

Insecticidal activity of crude extracts of four plants and their Ag Nano liquids against *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae)

Madasamy Mariappan

St Xavier's College

Sahayaraj Kitherian (✉ ksraj48@gmail.com)

St Xavier's College

Samy M Sayed

Taif University

Hosny Kesba

Taif University

Selvaraj Prathas

St Xavier's College

Madasamy Koilraj

St Xavier's College

Research Article

Keywords: Crude extract, AgNP's, vijaya neem, Cotton Mealybug

Posted Date: March 14th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1305717/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

A good quantity of global agricultural production is being devastated due to insect pests which need serious attention by the agricultural scientists and policy makers. The sole dependence on agrochemicals has increased over the past decades. However, adoption of botanical insecticides is gaining momentum as an ecofriendly alternative to the hazardous chemical pesticides. Applications of bionanoparticles are highly effective and may limit the risk of synthetic pesticide usage. Leaf dip and topical application toxicity bioassay were carried out to investigate the biological effects of aqueous leave extracts of *Annona squamosa*, *Justicia adathoda*, *Ipomea carnea* and *Pongamia glabura* at different concentrations (1.25, 2.5, 5 and 10%) against first, second, third nymphal instars and adult of *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae). The result suggest that the aqueous leaf extract of *P. glabura* ($LC_{50} = 9.673\%$) showed more impact after 144 hrs of exposure followed by *J. adathoda*, *I.carnea* and *A. squamosa* in leaf dip toxicity bioassay. In the topical application, *P. glabura* ($LC_{50} = 1.042\%$) recorded the highest insecticidal activity followed by other three tested plants. The AgNP's of *P. glabura*-AgNP's showed highest impacts ($LC_{50} = 4.750\%$) than other three tested nanoparticles i.e., *I.carnea*-AgNP's, *J. adathoda*-AgNP's, *A. squamosa*-AgNP's in addition to Vijay neem, respectively. From these experiments, it is evident that the *P. glabura*-AgNP's and *J.adathoda*-AgNP's possess more insecticidal activity and hence these products could be used for *P. solenopsis* management.

Introduction

The cotton mealy bug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) is a polyphagous insect pest. It feeds on more than 154 plant species including field crops, vegetables, ornamentals, fruit trees, weeds and bushes and causes economic damage mainly to vegetables, oilseed and ornamental plants.^{1,2} The infestations with mealy bugs on different host plants could be effectively controlled using biological control agents.^{1,3-5}

The evaluation of plant extracts for their deleterious effects on insects is one of the approaches used for the search of novel botanical insecticides.⁶ Botanical pesticides are biological products which are used as mixtures for preventing, fighting and killing a variety of pests such as weed, insects, rodents and microbes.⁷ Botanical pesticides are biodegradable⁸ and their use in crop protection could be an effective and sustainable alternative to synthetic chemicals. *Azadirachta indica*, *Ocimum sanctum*, *Calotropis gigantea*, *Nicotina tabacum* and *Alium sativum*⁹, *Thymus vulgaris* L. (Lamiaceae) (Whole plant), *Artemisia absinthium* L. (Asteraceae) (Seeds), *Pluchea dioscoridis* L. (Asteraceae) (Leaves), *Cyperus articulatus* L. (Cyperaceae) (Tubers), *Mentha longifolia* (L.) Huds. (Lamiaceae) (Leaves), *Anethum graveolens* L. (Apiaceae) (Aereal parts), *Lantana camara* L.(Verbenaceae) (Leaves), *Zingiber officinale* Rosc. (Zingiberaceae) (Roots), *Elettaria cardamomum* Maton (Zingiberaceae) (Seed pods), *Syzygium aromaticum* (L.) Merr. & Perry (Myrtaceae) (Buds)¹, *Mentha longifolia*, *Mentha piperita*, *Mentha spicata*, and *Nepeta cataria*¹⁰, *A. indica*, *Eucalyptus globules*, *Ocimum basilicum* L.¹¹ were used for the management of *P. solenopsis*. Further, neem oil and NSKE¹², biopesticides and biorationals insecticides¹³ or commercial need product (Neemosal)¹⁴ were also tested against *P. solenopsis*.

Proposed plants like *Annona squamosa*¹⁵, *Justicia adatoda*¹⁶, *Ipomea carnea*^{17,18} and *Pongamia glabra* (= *Pongamia pinnata*)^{11,19} showed insecticidal, repellent and antifeedant properties against various insect pests have been known for three decades. However, crude extracts of these plants have not tested against various life stages of *P. solenopsis*.

The nanotechnology has become one of the most promising new approaches for insect pest control.²⁰ Biological synthesis of nanoparticles has received increased attention due to a growing need to develop environmentally benign technologies in material synthesis.²¹ Various plant-based silver nanoparticles have been utilized in pest control like *Nerium oleander*²², *Sargassum muticum*²³, *Avicennia marina*²⁴, *Ficus religiosa* and *Ficus benghalensis*²⁵, *Punica granatum*²⁶, *Euphorbia prostrata*.²⁷ It was also stressed that silver nanoparticles (AgNPs) synthesized by using tested essential oils of *Cymbopogon citratus*, *Thymus vulgaris* and *Pelargonium graveolens* and were evaluated against female of *P. solenopsis*.^{28,29}

The literature review shows that so far no work has been undertaken on the utilization of *A. squamosa*, *J. adatoda*, *I. carnea* and *P. glabra* against any life stages of *P. solenopsis* either under laboratory or field conditions. Therefore, considering the lacuna, the present study attempts to evaluate the efficacy of some native botanicals against cotton mealy bug, *P. solenopsis*. The present study was aimed to develop a rapid and simple biosynthesis of silver nanoparticle by reducing the metal (AgNO₃), using the leaf extracts. Hence with the following objectives: 1) impact of crude extracts of *Annona squamosa*, *Justicia adathoda*, *Ipomea carnea* and *Pongamia glabra* and their bionanomaterials and commercial pesticides vijay neem on the mortality of *P. solenopsis* life stages and 2) to determine the lethal concentration (LC₃₀, LC₅₀ and LC₉₀) for the crude extracts and their bionanomaterials of selected botanicals and the vijay neem on the mortality of *P. solenopsis* life stages.

Material And Methods

Collection of botanicals. Permission has been obtained for collecting the botanicals namely *Annona squamosa* L., (Family: Annonaceae) was collected from Palayapettai, Tirunelveli (N 08° 73' 70.75" latitude and E 077° 66' 57.73" longitude) (Plate 1.1a), *Justicia adathoda* L., (Family: Acanthaceae) was collected from St. Xavier's College campus, Palayamkottai, Tirunelveli (N 08° 71' 80.23" latitude and E 077° 73' 81.22" longitude) (Plate 1.1b), *Ipomea carnea* J.(Family: Convolvulaceae) was collected from the Tirunelveli Town (N 08° 73' 05.34" latitude and E 077° 68' 75.15" longitude) (Plate 1.1c) and *Pongamia glabra* Ventenat. (Family: Papilionaceae) was collected from St. Xavier's College campus, Palayamkottai, Tirunelveli (N 08° 71' 74.48" latitude and E 077° 74' 01.66" longitude) (Plate 1.1d) Tamil Nadu, India. and The collected plant materials were washed twice with fresh water and ones with distilled water to remove the dust and debris.

Preparation of botanical crude extracts. Five gram of fresh and healthy leaves was chopped into small pieces and was taken in a 250 ml conical flask. 100 ml of distilled water was added to it and was heated in a water bath at 72°C for 1 hour. The decoction was then cooled and filtered through Whatman No. 1 filter paper. The filtrate was collected in a 50 ml sterilized standard flask and was used for further studies. **All**

experimental protocols involving plant materials were conducted in accordance with institutional, national, and international guidelines and legislation.

Preparation of silver nanoparticles. Silver nitrate was purchased from Hi-Media (RM409-25G) with 99.9% purity was used for the preparation. 17 mg silver nitrate was taken and dissolved in the 100 ml of double distilled water (10^{-3} M AgNO_3). To induce the synthesis of silver nanoparticles, 20 μl of plant extract was added at time interval 1 minute regularly. The finally synthesized pale yellow to reddish brown color and its store for 100 ml in sterilized standard flask used for this experimental study.

Preparation of commercial Vijay neem. Indian farmers have been using a neem- based insecticides Vijay neem (Madras Fertilizers Limited, Chennai) was purchased from local agrochemical market and field recommended dose of 0.03% was prepared (30 μl /100 ml water) and used for this experiment.

Collection and rearing of *Phenacoccus solenopsis*. Life stages of *P. solenopsis* were collected from cotton field of Palayapettai (N 08° 73' 70.75" latitude and E 077° 66' 57.73" longitude) and Savalaperi (N 08° 49' 55.6" latitude and E 077° 51' 34.8" longitude) Tirunelveli, Tamil Nadu, India. The collected *P. solenopsis* cultures were maintained on cotton plant and also using newly sprouted potatoes shoots in plastic tray (height 30 cm $\times$ 24 cm width, depth 5 cm) under the laboratory conditions (height 185 cm $\times$ 89 cm width) at room temperature ($29 \pm 2^\circ \text{C}$), 70–80% RH and 11 L: 13 D hours.⁹ The laboratory emerged insects were also maintained continuously for the experiment. Laboratory emerged first, second, third instar nymphs and adult *P. solenopsis* were used for the experimental study.

Toxicity bioassay -Leaf dip method. Leaf dip method was performed in plastic containers (4 cm diameter and 5 cm height). Four different concentrations (1.25, 2.5, 5 and 10%) of the aqueous extracts of used for the leaf dip method of toxicity bioassay. Fresh and healthy cotton leaves (KC-II) was cut chopped into small pieces of 2 $\times$ 2 cm size and then soaked for five minutes in the experimental solutions and were then shade dried for ten minutes.^{46,47} The shade dried leaves were fed to the nymphs and adults of *P.solenopsis*. The six replications each with ten nymphs, adult per treatment were used for the experiment. Control category was treated with distilled water and teepol (100 ml + 50 μl). The animals were maintained as mentioned above. Mortality was recorded after 24, 48, 72, 96, 120 and 144 hrs and corrected according to the following formula.

% of the animals killed in treated - % of the animal killed in the control

$$\text{Corrected percent mortality (CPM)} = \frac{\text{---} \times 100}{100 - \% \text{ of the animals killed in the control}}$$

Toxicity bioassay -Topical application. All the four different concentrations of the experimental solutions were sprayed separately on different sets of *P.solenopsis* nymphs and adults using a hand sprayer (capacity of 100 ml). The experimental animals were then introduced into the experimental rearing tray (23 cm width; 30 cm length and 5 cm height). Observations were made on the mortality of the animals at 24 hours intervals up to 144 hours after treatment (24, 48, 72, 96, 120 and 144 hrs).⁴⁸

Statistical analysis. Corrected mortality of insect was calculated using Abbott correction formula for natural mortality in untreated control.⁴⁹ The corrected mortality data was subjected to further analysis using SPSS.⁵⁰ The probit analysis (LC_{30} , LC_{50} and LC_{90}), df-values, chi-square values and significance values were recorded. The mortality data were subjected to one way Analysis of Variance (ANOVA); the mean values compared by Tukey test ($P = 0.005$ and 'P' values arrived at to assess the statistical significance of values less than 0.005 were considered as significant using statistical package.⁵⁰

Results

Insecticidal activity of crude extracts. Results of the experiments shows that significantly highest nymphal mortality was observed ($df = 4,10$; $F = 12.659$; $p < 0.005$; $LC_{50} = 9.673\%$ and $df = 4,10$; $F = 22.861$; $p < 0.005$; $LC_{50} = 1.042\%$ for leaf dip bioassay and topical application toxicity bioassay respectively) in *P. glabura* treated insects at 144 hrs against first instar nymphs of *P. solenopsis* (Tables 1 and 2; Fig. 1). In the second nymphal instar, leaf dip toxicity bioassay showed the highest nymphal mortality in *P. glabura* (93.33%; $df = 4,10$; $F = 95.583$; $p < 0.005$ and $LC_{50} = 5.145\%$) on 144 hrs against second nymphs instar *P. solenopsis*. However, topical application toxicity bioassay show that the highest nymphal mortality was recorded in *J. adathoda* on 144 hrs (76.66%; $df = 4,10$; $F = 12.855$; $p < 0.005$ and $LC_{50} = 3.295\%$) (Table 2, Fig. 2).

Table 1
Probit analysis of leaf dip bioassay using plant extracts on *Psolenopsis*.

Plant Extract	Life stages	LC ₅₀	Chi-square	Significance
<i>A.squamosa</i>	First instar	1.245	1.797	0.407
	Second instar	1.495	0.894	0.344
	Third instar	8.277	3.561	0.059
	Adult	2.318	0.005	0.996
<i>J.adathoda</i>	First instar	7.408	1.326	0.515
	Second instar	1.376	0.575	0.448
	Third instar	1.366	3.255	0.071
	Adult	1.716	1.062	0.303
<i>I.carnea</i>	First instar	8.036	1.277	0.528
	Second instar	1.429	0.822	0.365
	Third instar	1.488	1.223	0.269
	Adult	1.892	0.441	0.506
<i>P.glabura</i>	First instar	9.673	0.06	0.970
	Second instar	5.145	0.874	0.350
	Third instar	4.541	0.316	0.574
	Adult	1.193	0.371	0.542

Table 2
Probit analysis of topical application bioassay using plant extract on
P.solenopsis

Plant Extract	Life stages	LC ₅₀	Chi-s quare	Significance
<i>A.squamosa</i>	First instar	1.139	1.567	0.457
	Second nstar	5.896	1.014	0.602
	Third instar	4.248	1.36	0.506
	Adult	6.317	2.858	0.204
<i>J.adathoda</i>	First instar	3.534	0.829	0.661
	Second nstar	3.295	0.656	0.72
	Third instar	4.201	1.298	0.523
	Adult	5.137	0.244	0.885
<i>I.carnea</i>	First instar	1.242	1.797	0.407
	Second nstar	7.408	1.326	0.515
	Third instar	8.036	1.277	0.528
	Adult	9.673	0.06	0.97
<i>P.glabura</i>	First instar	1.042	0.879	0.644
	Second nstar	8.925	0.506	0.777
	Third instar	5.863	1.435	0.488
	Adult	8.330	0.678	0.713

In the leaf dip toxicity bioassay of the laboratory experiment shows that the highest nymphal mortality was observed in *P. glabura* (90.00%; df = 4,10; F = 38.643; p < 0.005 and LC₅₀ = 4.541%) on 144 hrs followed by *J. adathoda* (76.66%; df = 4,10; F = 13.617; p < 0.005 and LC₅₀ = 1.366%), *A. squamosa* (76.66%; df = 4,10; F = 10.375; p < 0.001 and LC₅₀ = 8.277%) (Fig. 3) and *I.carnea* (66.66%; df = 4,10; F = 20.088; p < 0.005 and LC₅₀ = 1.488%) (Fig. 4) against third instar nymphs of *P. solenopsis*. But in the topical application bioassay the highest nymphal mortality was recorded in *J. adathoda* (df = 4,10; F = 15.967; p < 0.005 and LC₅₀ = 4.201%) followed by *P. glabura* (df = 4,10; F = 24.328; p < 0.002 and LC₅₀ = 5.863%) against third instar nymphs of *P. solenopsis*, after 144 hrs of exposure.

In leaf dip toxicity bioassay the highest adults mortality was recorded in *P. glabura* (100%; df = 4,10; F = 85.812; p < 0.005 and LC₅₀ = 1.193%) on 144 hrs against adult of *P. solenopsis*. The result of topical application toxicity bioassay presented in the Table 2 and Fig. 2 recorded the highest nymphal mortality in *J. adathoda* (83.00%; df = 4,10; F = 53.056; p < 0.005 and LC₅₀ = 5.137%) on 144 hrs.

The overall comparison of the nymphicidal activity of the experimental crude extracts using the cumulative average mortality of first, second, third nymphal instars and adult *P. solenopsis* the leaf dip method (86.666%) recorded the highest mortality in *P. glabura* followed by *J. adathoda* (78.33%), *A. squamosa* (71.66%) and *I. carnea* (69.99%) and the topical application bioassay recorded the maximum mortality (77.49%) in *J. adathoda* followed by *P. glabura* (73.33%), *A. squamosa* (62.49%) and (56.66%) in *I. carnea* (Table 3)

Table 3
Cumulative average mortality (%) of first, second, third nymphal instar and adult *P. solenopsis*.

Botanicals/AgNP's	Average (%)	
	Leaf dip method	Topical application method
<i>A. squamosa</i>	71.66	62.49
<i>J. adathoda</i>	78.33	77.49
<i>I. carnea</i>	69.99	56.66
<i>P. glabura</i>	86.66	73.33
<i>A. squamosa</i> – AgNP's	86.66	88.33
<i>J. adathoda</i> – AgNP's	90.83	89.99
<i>I. carnea</i> – AgNP's	85.83	79.66
<i>P. glabura</i> – AgNP's	95.83	86.66
Vijay neem	86.24	76.66

Insecticidal activity of AgNP's. Insecticidal activity of AgNP's on the first nymphal instar of *P. solenopsis* mortality was recorded for the experimental nanoparticles using in leaf dip and topical application toxicity bioassays (Tables 3 and 4; Figs. 5–8). Result of the experiments presented in Table 3, 4 and Figs. 5–8 shows that the highest nymphal mortality was observed in *P. glabura*-AgNP's (86.66%; df = 4,10; F = 28.278; $p < 0.005$ and $LC_{50} = 4.750\%$) (Fig. 5) on 144 hrs in leaf dip method of toxicity bioassay against the first instar nymphs of *P. solenopsis*. However, in topical application toxicity bioassay showed highest nymphal mortality (90.00%; df = 4,10; F = 87.357; $p < 0.005$ and $LC_{50} = 1.276\%$) in *A. squamosa*-AgNP's on 144 hrs (Fig. 6).

Table 4
Probit analysis of leaf dip bioassay using bionanoparticles on *P.solenopsis*.

Bionano particles	Life stages	LC ₅₀	Chi-square	Significance
AS-AgNps	First instar	1.734	0.122	0.941
	Second instar	6.433	0.36	0.849
	Third instar	1.491	0.792	0.373
	Adult	1.892	0.441	0.506
JA-AgNps	First instar	1.151	1.637	0.441
	Second instar	6.440	0.318	0.573
	Third instar	1.188	0.105	0.764
	Adult	1.641	2.147	0.143
IC-AgNps	First instar	5.951	0.033	0.984
	Second instar	9.029	0.373	0.541
	Third instar	1.714	0.635	0.425
	Adult	3.577	8.276	0.005
PG-AgNps	First instar	4.750	0.141	0.932
	Second instar	4.928	8.077	0.004
	Third instar	1.434	0.837	0.36
	Adult	1.894	0.681	0.409
Vijay neem	First instar	4.949	1.394	0.498
	Second instar	9.601	0.831	0.362
	Third instar	1.466	0.160	0.690
	Adult	6.812	0.273	0.601

In the second instars, significantly highest nymphal mortality (100%; df = 4,10; F = 86.75; p < 0.005 and LC₅₀ = 4.928%) in *P. glabura*-AgNP's in leaf dip toxicity. Similar results was also recorded in the topical application toxicity test (df = 4,10; F = 219.167; p < 0.005 and LC₅₀ = 1.141%). Similar results was also observed for the third instar nymphs of *P. solenopsis* (df = 4,10; F = 52.293; p < 0.005 and LC₅₀ = 1.434%) when *P. glabura*-AgNP's applied as leaf dip bioassay (Table 4). But the topical application bioassay showed the highest nymphal mortality (df = 4,10; F = 124.7; p < 0.005 and LC₅₀ = 1.434%) in *J. adathoda*-AgNP's (Table 5; Fig. 7).

Table 5
Probit analysis of topical application bioassay using bionanoparticles on
P.solenopsis.

Bionano particles	Life stages	LC ₅₀	Chi-square	Significance
<i>AS-gNps</i>	First instar	1.276	4.871	0.088
	Second instar	1.226	2.258	0.323
	Third instar	1.508	2.17	0.338
	Adult	1.848	0.094	0.954
<i>JA-AgNps</i>	First instar	1.034	0.106	0.948
	Second instar	1.141	0.996	0.608
	Third instar	1.434	2.667	0.264
	Adult	1.795	0.414	0.813
<i>IC-AgNps</i>	First instar	1.734	0.122	0.941
	Second instar	1.151	1.637	0.441
	Third instar	5.951	0.033	0.984
	Adult	4.750	0.141	0.932
<i>PG- AgNps</i>	First instar	7.399	1.328	0.515
	Second instar	4.229	0.013	0.993
	Third instar	8.812	0.028	0.986
	Adult	3.738	1.133	0.568
Vijay neem	First instar	7.048	3.19	0.203
	Second instar	1.076	7.522	0.023
	Third instar	4.949	1.394	0.498
	Adult	6.595	1.886	0.397

Result of the leaf dip toxicity bioassay recorded significantly highest (df = 4,10; F = 0.886; p < 0.005 and LC₅₀ = 1.894%) adult mortality in *P. glabura* -AgNP's. Similar results was also recorded for topical application toxicity bioassay (df = 4,10; F = 76.062; p < 0.005 and LC₅₀ = 3.738%) for *P. glabura* -AgNP's. *I.carnea* -AgNP's (86.66%; df = 4,10; F = 28.278; p < 0.005 and LC₅₀ = 4.750%) showed least impacts to the adult of *P.solenopsis* (Fig. 8).

Overall comparison of nymphicidal activity of the experimental AgNP's using the cumulative average mortality of first, second, third nymphal instar and adult *P. solenopsis* the leaf dip toxicity bioassay using *P.*

glabura-AgNP's (95.83%) followed by *J. adathoda*-AgNP's (90.83%), *A. squamosa*-AgNP's (86.66%) and *I. carnea*-AgNP's (85.83%) and followed by topical application bioassay recorded in *J. adathoda*-AgNP's (89.99%), *A. squamosa*-AgNP's (88.33%), *P. glabura*-AgNP's (86.66%) and (79.66%) in *I. carnea*-AgNP's (Table 3).

Insecticidal activity of vijayneem. The mortality was recorded for experimental sample vijayneem using in leaf dip and topical application toxicity bioassays. Results of the experiments presented in the (Fig. 9) shows that the highest nymphal mortality (80.00%; df = 4,10; F = 39.5; p < 0.005 and LC₅₀ value = 4.949%) was recorded in vijayneem on 144 hrs in leaf dip toxicity bioassay followed by first instar nymphs of *P. solenopsis* and (83.33%; df = 4,10; F = 34.726; p < 0.005 and LC₅₀ = 9.601%), against second instar nymphs of *P. solenopsis* and followed by (81.66%; df = 4,10; F = 24.236; p < 0.005 and LC₅₀ = 1.466%), against third instar nymphs of *P. solenopsis* and then (100.00%; df = 4,10; F = 61.837; p < 0.005 and LC₅₀ = 6.812%), against adult of *P. solenopsis*.

Figure 9 shows that the highest nymphal mortality (80.00%; df = 4,10; F = 41.167; p < 0.005 and LC₅₀ = 7.048%) was observed in vijayneem on 144 hrs in topical toxicity bioassay followed by first instar nymphs of *P. solenopsis* and then (50.00%; df = 4,10; F = 19.206; p < 0.001 and LC₅₀ = 1.076%), against second instar nymphs of *P. solenopsis* and followed by (80.00%; df = 4,10; F = 39.5; p < 0.005 and LC₅₀ = 4.949%), against third instar nymphs of *P. solenopsis* and adult (76.66%; df = 4,10; F = 31.179; p < 0.005 and LC₅₀ = 6.595%) of *P. solenopsis*.

Among the cumulative average mortality of vijayneem in leaf dip toxicity bioassay recorded (86.24%) mortality followed by topical application toxicity bioassay (76.66%) (Table 3). Over all the leaf dip and topical application toxicity bioassay of highest insecticidal activity was recorded in nanomaterials, vijayneem and followed by crude extract in high concentration (10%) by insecticidal activity of first, second, third nymphal instars and adult *P. solenopsis*.

Discussion

The plants have evolved a plethora of secondary metabolites which play a major role in insecticidal activity.^{16,30} The recent research was highly drifted on screening and characterization of green chemistry with potential target insecticidal agents.³¹ The recent studies have demonstrated the insecticidal properties of chemicals derived from plants that are active against specific target species, biodegradable to non-toxic products and potentially suitable for use in integrated pest management programs.³²

The AgNPs of *PG*, *JA*, *AS* and *IC* showed 100% mortality at various exposure periods, which indicates that there are lots of scope for reducing the nanoparticles level in *P. solenopsis* management and that this can be extended to other crops too. Results also shows does dependent mortality as observed for plant extracts to *P. solenopsis*.^{1,33,34} The present study also reveals that AgNPs synthesized using aqueous leaf extract of *A. squamosa*, *J. adathoda*, *I. carnea* and *P. glabura* showed insecticidal effects on *P. solenopsis*. The

recent research on insecticidal action of plant materials specially secondary metabolites and essential oils resulted that they are eco-friendly, biodegradable and species specific.^{35–38}

Compared with the LC₅₀ values was shown to be 3.4, 2.9, 2.2 and 2.0 times lower for AG-AgNPs, JA-AgNPs, PG-AgNPs and AC-AgNPs than for the *A. squamosa*, *J. adathoda*, *P. glabura* and *I. carnea* crude extract exposure. An opposite trend was recorded when essential oils of *C. citratus*, *T. vulgaris* and *P. graveolens* AgNPs were evaluated against *P. solenopsis*.²⁹ Therefore, JA-AgNPs, AG-AgNPs, PG-AgNPs and AC-AgNPs were 3.7, 3.6, 1.8 and 1.4 times more toxic than the commercial formulation (vijaynem) respectively, which suggested that the biological activity was considerably improved through using AgNPs, and bionano materials have significantly better insecticidal toxicity than that of the crude extracts and commercial formulation. Moreover, bio-nanomaterials can save the manpower through decreasing the number of pesticide applications needed, and pesticide dosage compared to traditional pesticide. Furthermore, nanoparticles size of *A. squamosa*-AgNPs, *J. adathoda*-AgNPs, *I. carnea*-AgNPs and *P. glabura*-AgNPs is 1.56–3.13 nm, 1.56–3.13 nm, 1.56–3.13 nm and 1.56–3.13 nm respectively in AFM analysis. These small size can increase the penetration and absorption of the active ingredients by the pest.^{389–41}

Many factors influencing the effectiveness of plant extracts and or bio-nano materials to control insect pests are different plant species, part of plant, tested insects, methods of extraction, type of solvents, and method of application. Plant extracts coupled with nanotechnology that are active at reducing pest populations at the insect stage can provide many associated benefits to pest control. AS, JA, IC and PG-AgNPs was considered to be potential agents for various biological applications including insecticidal agents.⁴² The results indicate that different concentration of all nanoparticles caused higher mortality in leaf dip method toxicity and topical application method toxicity bioassays. This indicates that the all-AgNPs in the intracellular space can bind to sulphur containing proteins or to phosphorus containing protein compounds like DNA, leading to the denaturation of some organelles and enzymes⁴³ causing death. Further, the mechanism which causes the death of the insect could be the ability of the nanoparticles to penetrate through the insect membranes^{21,44} reported that half-life values for rotenone concentration with ZnO nanoparticle under UV radiation were found to be higher than those of rotenone alone. It may also be applied here, but it requires future studies. Under natural conditions, the released insect moved to various places for hiding during afternoons.

Insecticide based on Neem oil works as a systemic insecticide for many plants when applied as a soil drench. Once the product is in the plant's vascular system, insects take it in during feeding. The commercial neem-based biopesticide at different concentration showed drastic mortality when combined with monocrotophos in contact toxicity. This proves the statement, "The chemical pesticides will continue to be the main weapon against *P. solenopsis* for the near future". Mortality was varied according to the developmental stage, and type of bioassays used⁴⁵ as observed in the present study.

The present study illustrates that the botanicals *A.squamosa*, *J.adathoda*, *I.carnea* and *P.glabura* is a potential one for the eco-congenial nymphicide development as an alternate to chemical insecticides that are being currently used in cotton pest management programs. Our results reveal that the nymphal

mortality and adult mortality is high in leaf dip method of toxicity bioassay followed by topical application. Mortality was enhanced while crude extracts combined with the bionanoparticles, vijay neem and they are considered most suitable for environment friendly pest management. Perhaps to reduce the quantity of chemical pesticides, farmers can be encouraged to use the concentrations for the pest management programme.

Declarations

Acknowledgement

This research publication was financed by Taif University Researchers Supporting Project number (TURSP -2020/92), Taif university, Taif, Saudi Arabia.

Author contributions

Kitherian Sahayaraj: Conceptualization; Investigation; Methodology; Funding acquisition; Supervision; Project administration; Data curation; Writing - original draft; Writing - review & editing.

Madasamy, M : Investigation; Methodology; Data curation; Software, Visualization, Writing - original draft, review & editing.

Samy Sayed: Funding acquisition, Resources; Data processing; Writing - review & editing.

Hosny Kesba: Funding acquisition, Resources; Data processing; Writing - review & editing

Prathas Selvaraj: Conceptualization; Methodology; Co-Supervision; Project administration; Data curation; Writing - review & editing.

Madasamy, K: Data curation; Software; Visualization; Writing - review & editing.

Conflict interest statement

The authors declared they have no conflict of interest.

Data availability statement

The datasets generated and/or analyzed during the current study are not publicly available due reserved for filing a patent but are available from the corresponding author on reasonable request.

References

1. Mostafa, M.E., Youssef, N.M. & Abaza, A.M. Insecticidal activity and chemical composition of plant essential oil against cotton mealy bug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae). Entomol. Zool. Stud. **6** (2), 539-543.
<https://www.entomoljournal.com/archives/2018/vol6issue2/PartH/6-1-239-777.pdf> (2018)

2. Wang, Y., Watson, G.W. & Zhang, R. The potential distribution of an invasive mealybug *Phenacoccus solenopsis* and its threat to cotton in Asia. *Agri. Forest Entomol*, **30**, 317-320.
<https://doi.org/10.1111/j.1461-9563.2010.00490.x> (2010).
3. S. Cariri Lopes, J. Oliveira, J.M. Oliveira, M.D. Oliveira, & Souza, A.M. Host plants for mealybugs (Hemiptera: Pseudococcidae) in grapevine crops. e-ISSN 1983-4063 - www.agro.ufg.br/pat - Pesq. Agropec. Trop., Goiânia, **49** e54421. <https://doi.org/10.1590/1983-40632019v4954421> (2019).
4. El-Zahi, S.E., Aref, S.A. & Korish, S.K.M. The cotton mealy bug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) as a new menace to cotton in Egypt and its chemical control. *J. Plant Prot. Res.* **56** (2), 111-115. <https://doi.org/1515/JPPR-2016-0017> (2016).
5. Kousar, T., Sahito, H.A., Jatoti, F.A., Shah, Z.H. & Mangrio, W.M. Resistant insecticides of cotton mealy bug, *Phenacoccus solenopsis* (Tinsley) under laboratory conditions. *J. Entomol. Zool. Stud.* **4**(6), 355-359. <https://www.entomoljournal.com/archives/2016/vol4issue6/PartE/4-6-22-507.pdf> (2016).
6. Isman, M.B. Leads and prospects for the development of new botanical insecticides. *Pestic. Toxicol.* **3**, 1-20. <https://ci.nii.ac.jp/naid/10009699412> (1995).
7. Ismail, T., & Walaa, M.E. Prevalence of some pesticides residues in buffalo's milk with refer to impact of heating. *J. Vet. Sci.* **48** (2), 113-123. <https://doi.org/10.5455/ajvs.215565> (2016).
8. Delvin, J.F. & T. Zettel, *Ecoagriculture: Initiatives in Eastern and Southern Africa*. Weaver Press, Harare (1999).
9. Prishanthini, M. & Vinobaba, M. Efficacy of some selected botanical extracts against the Cotton mealybug *Phenacoccus solenopsis* (Tinsley) (Hemiptera: Pseudococcidae). *J. Sci. Res.* **4** (3), 1-6.
<http://www.ijsrp.org/research-paper-0314/ijsrp-p2738.pdf> (2014)
10. Roozdar, E.B., Habibpour, M.S., Mossadegh, & Mahmoodi S.M. 2019. Evaluation of biological effects of essential oils of 14 plants species against cotton mealybug, *Phenacoccus solenopsis* (Hem.: Pseudococcidae) under laboratory conditions." *Plant Protection* **42**(3): 71-86.
11. Singh, A., Bhatt,G., Gujre,N., Mitra, S., Swaminathan, , Limaye, A.M. & Rangan, L. Karanjin. *Phytochemistry*, **183**, PP. 11264. <https://doi.org/10.1016/j.phytochem.2020.112641>
12. Bharathi, K. & Muthukrishnan, N. Evaluation of Botanicals against Cotton Mealy Bug, *Phenacoccus solenopsis* Tinsley (Psuedococcidae: Hemiptera) *Int. J. Curr. Microbiol. App. Sci* , **6** (12), 1055-1061.
<https://doi.org/10.20546/ijcmas.2017.612.119> (2017).
13. Kumar, R., Mukesh, N., Rahul, C., Vijender, P. & Kranthi, R. Evaluation of ecofriendly control methods for management of mealybug, *Phenacoccus solenopsis* Tinsley in cotton. *J. Entomo.* **9**(1), 32-40.
<https://doi.org/10.3923/je.2012.32.40> (2012).
14. Ahmad,F., W. Akram, A. Sajjad, & Imran, A.U. Management practices against cotton mealybug, *Phenacoccus Solenopsis* (hemiptera: pseudococcidae). *Int. J. Agric. Biol.* **13** 547–552 <https://doi.org/10-448/SVI/2011/13-4-547-552> (2011).
15. Katole, R.M., Sharma, K. & Joshi, C.K. *Annona squamosa* L. As a potential natural botanical pesticide and its futuristic research scope: a review. *Plant Cell Biotechnol. Mol. Biol.* **22**(41-42) 75-98.
<https://www.ikpress.org/index.php/PCBMB/article/view/6683> (2021).

16. Khan, I., Ahmad, S. Azam, F. Hassan, A. Aziz, N. Rehman, F. Ullah, & Z. Liaqat, Pharmacological activities of *Justicia adhatoda*, Pak. J. Pharm. Sci., **31**(2), 371-377 : <https://www.researchgate.net/publication/323840337> (2018).
17. Kunal, Vishal, C. Singla, A. Sharma, & A. Dhiman, An update on phytochemistry and therapeutic properties of *Ipomoea carnea*, pharmacogn. phytochem. **10**(1), 01-06. <http://dx.doi.org/10.22271/phyto/https://www.phytojournal.com/archives/2021/vol10issue1/PartA/9-6-384-266.pdf> (2021).
18. Wadnerwar, N. & Deogade, M. Future perspectives of Therapeutic Claims of an Ethnopharmacological Drug *Ipomoea carnea*-A Critical Review. Int. J. Ayurveda Res., **12**(2), 177-184. <https://doi.org/10.47552/ijam.v12i3.1884> (2021).
19. Fugare, A.G., Shete, R.V. & Adak, S. A Review on *Pongamia pinnata* (L.): Traditional Uses, Phytochemistry and Pharmacological Properties. J. Drug Deliv. ther. **11**(supply 1), 207-211. <https://doi.org/10.22270/jddt.v11i1-s.4522> (2021).
20. Bhattacharyya, A., A. Bhaumik, P.U. Rani, S. Mandal, & Epidi, T.T. Nano-particles- A recent approach to insect pest control. Afr. J. Biotechnol, **9**, 3489-3493. <http://www.academicjournals.org/AJB> (2010).
21. Ramar, G., Suman, T., Elangomathavan, R. & Jeyasanjkar, Larvicidal activity of biologically synthesised silver nanoparticles against dengue vector *Aedes aegypti* (Culicidae). Discovery, **9** (23), 65-68. <http://www.discovery.org.in/d.htm> (2014).
22. Rouhani, M., M.A. Samih, & S. Kalantari, Insecticide effect of silver and zinc nanoparticles against *Aphis nerii* Boyer De Fonscolombe (Hemiptera: Aphididae). Chil J. Agric. Res. **72**, 590–594. <http://dx.doi.org/10.4067/S0718-58392012000400020> (2012).
23. Vinayaga Moorthi, P., C. Balasubramanian, & Mohan, S. An improved insecticidal activity of silver nanoparticle synthesized by using *Sargassum muticum*. Appl. Biochem. Biotechnol. **175**(1), 135-140. <https://doi.org/10.1007/s12010-014-1264-9> (2015).
24. Sankar, M.V. & Abideen, S. Pesticidal effect of green synthesized silver and lead nanoparticles using *Avicennia marina* against grain storage pest *Sitophilus oryzae*. I. Nanomater. Biostructures, **5** (3), 32-39. <https://urpjournals.com> (2015).
25. Kantrao, S., M.A. Ravindra, S.M.D. Akbar, P.D.K. Jayanthi, & A. Venkataraman, Effect of biosynthesized silver nanoparticles on growth and development of *Helicoverpa armigera* (Lepidoptera: Noctuidae): interaction with midgut protease. J. Asia. Pac. Entomol. **20**, 583–589. <https://doi.org/10.1016/j.aspen.2017.03.018> (2017).
26. Bharani, R.S.A. & Namasivayam, S.K.R. Biogenic silver nanoparticles mediated stress on developmental period and gut physiology of major lepidopteran pest *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) –an eco-friendly approach of insect pest control. J. Environ. Chem. Eng. **5**, 453–467. <https://doi.org/10.1016/j.jece.2016.12.023> (2017).
27. Zahir, A.A., Bagavan, A., Kamaraj, C., Elango, G. & Rahuman, A.A. Efficacy of plant-mediated synthesized silver nanoparticles against *Sitophilus oryzae*. J. Biopestic. **5**, http://www.jbiopest.com/users/lw8/efiles/vol_5_0_95_102f.pdf (2012)

28. Rikta, S.Y. & Rajiv, P. Applications of silver nanomaterial in agricultural pest control. In Silver Nanomaterials for Agri-Food Applications, Elsevier, pp. 453-470. <https://doi.org/10.1016/C2019-0-05215-6> (2021).
29. Sawsan, M.A., A. Afaf, H. Horeia, A.A. Naema, Enayat, & M. Mohammed, Nano Bioinsecticides based on Essential oils Against *Phenacoccus solenopsis*. Egypt. Acad. J. Biol. Sci., A Entomol. **11**(5), 1-12. <https://doi.org/10.21608/eajbsa.2018.17738> (2018).
30. Ayil-Gutiérrez, B.A., L.F. Sánchez-Teyer, F. Vazquez-Flota, M. MonforteGonzález, Y. Tamayo-Ordóñez, M.C. Tamayo-Ordóñez, & G. Rivera, Biological effects of natural products against Spodoptera spp. Crop Prot **114**, 195–207. <https://doi.org/10.1016/j.cropro.2018.08.032> (2018).
31. Giongo, A.M.M., Vendramim, J.D. & Forim, M.R. Evaluation of neem based nano-formulations as alternative to control fall armyworm. Cienc. E. Agrotecnol. **40**, 26–36. <https://doi.org/10.1590/S1413-70542016000100002>(2016).
32. Isman, M.B. Botanical insecticides, deterrents and repellents in modern agriculture and an increasingly regulated world, Annu. Rev. Entomol, **51**, 45-66. <https://doi.org/10.1146/annurev.ento.51.110104.151146> (2006).
33. Saad, L.H.E. , S.El. Negm, A.A. Saleh, & A.M.E. Abd El-Mageed, Essential Oils as Green Insecticides: GC/MS Analysis and Toxicological Studies on Cotton Mealybugs *Phenacoccus solenopsis* (Tinsley) (Hemiptera: Pseudococcidae). International Journal of Scientific Research and Sustainable Development, **4**(1), 1-26. <https://doi.org/10.21608/IJSRSD.2021.177036> (2021).
34. Sardar, M.U., M.M. Rashid, & M. Naeem. Entomocidal Efficacy of Different Botanical Extracts against Cotton Mealybug, *Phenacoccus solenopsis* Tinsley (Sternorrhyncha: Pseudococcidae), J. Entomol. Zool. Stud, **6**(5), 2078-2084. <https://www.entomoljournal.com/archives/2018/vol6issue5/PartW/6-4-279-737.pdf> (2018).
35. Rattan, R.S. Mechanism of action of insecticidal secondary metabolites of plant origin Crop Protection, **29** (9) 913-920. <https://doi.org/10.1016/j.cropro.2010.05.008> (2010).
36. Senthil-Nathan, S., Hisham, A. & Jayakumar, G. Larvicidal and growth inhibition of the malarial vector *Anopheles stephensi* by triterpens from *Dysoxylum* (Meliaceae). Fitoterapia, **76**, 106-111. <https://doi.org/10.1016/j.fitote.2007.07.013> (2008).
37. Senthil-Nathan, S., M.Y. Choi, C.H. Paik, & H.Y. Seo, Food consumption and detoxification enzyme activity of the rice leaf folder larvae after treatment with *Dysoxylum triterphenes*. Biochem. Physiol, **88**, 260–267. <https://doi.org/10.1016/j.pestbp.2006.12.004> (2007).
38. Senthil-Nathan, S. Kalaivani, & K. Sehoon, Effects of *Dysoxylum malabaricum* Bedd. (Meliaceae) extract on the malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae). Bioresources Technology, **97**(16), 2077-2083. <https://doi.org/10.1016/j.biortech.2005.09.03> (2006a).
39. Elabasy, A. Shoaib, M. Waqas, Z. Shi, & M. Jiang, Cellulose nanocrystals loaded with thiamethoxam: fabrication, characterization, and evaluation of insecticidal activity against *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae). Nanomaterials, **10**(4), 788. <https://doi.org/10.3390/nano10040788> (2010)

40. Saini, P., Gopal, M., Kumar, R. & Srivastava, Development of pyridalyl nanocapsule suspension for efficient management of tomato fruit and shoot borer (*Helicoverpa armigera*). J. Environ. Sci. Health B, **49**, 344–351. <https://doi.org/10.1080/03601234.2014.882168> (2014).
41. Anjali,C., Y. Sharma, A. Mukherjee, & N. Chandrasekaran, Neem oil (*Azadirachta indica*) nanoemulsion— A potent larvicidal agent against *Culex quinquefasciatus*. Pest Manag. Sci. **68**, 158–163. <https://doi.org/10.1002/ps.2233>. Epub 2011 Jul 1 (2012).
42. Parashar, V., R. Parashara, B. Sharmab, A. Avinash, & C. Pandey, Parthenium leaf extract mediated synthesis of silver nanoparticles: a novel approach towards weed utilization. Dig. J. Nanomater. Biostructures, **4**(1), 45–50. <https://doi.org/1.1.473.7183&rep=rep1&type=pdf> (2009).
43. Rai,M., A. Yadav, & A. Gade, Silver nanoparticles as a new generation of antimicrobials. Biotechnology Advances, **27**, 76-83. <https://doi.org/1016/j.biotechadv.2008.09.002> (2009).
44. Chen, X.J., Zhi, Y.M. , Ya, J.R., Hao, T.G. & Chun, L.L. Effects of ZnO nanoparticle on photo-protection and insecticidal synergism of rotenone. Journal of Agricultural Science, **8**, (2) 16-20. <https://doi.org/1016/j.plaphy.2021.03.053>. Epub 2021 Mar 30 (2016).
45. Nana,P., S. Ekesi, F. Nchu, & N.K. Maniania, Compatibility of *Metarhizium anisopliae* with *Calpurnia aurea* leaf extracts and virulence against *Rhipicephalus pulchellus*. Appl. Entomol. **140**, 590–597, <https://doi.org/10.1111/jen.12289> May 2016 (2016).
46. Mansour, S.A., F. Bakr, S.A. Hamouda, & R.I. Mohamed, Toxic and synergistic properties of several botanical extracts against larval and adult stages of the mosquito, *Anopheles pharoensis*. Biopestic. Int **6**(2), 129-145. <http://www.koulresearch.org> (2010).
47. Liu, X. & A.P. Stansly, Deposition and bioassay of insecticides applied by leaf dip and spray tower against *Bemisia argentifolii* nymphs. Pesticidal Science, **44**, 317-322. https://swfrec.ifas.ufl.edu/docs/pdf/entomology/publications/ref_0020.pdf (1995).
48. Mansour, S.A., A. El-Sharkawy, & N.A. Abdel- Hamid, Toxicity of essential plant oils, in comparison with conventional insecticides, against the desert locust, *Schistocerca gregaria* (Forsk.) . Industrial Crops and Products, **63**, 92-99. <https://doi.org/1016/j.indcrop.2014.10.038> (2015).
49. Abbott, W.S. A method of computing the effectiveness of an insecticide. Econ. Entomol. **18** 65-266 (1925).
50. Finney, D.J. Probit analysis Cambridge University Press, London, pp.68-78 (1971).

Figures

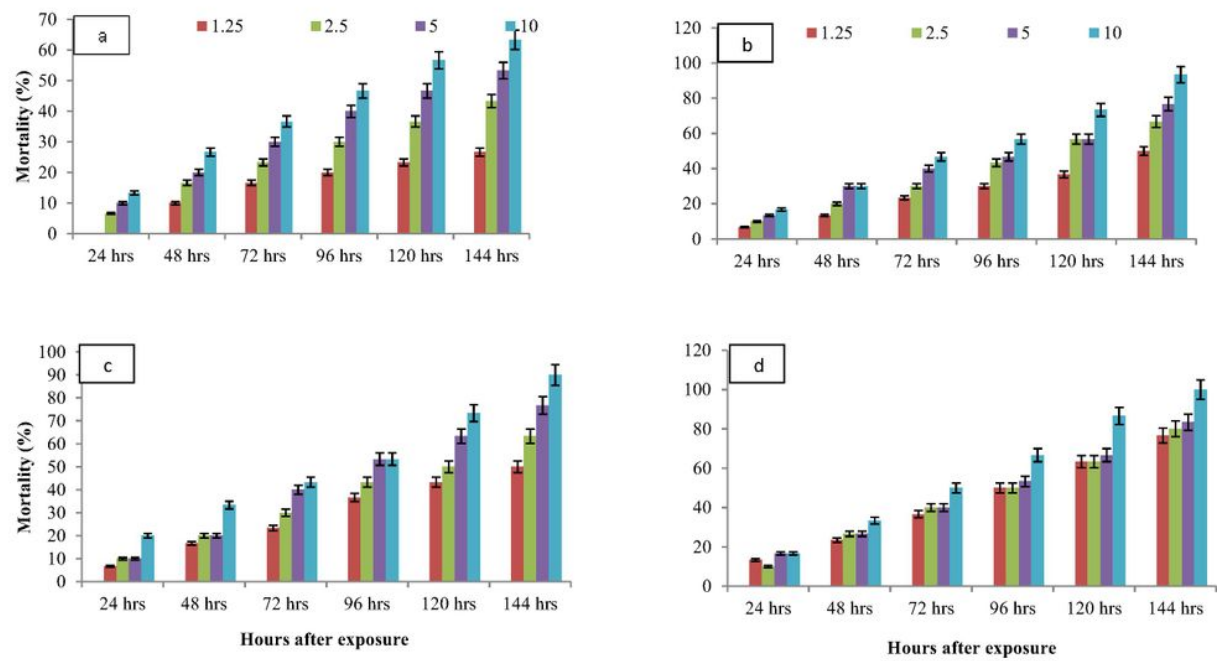


Figure 1. Impact of *Pongamia glabura* crude extracts (1.25, 2.5, 5 and 10 %) on the mortality of *P. solenopsis* first instar (a), second instar (b), third instar (c) nymphs and adults (d)

Figure 1

Impact of *Pongamia glabura* crude extracts (1.25, 2.5, 5 and 10 %) on the mortality of *P. solenopsis* first instar (a), second instar (b), third instar (c) nymphs and adults (d) *P. solenopsis*

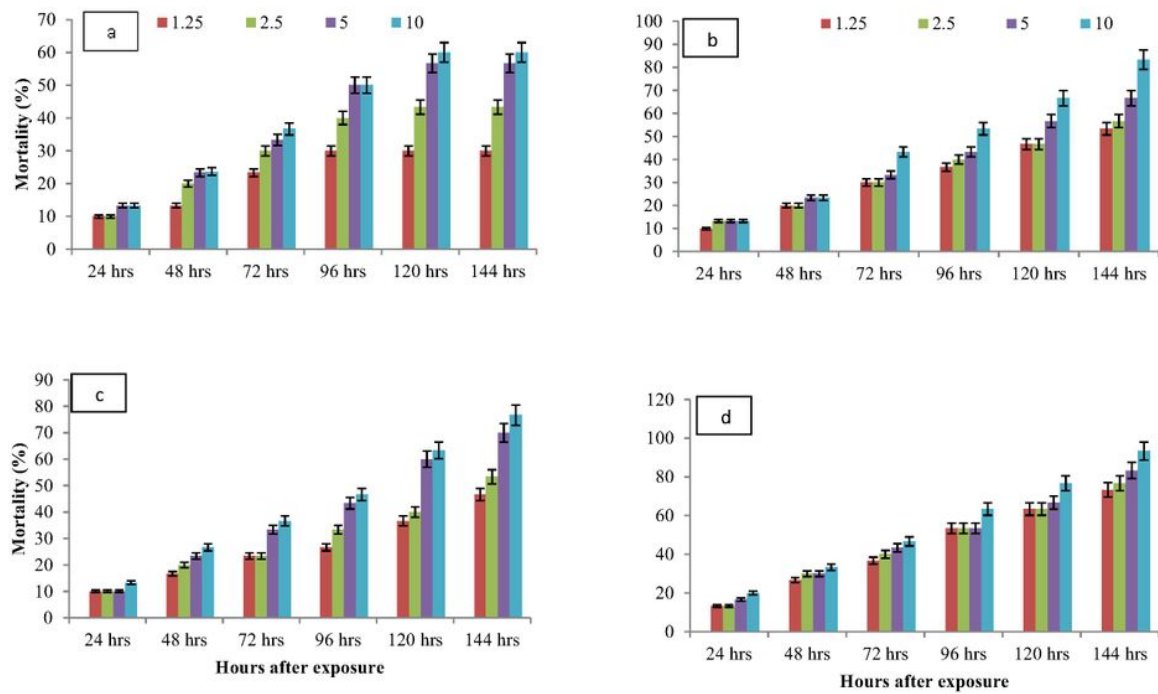


Figure 2. Impact of *Justicia adathoda* crude extracts (1.25, 2.5, 5 and 10 %) on the mortality of first instar (a), second instar (b), third instar (c) nymphs and adults (d) *P. solenopsis*.

Figure 2

Impact of *Justicia adathoda* crude extracts (1.25, 2.5, 5 and 10 %) on the mortality of first instar (a), second instar (b), third instar (c) nymphs and adults (d) *P. solenopsis*

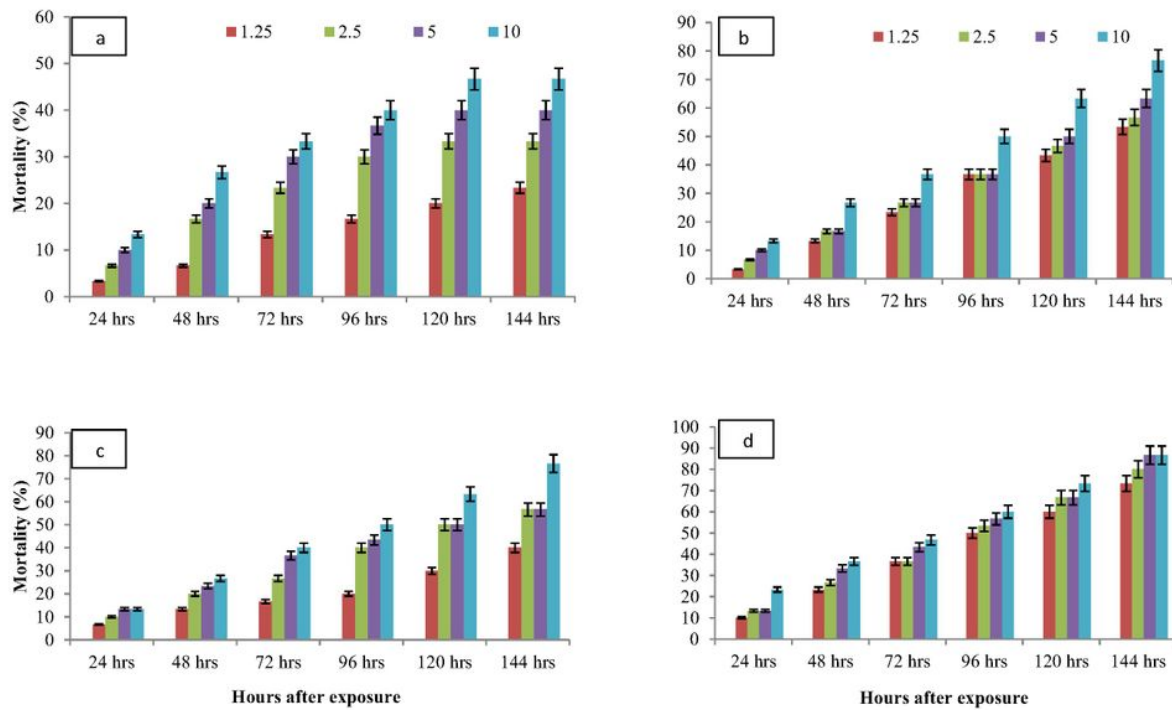


Figure 3. Impact of *Annona squamosa* crude extracts (1.25, 2.5, 5 and 10 %) on the mortality of *P. solenopsis* first instar (a), second instar (b), third instar (c) nymphs and adults (d)

Figure 3

Impact of *Annona squamosa* crude extracts (1.25, 2.5, 5 and 10 %) on the mortality of *P. solenopsis* first instar (a), second instar (b), third instar (c) nymphs and adults (d)

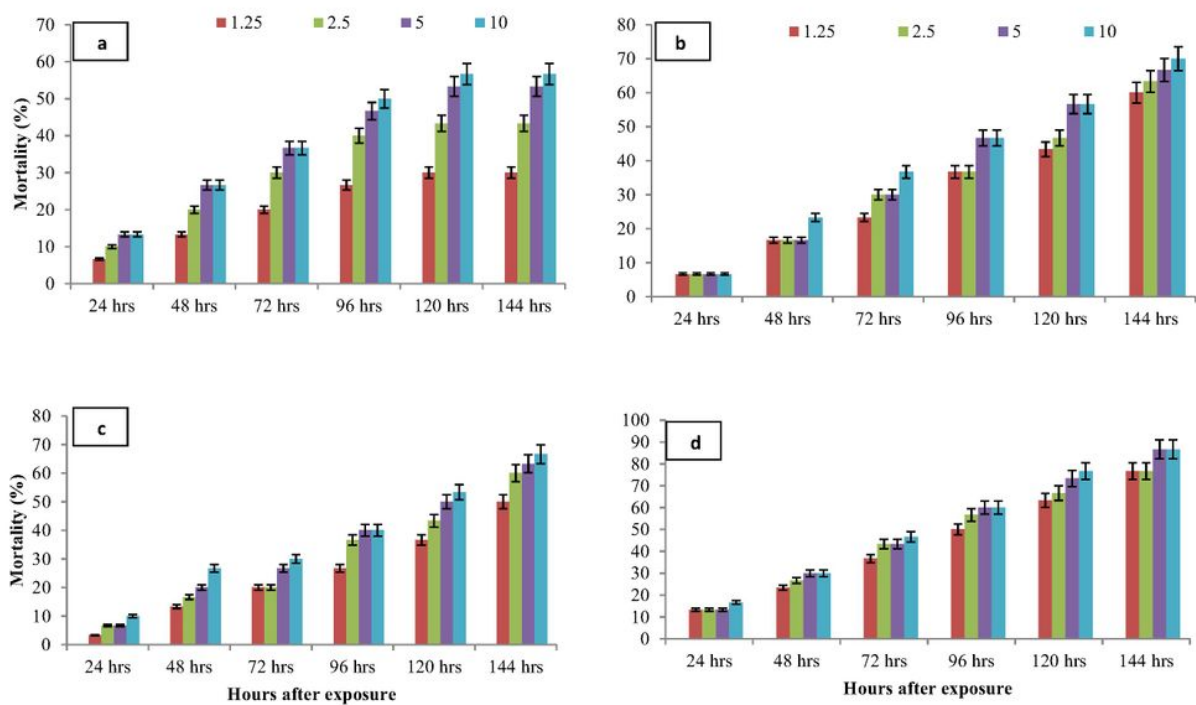


Figure 4. Impact of *Ipomea carnea* crude extracts (1.25, 2.5, 5 and 10 %) on the mortality of *P. solenopsis* first instar (a), second instar (b), third instar (c) nymphs and adults (d) of *P. solenopsis*

Figure 4

Impact of *Ipomea carnea* crude extracts (1.25, 2.5, 5 and 10 %) on the mortality of *P. solenopsis* first instar (a), second instar (b), third instar (c) nymphs and adults (d) of *P. solenopsis*

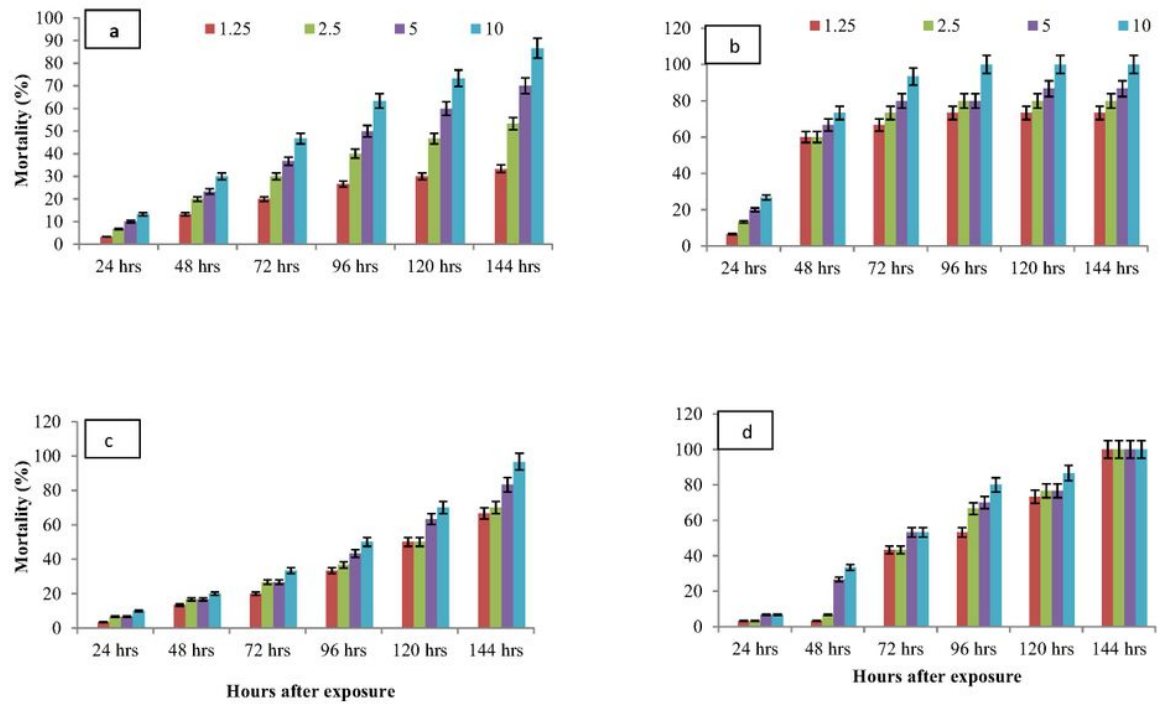


Figure 5. Impact of *Pongamia glabura*-AgNP's (1.25, 2.5, 5 and 10 %) on the mortality of *P. solenopsis* first instar (a), second instar (b), third instar (c) nymphs and adults (d)

Figure 5

Impact of *Pongamia glabura*-AgNP's (1.25, 2.5, 5 and 10 %) on the mortality of *P. solenopsis* first instar (a), second instar (b), third instar (c) nymphs and adults (d)

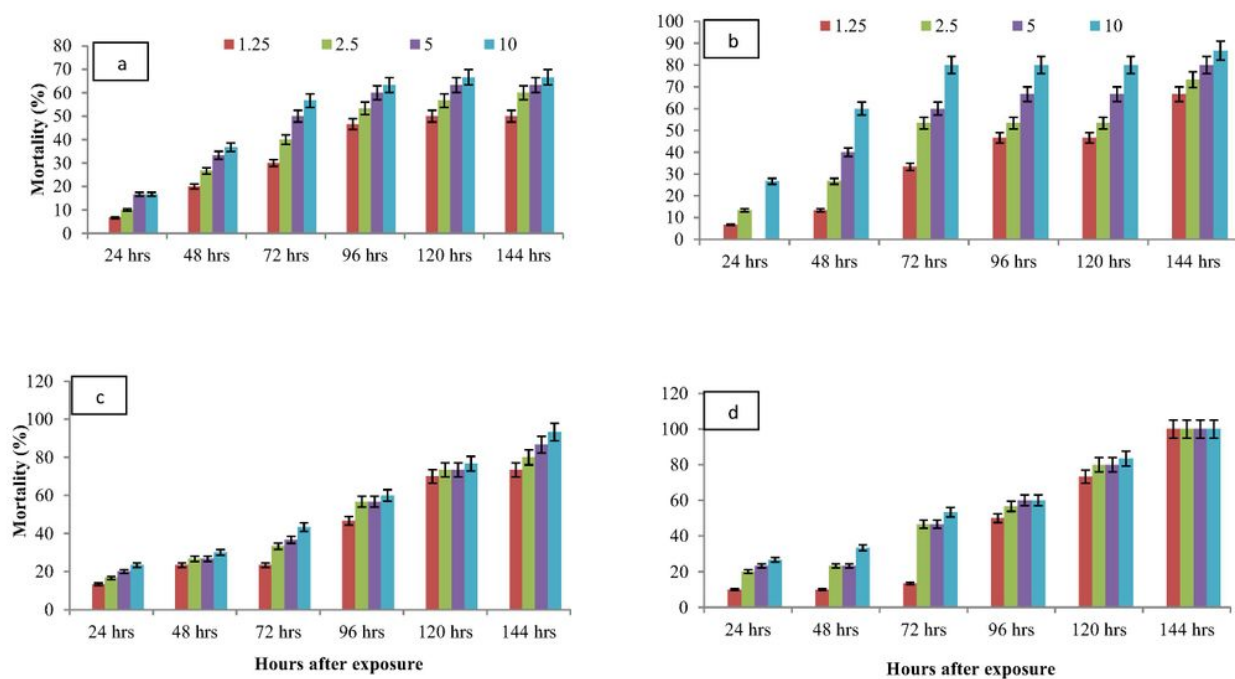


Figure 6. Impact of *Annona squamosa*-AgNP's (1.25, 2.5, 5 and 10 %) on the mortality of *P. solenopsis* first instar (a), second instar (b), third instar (c) nymphs and adults (d)

Figure 6

Impact of *Annona squamosa*-AgNP's (1.25, 2.5, 5 and 10 %) on the mortality of *P. solenopsis* first instar (a), second instar (b), third instar (c) nymphs and adults (d)

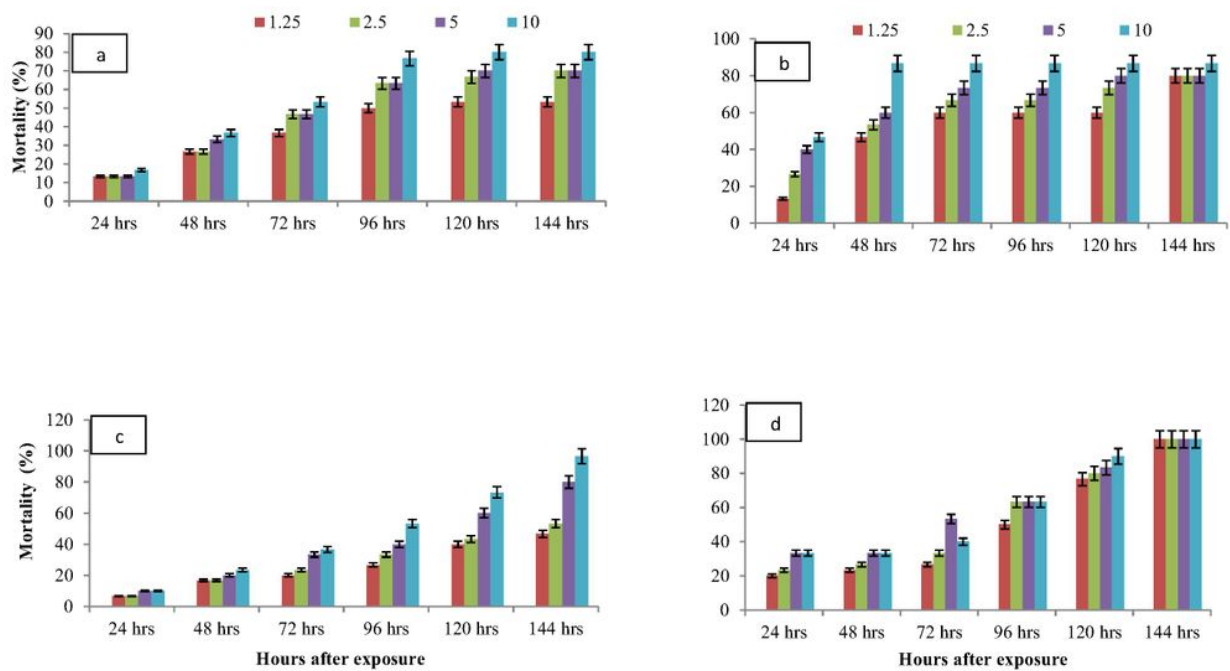


Figure 7. Impact of *Justicia adathoda*-AgNP's (1.25, 2.5, 5 and 10 %) on the mortality of *P. solenopsis* first instar (a), second instar (b), third instar (c) nymphs and adults (d)

Figure 7

Impact of *Justicia adathoda*-AgNP's (1.25, 2.5, 5 and 10 %) on the mortality of *P. solenopsis* first instar (a), second instar (b), third instar (c) nymphs and adults (d)

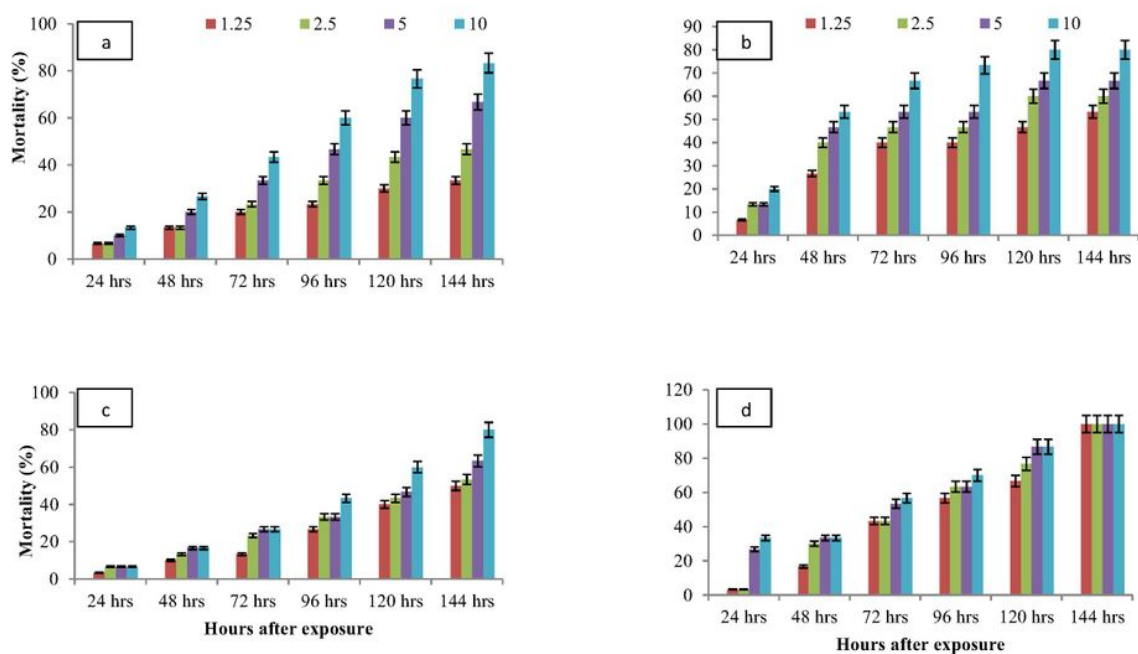


Figure 8. Impact of *Ipomea carnea*-AgNP's (1.25, 2.5, 5 and 10 %) on the mortality of *P. solenopsis* first instar (a), second instar (b), third instar (c) nymphs and adults (d)

Figure 8

Impact of *Ipomea carnea*-AgNP's (1.25, 2.5, 5 and 10 %) on the mortality of *P. solenopsis* first instar (a), second instar (b), third instar (c) nymphs and adults (d)

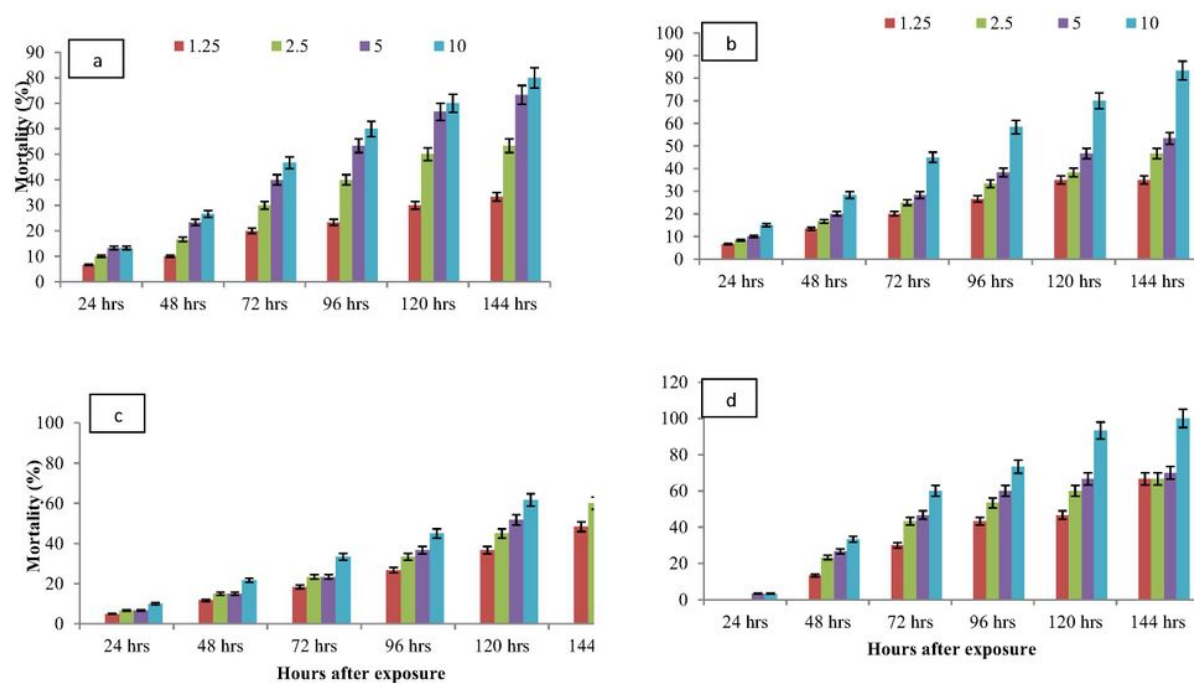


Figure 9. Impact of Vijay neem (1.25, 2.5, 5 and 10 %) on the mortality of *P. solenopsis* first instar (a), second instar (b), third instar (c) nymphs and adults (d)

Figure 9

Impact of Vijay neem(1.25, 2.5, 5 and 10 %) on the mortality of *P. solenopsis* first instar (a), second instar (b), third instar (c) nymphs and adults (d)