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RESEARCH ARTICLE

Synergistic efficacy of insecticides and phytochemicals against rugose spiraling whitefly (*Aleurodicus rugioperculatus*) in India

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Abstract

The rugose spiraling whitefly (*Aleurodicus rugioperculatus* Martin), an invasive pest impacting coconut and banana crops in India, exhibits adaptability and resistance to conventional insecticides, posing significant agricultural challenges. This study assessed theefficacy of imidacloprid, spiromesifen and thiodicarb, alone and combined with synergists (piperonyl butoxide, PBO, diethyl malate, DEM, propargyloxy phthalimide, PP) and plant extracts, against RSW populations from Bhubaneswar, India, in 2024. Leaf dip bioassays determined LC_{50} values, synergistic ratios (SRs) and 24 hr mortality rates. Spiromesifen demonstrated the highest efficacy (LC_{50} 0.431 ppm in January), followed by imidacloprid (0.458 ppm) and thiodicarb (0.528 ppm). Synergism with PBO markedly enhanced potency, reducing spiromesifen's LC_{50} to 0.046 ppm (SR 9.956), imidacloprids' to 0.108 ppm (SR 3.990) and thiodicarb's to 0.140 ppm (SR 3.771). Plant extract combinations yielded up to 75 % mortality with spiromesifen and Pongamia oil, 70 % with thiodicarb and Pongamia oil and 60 % with imidacloprid and sesamum oil. Seasonal resistance increased LC_{50} values by 34-64 % from January to May (e.g., thiodicarb 0.865 ppm), reflecting heightened resistance in warmer conditions. These results highlight that integrating synergists and phytochemicals with insecticides significantly boosts rugose spiraling whitefly (RSW) control, reducing required doses by up to 92 % and mitigating resistance. This approach offers a sustainable pest management strategy for Indian agriculture, particularly in coastal regions where RSW thrives. Field trials are recommended to validate these laboratory findings and refine integrated pest management practices.

Keywords: insecticides; phytochemicals; rugose spiraling whitefly; spiromesifen; synergists

Introduction

The rugose spiraling whitefly (RSW), *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae), is an invasive polyphagous pest that has become a significant concern in India since its initial detection in 2016 (1). Originating from Central America, RSW has spread rapidly across tropical regions, severely affecting economically critical crops such as coconut (*Cocos nucifera*) and banana (*Musa* spp.) in states including Tamil Nadu, Karnataka, Kerala and Andhra Pradesh (1). Field surveys in Tamil Nadu have revealed extensive infestations, with all life stages-eggs, nymphs and adults-present on host plants, causing nutrient depletion, water stress and black sooty mould growth due to honeydew secretion (2). Although RSW rarely kills its hosts, its feeding reduces photosynthetic capacity and crop yields, threatening India's agricultural economy. Coconut, a vital crop contributing significantly to coastal livelihoods, faces increasing losses, with production valued at over ₹ 30000 crore annually. Predictive models suggest high habitat suitability along India's eastern and western coastal regions, underscoring the need for robust control strategies (3).

RSW's ability to infest over 100 host species, including ornamentals and fruit trees, complicates management efforts (2). Its resilience is driven by detoxification enzymes, such as cytochrome P_{450} and glutathione S-transferases, which break down xenobiotics like insecticides, enabling resistance development (2). This adaptability means control measures effective on one host may fail on another, as noted in Kerala field observations (1). Insecticides such as imidacloprid, spiromesifen and thiodicarb have been widely used against whiteflies, but their efficacy against RSW is diminishing due to resistance and sub-lethal effects (4, 5). In India, repeated applications have heightened environmental risks, including impacts on pollinators and soil ecosystems, necessitating sustainable alternatives.

The need for this research stems from the escalating resistance of RSW to conventional insecticides, the associated environmental hazards and the underexplored potential of integrated approaches in Indian agricultural contexts, particularly for key crops like coconut and banana. This study was conducted to bridge these gaps by evaluating combined strategies that enhance efficacy while promoting sustainability. Synergists like piperonyl butoxide (PBO) enhance insecticide toxicity by inhibiting detoxifying enzymes, offering a means to overcome resistance (6). Meanwhile, phytochemicals from plants such as *Pongamia pinnata* and *Terminalia arjuna* provide eco-friendly options, leveraging natural insect-repellent or toxic

properties (7). Despite their potential, the combined efficacy of insecticides, synergists and plant extracts against RSW remains underexplored in India. This study aimed to assess the susceptibility of RSW populations from Bhubaneswar, India, to imidacloprid, spiromesifen and thiodicarb; evaluate their synergistic effects with PBO, diethyl malate (DEM) and propargyloxy phthalimide (PP); and investigate the efficacy of combining these insecticides with plant extracts from *Alstonia scholaris*, *Terminalia arjuna* and other local species. Conducted in 2024 at the Orissa University of Agriculture and Technology (OUAT), this research seeks to inform integrated pest management (IPM) strategies, addressing efficacy, resistance and sustainability for RSW control in India's agricultural systems.

Materials and Methods

Insect rearing

Rugose spiraling whitefly (RSW) populations were collected from infested coconut leaflets at the Orissa University of Agriculture and Technology (OUAT) Farm, Bhubaneswar, India. Rearing method was followed with modifications (8). Leaflets were maintained under ambient conditions (25-30 °C, 60-70 % relative humidity), tied to coconut saplings and covered with shade nets. Egg spirals appeared within 2-3 days, followed by crawlers (3-6 days), second instars (5-7 days), third instars (5-7 days), pupae (10-14 days) and adults (7 days). This lifecycle was continuously monitored to ensure a steady supply of test insects.

Insecticides and synergists

Three technical-grade insecticides-imidacloprid, spiromesifen and thiodicarb- were prepared in acetone using serial dilutions per (9). Synergists included piperonyl butoxide (PBO), diethyl malate (DEM) and propargyloxy phthalimide (PP). Non-toxic doses of synergists were determined through preliminary dose-response bioassays, with toxicity tested at concentrations of 500 ppm for piperonyl butoxide (PBO), diethyl malate (DEM) and plant extracts and 1000 ppm for propargyloxy phthalimide (PP) (9, 10) (Table 2).

Plant extract preparation

Fresh leaves of *Alstonia scholaris* and *Nyctanthes arbor-tristis*, bark of *Terminalia arjuna* and seeds of *Linum usitatissimum*, *Jatropha gossypiifolia*, *Sesamum indicum* and *Millettia pinnata* were collected from OUAT Farm or nearby areas. Plant parts were thoroughly washed with distilled water to remove contaminants, dried in an oven at 100 °C for 7 days to ensure complete dehydration, ground into a fine powder using a mechanical grinder and stored in airtight containers to prevent moisture absorption or degradation, following the protocol with modifications (11). Extracts were obtained through exhaustive extraction using a Soxhlet apparatus with hexane or petroleum ether (boiling range 60-70 °C) as the solvent, continuing until the siphon tube solvent appeared colourless. The solvent was subsequently removed under reduced pressure via rotary evaporation to yield concentrated crude extracts, which were then diluted with acetone or other suitable solvents as required for the bioassays.

Bioassay methods

To evaluate insecticide efficacy, leaf dip bioassays were performed. Coconut leaflets were immersed in test solutions for 10-15 min, removed and drained on absorbent paper before air-drying. The treated leaflets were then placed on moist tissue paper in Petri dishes to maintain humidity. A standardized number of rugose spiraling whitefly (*Aleurodicus rugioperculatus*) nymphs or adults were carefully transferred to the leaflets using a fine camel-hair brush. Petri dishes were sealed to ensure high humidity with minimal air exchange and incubated at 25-30 °C. Mortality was assessed after 24 hr and corrected using Abbott's formula (12). LC_{50} values were calculated from serial dilutions designed to produce mortality rates between 20 % and 80 %.

Synergism studies

Insecticides were combined with non-toxic doses of synergists (PBO, DEM, PP) or plant extracts and applied via leaf dip assay to nymphal populations observed under a trinocular microscope. Mortality was assessed after 24 hr. Synergistic ratios (SRs) were calculated as the ratio of the LC_{50} of the insecticide alone to the LC_{50} of the combination.

Statistical analysis

LC_{50} values and their 95 % fiducial limits were calculated using maximum likelihood estimation in Pololura software. Probit analysis, was applied to bioassay data to derive dosage-mortality regression lines and LC_{50} values (13). Model fit was verified using chi-square tests to ensure statistical reliability.

Results and Discussion

Insecticide susceptibility and seasonal variation

Leaf dip bioassays conducted in January and May 2024 on *Aleurodicus rugioperculatus* Martin nymphs from Bhubaneswar, India, revealed varying susceptibility to imidacloprid, spiromesifen and thiodicarb. In January, spiromesifen exhibited the highest efficacy (LC_{50} 0.431 ppm), followed by imidacloprid (0.458 ppm) and thiodicarb (0.528 ppm) (Table 1). By May, LC_{50} values increased, indicating seasonal resistance: imidacloprid to 0.628 ppm (37 % increase), spiromesifen to 0.576 ppm (34 % increase) and thiodicarb to 0.865 ppm (64 % increase) (Table 1). Slope values (±SE) ranged from 0.500 ± 0.350 to 1.001 ± 0.206, with chi-square values (0.987-0.999) confirming probit model fit. Spiromesifen's superior performance aligns with its action as a lipid biosynthesis inhibitor, targeting nymphal development more effectively than the neonicotinoid imidacloprid or the carbamate thiodicarb (14). Imidacloprid's moderate efficacy reflects growing

Table 1. Susceptibility of *Aleurodicus rugioperculatus* to insecticides (January and May 2024)

Insecticide	Period	LC_{50} (ppm)	FL (Lower–Upper)	Slope ± SE	Chi-square
Imidacloprid	Jan	0.458	0.127–1.456	0.697 ± 0.270	0.999
	May	0.628	0.227–1.462	1.001 ± 0.206	0.998
Spiromesifen	Jan	0.431	0.950–2.219	0.500 ± 0.350	0.987
	May	0.576	0.248–1.590	0.981 ± 0.206	0.999
Thiodicarb	Jan	0.528	0.198–1.406	0.931 ± 0.217	0.999
	May	0.865	0.327–2.291	0.866 ± 0.216	0.997

*Note: LC_{50} = Lethal concentration for 50 % mortality (ppm = parts per million); FL = Fiducial limits; SE = Standard error

resistance, consistent with reduced long-term control in whitefly populations due to sub-lethal effects like diminished fecundity (4). Thiodicarb's lower efficacy, with the largest LC_{50} increase, suggests rapid detoxification by enzymes like esterases (2, 5). The seasonal rise in LC_{50} values, particularly pronounced in warmer May conditions, mirrors findings linking increased whitefly resistance to drought-stressed plants, which enhance pest survival through concentrated sap (15). Recent genomic studies further confirm RSW's resistance, identifying expanded gene families for insecticide metabolism. Targeting nymphs proved effective due to their immobility, supporting strategies prioritizing developmental stages (16).

Synergistic effects with enzyme inhibitors

Synergism was evaluated by combining insecticides with non-toxic doses of piperonyl butoxide (PBO), diethyl malate (DEM) and propargyloxy phthalimide (PP), determined via preliminary bioassays (Table 2). For imidacloprid (May LC_{50} 0.628 ppm), PBO reduced the LC_{50} to 0.108 ppm (SR 3.990), DEM to 0.221 ppm (SR 1.950) and PP to 0.355 ppm (SR 1.214) (Table 3). Spiromesifen (May LC_{50} 0.576 ppm) showed the strongest synergism with PBO (LC_{50} 0.046 ppm, SR 9.956), followed by DEM (0.149 ppm, SR 3.070) and PP (0.175 ppm, SR 1.136). Thiodicarb (May LC_{50} 0.865 ppm) yielded LC_{50} values of 0.140 ppm with PBO (SR 3.771), 0.430 ppm with DEM (SR 1.227) and 0.504 ppm with PP (SR 1.047) (Table 3). Chi -square values (0.923-0.998) validated model fits. PBOs' marked enhancement, particularly with spiromesifen (92 % LC_{50} reduction), stems from its inhibition of cytochrome P450 enzymes, which detoxify insecticides in resistant whiteflies (6). DEM and PP were less effective, aligning with findings on hemipterans (17). These reductions support synergists' role in lowering doses and delaying resistance, contrasting with unabated resistance without such interventions (18, 19). PBOs' broad-spectrum enzyme inhibition makes it a valuable tool for resistance management, as recent studies on whitefly control emphasize (20).

Synergism with plant extracts

Insecticides at LC_{50} concentrations were combined with non-toxic doses of plant extracts (Table 2) and tested against *A. rugioperculatus* nymphs. Imidacloprid achieved 60 % mortality with sesamum oil (12/20 nymphs), 55 % with linseed oil (11/20), 50 % with *Nyctanthes arbor-tristis* leaf (10/20), 45 % with jatropha oil (9/20), 40 % with Pongamia oil (8/20), 35 % with *Terminalia arjuna* bark (7/20) and 25 % with *Alstonia scholaris* leaf (5/20) (Table 4). Spiromesifen recorded higher mortality: 75 % with Pongamia oil (15/20), 70 % with linseed oil (14/20), 65 % with jatropha oil (13/20), 50 % with sesamum oil (10/20), 40 % with *T. arjuna* bark and *N. arbor-tristis* leaf (8/20 each) and 30 % with *A. scholaris* leaf (6/20). Thiodicarb combinations yielded 70 % with Pongamia oil (14/20), 65 % with *T. arjuna* bark (13/20), 60 % with *N. arbor-tristis* leaf (12/20), 55 % with jatropha oil (11/20), 50 % with linseed oil (10/20), 45 % with *A. scholaris* leaf (9/20) and 40 % with sesamum oil (8/20) (Table 4). Control mortality (acetone) was 5 % (1/20). Pongamia oil's high efficacy (75 % with spiromesifen, 70 % with thiodicarb) reflects its bioactive compounds like karanjin, supporting botanical oils' insecticidal properties (7). Sesamum oil and *T. arjuna* bark also enhanced efficacy, aligning with recent findings on biopesticides for moderate RSW infestations (16). The leaf dip assay's success underscores topical applications against whiteflies (20, 21). However, efficacy varied by insecticide, suggesting compatibility differences needing further mechanistic study. Seasonal resistance trends highlight the need for adaptive

Table 2. Non-toxic dose fixation of synergists and plant extracts (500 ppm)

Treatment	Mortality (%)	Corrected Mortality (%)	Toxic/Non-Toxic
PBO	20	15.79	Toxic
DEM	25	21.05	Toxic
PP (1000 ppm)	50	47.37	Toxic
N. arbor-tristis	20	15.38	Toxic
A. scholaris	25	18.87	Toxic
T. arjuna	20	15.38	Toxic
Linseed oil	25	18.87	Toxic
Sesamum oil	35	25.93	Toxic
Pongamia oil	45	33.33	Toxic
Jatropha oil	30	22.22	Toxic

Table 3. Synergistic effects of insecticides with enzyme inhibitors (May 2024)

Insecticide + Synergist	LC_{50} (ppm)	FL (Lower–Upper)	SR	Chi-square
Imidacloprid + PBO	0.108	0.063–0.790	3.99	0.923
Imidacloprid + DEM	0.221	0.108–1.171	1.95	0.992
Imidacloprid + PP	0.355	0.606–0.811	1.214	0.978
Spiromesifen + PBO	0.046	0.061–1.985	9.956	0.983
Spiromesifen + DEM	0.149	0.075–1.595	3.07	0.995
Spiromesifen + PP	0.175	0.095–1.542	1.136	0.998
Thiodicarb + PBO	0.14	0.104–1.113	3.771	0.991
Thiodicarb + DEM	0.43	0.142–1.306	1.227	0.988
Thiodicarb + PP	0.504	0.384–1.457	1.047	0.973

Note: LC_{50} = Lethal concentration for 50 % mortality (ppm = parts per million); FL = Fiducial limits; SR = Synergistic ratio; PBO = Piperonyl butoxide; DEM = Diethyl malate; PP = Propargyloxy phthalimide

Table 4. Mortality from insecticides with plant extracts (May 2024)

Botanical	Imidacloprid (%)	Spiromesifen (%)	Thiodicarb (%)
N. arbor-tristis	50 (10/20)	40 (8/20)	60 (12/20)
A. scholaris	25 (5/20)	30 (6/20)	45 (9/20)
T. arjuna	35 (7/20)	40 (8/20)	65 (13/20)
Sesamum oil	60 (12/20)	50 (10/20)	40 (8/20)
Linseed oil	55 (11/20)	70 (14/20)	50 (10/20)
Jatropha oil	45 (9/20)	65 (13/20)	55 (11/20)
Pongamia oil	40 (8/20)	75 (15/20)	70 (14/20)
Control (acetone)	5 (1/20)	5 (1/20)	5 (1/20)

application timing, as dry conditions exacerbate infestations (15, 22).

Integrated control potential

Spiromesifen with PBO and Pongamia oil offers a potent, low-impact strategy, reducing LC_{50} by 92 % and achieving 75 % mortality. Integrating synergists and phytochemicals counters seasonal resistance (34-64 % LC_{50} increase) and reduces chemical reliance, supporting sustainable IPM. Field trials are critical to validate these findings under natural conditions, as laboratory results may vary with environmental factors like humidity and host diversity (2, 22).

Conclusion

This study establishes spiromesifen as the most effective insecticide against *Aleurodicus rugioperculatus*, with an LC_{50} of 0.431 ppm in January 2024, significantly enhanced by piperonyl butoxide (LC_{50} 0.046 ppm, SR 9.956) and Pongamia oil (75 % mortality). Combinations with synergists and plant extracts improved the moderate efficacy of imidacloprid and thiodicarb, reducing required doses by up to 92 % and countering seasonal resistance increases of 34-64 % by May. These findings highlight a sustainable, integrated approach to managing this invasive pest in India, particularly for coconut and banana crops. Field trials are essential to validate these laboratory results and refine practical applications for Indian agriculture.

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Authors' contributions

SR conducted the leaf dip bioassays, collected RSW samples, and drafted the initial manuscript. SSD conceived the study, designed the experimental framework, supervised the research and revised the manuscript. MKT participated in insect rearing, performed statistical analyses using Pololura software and contributed to data interpretation. MRK prepared the plant extracts, assisted in bioassay execution and provided insights on phytochemical applications. PM participated in the design of synergism studies, conducted probit analysis and finalized the statistical validation. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors have no conflicts of interest to declare.

Ethical issues: None

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