-461.704	223.125 165.692-289.187	142.234 100.356-184.683
Garlic	1228.029 956.200-1698.610	945.351 736.826-1272.294	645.337 496.052-844.486
Eucalyptus	1098.151 1405.515-1902.599	1070.299 838.744-1401.620	705.845 534.915-912.892

Table 18: Comparison of LC50 values of plant extracts at 3 time intervals against 3rd instar of *Planococcus citri*.

Plant extracts (mg/100 ml)	LC50 with fiducial limit		
	24 hours	48 hours	72 hours
Ginger	68.259 53.432-92.554	53.192 41.658-70.269	35.843 27.456-46.360
Neem	145.420 112.468-200.834	104.614 80.513-139.758	68.954 51.710-89.759
Garlic	430.919 336.322-574.706	290.016 221.924-377.105	208.093 158.038-263.999
Eucalyptus	1463.312 1132.499-2075.940	1088.628 828.403-1550.379	821.253 633.805-1104.400

LC50 values for plant based AgNPs against 2nd and 3rd instars of *Planococcus citri*: Table 19 and 20 show LC50 values along with their fiducial limits at the 95% for plant based silver nanoparticles of ginger, garlic, neem, and eucalyptus against 2nd and 3rd instars of *P. citri*. Ginger AgNPs gave the best results and the

lowest LC50 values by a large margin followed by neem. On the other hand, garlic and eucalyptus gave significantly poorer results as compared to the first two plants. The required concentration for killing 50% of the nymphal population was observed to decrease with the passage of time.

Table 19: Comparison of LC50 values of plant based AgNPs at 3 time intervals against 2nd instar of *Planococcus citri*.

Plant based AgNPs (mg/100 ml)	LC50 with fiducial limit		
	24 hours	48 hours	72 hours
Ginger	68.259 53.432-92.554	53.192 41.658-70.269	35.843 27.456-46.360
Neem	145.420 112.468-200.834	104.614 80.513-139.758	68.954 51.710-89.759
Garlic	430.919 336.322-574.706	290.016 221.924-377.105	208.093 158.038-263.999
Eucalyptus	1463.312 1132.499-2075.940	1088.628 828.403-1550.379	821.253 633.805-1104.400

Table 20: Comparison of LC50 values of plant based AgNPs at 3 time intervals against 3rd instar of *Planococcus citri*.

Plant based AgNPs (mg/ 100ml)	LC50 with fiducial limit		
	24 hours	48 hours	72 hours
Ginger	68.259 53.432-92.554	53.192 41.658-70.269	35.843 27.456-46.360
Neem	145.420 112.468-200.834	104.614 80.513-139.758	68.954 51.710-89.759
Garlic	430.919 336.322-574.706	290.016 221.924-377.105	208.093 158.038-263.999
Eucalyptus	1463.312 1132.499-2075.940	1088.628 828.403-1550.379	821.253 633.805-1104.400

DISCUSSION

The main observations of the present study were that green synthesized AgNPs outperformed their plant extract counterparts in causing mortality of *P. citri* nymphs. Another important finding was that the 3rd instar larvae were found to be more resistant than the 2nd instar larvae irrespective of the treatments either with plant based extracts or green synthesized silver nanoparticles. It was also observed that mean percent mortality of the 2nd and 3rd instar larvae of *P. citri* increased with the passage of time and subsequently the required LC₅₀ decreased as the time interval increased. Many researchers have reported effectiveness of plant extracts and their silver nanoparticles in earlier studies. Eugenia et al. (2019) evaluated the effectiveness of two plant based copper nanoparticles

against cotton mealybug *Phenacoccus solenopsis* and proved that green synthesized CuNPs had a significant toxic effect after 96 hours. A similar study was conducted by Pavitra et al. (2018) in which the toxicity of rice based silica nanoparticles against cotton mealybug was tested. The results showed that rice based green silica NPs were effective in managing *P. solenopsis* populations. These particles were equally effective against the cotton aphid. Al-Shammari and Al-Zubaidi (2016) assessed the effectiveness of eucalyptus based silver nanoparticles against *P. citri*, and found effective against different life stages of *P. citri* i.e. eggs, instars, and adult females.

The finding of these researchers was not in line with those of the present study. The reason that in the present study, four plant based silver nanoparticles

were tested while Al-Shammari and Al-Zubaidi (2016) studied the effectiveness of only eucalyptus. Neem was the second best performer in this study in terms of inducing mortality in *P. citri* instars. Similarly, Islam and Ashraful (2015) compared the efficacy of extracts of seven plants including neem for their toxicity against mango mealybug. Neem was found to be the best in causing mortality of the insect pest.

Different plant extracts and their silver based nanoparticles were also found toxic to mosquitoes. The percentage of adult emergence reduced when *Culex pipiens* larvae were treated with the aqueous leaf extract of neem and their green AgNPs. Similarly, low percentage of adult emergence of *Aedes aegypti* and *C. quinquefasciatus* was obtained when treated with aqueous leaf extract of *Adiantum raddianum* and green synthesized AgNPs (Govindarajan et al., 2017). The adult longevity of male and female mosquitoes reduced when treated with *Momordica charantia* AgNPs and plant extract of *M. azedarach*. The reduction in adult longevity was the same as reported by Velayutham et al. (2013).

In the present study, the lethal and sub lethal values decreased with the exposure time and concentration. The minimum lethal and sub lethal values were recorded at high concentration and at high exposure time for both the larval instars after the application with each treatment. The findings are similar to those described by Benelli et al. (2018) who studied the lethal and sub lethal toxicity of *Mentha piperita*, *M. spicata*, *Ocimum basilicum*, *Helichrysum italicum*, *Achillea ligustica*, *Pelargonium odoratissimum*, *Cinnamomum verum* and *Lippia alba* extracts against 4th larval instar of *C. quinquefasciatus* and adults of *Musca domestica*. Results of the current study showed that the leaves of neem and ginger have shown toxicity against citrus mealybug because their leaves are highly rich in metabolic compounds as reported by Poopathi et al. (2015). It is concluded from this study that green silver nanoparticles of ginger, neem, garlic and eucalyptus are more toxic than the plant extracts against *P. citri*.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

MSB, MT and AG designed the study, MSB and HS, conducted the experiments and collected data, MT and ZRM analyzed the data, MT supervised the work, MSB and MT wrote the manuscript and all the authors edited and approved the final manuscript.

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