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**COMPARATIVE EFFICACY OF AZADIRACHTIN-BASED FORMULATION
IN MANAGING MEALYBUG (*Nipaecoccus nipae* MASKELL)
INFESTATION IN OIL PALM (*Elaeis guineensis* JACQ.)**

Mohamad Firdaus Ahmad^{1,2}, Roz Nuqman Rozabil¹ & Norhayu Asib^{1,2*}

¹DB Bioscience Sdn. Bhd.,
No 12, Jalan Perindustrian Mantin 2,
Kawasan Perindustrian Ringan Mantin,
Batu 8, 71700 Mantin, Negeri Sembilan.

²Department of Plant Protection,
Faculty of Agriculture,
Universiti Putra Malaysia,
43400 Serdang, Selangor Darul Ehsan
*Corresponding author: norhayuasib@upm.edu.my

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ABSTRACT

Mealybug (*Nipaecoccus nipae*) has recently emerged as a major pest in oil palm (*Elaeis guineensis* Jacq.) plantations in Southeast Asia, particularly in Sabah, Malaysia, where outbreaks have been associated with severe sooty mould development, impaired photosynthesis, and yield decline. Sustainable alternatives to synthetic insecticides are urgently needed to mitigate resistance development and environmental risks. This study evaluated the efficacy of an azadirachtin-based formulation combined with a plant-derived extract against *N. nipae* under laboratory and field conditions. Laboratory bioassays compared untreated control, azadirachtin alone, plant extract alone, and the formulation, DBMARK 30 EC with mortality assessed up to 96 h after treatment. Field trials were conducted at a commercial oil palm estate in Sabah using a complete randomized design (CRD) comparing DBMARK 30 EC, a commercial bioinsecticide, a chemical standard (imidacloprid + white oil), and an untreated control. Mealybug mortality, live population density, and visible sooty mould incidence were evaluated over 21 days after application (DAA). DBMARK 30 EC exhibited a strong synergistic effect in laboratory bioassays, achieving complete mortality by 96 h and significantly outperforming single-component treatments ($P < 0.05$). In the field, DBMARK 30 EC reduced live mealybug populations by up to 86.2% at 21 DAA, comparable to the chemical standard, while markedly reducing sooty mould coverage on treated fronds. These findings demonstrate that DBMARK 30 EC is an effective and environmentally compatible bioinsecticide with strong potential for integration into integrated pest management (IPM) programs in oil palm plantations.

Keywords: Oil palm; *Nipaecoccus nipae*; azadirachtin; botanical insecticide; integrated pest management

ABSTRAK

Koya putih (*Nipaecoccus nipae*) telah muncul sebagai perosak utama di ladang kelapa sawit (*Elaeis guineensis* Jacq.) di Asia Tenggara, khususnya di Sabah, Malaysia. Serangan perosak ini sering dikaitkan dengan pembentukan kulapuk jelaga yang teruk, penurunan kecekapan fotosintesis, serta kemerosotan hasil. Oleh itu, alternatif lestari kepada racun serangga sintetik amat diperlukan bagi mengurangkan risiko pembentukan rintangan dan kesan negatif terhadap alam sekitar. Kajian ini menilai keberkesanan DBMARK 30 EC, iaitu bioinsektisid botani bersinergi yang diformulasikan dengan azadiraktin dan ekstrak tumbuhan, terhadap *N. nipae* di bawah keadaan makmal dan lapangan. Ujian bioesei makmal membandingkan rawatan kawalan tanpa rawatan, azadiraktin sahaja, ekstrak tumbuhan sahaja dan formulasi DBMARK 30 EC, dengan kadar kematian dinilai sehingga 96 jam selepas rawatan. Percubaan lapangan dijalankan di sebuah ladang kelapa sawit komersial di Sabah menggunakan reka bentuk rawak lengkap (CRD) yang membandingkan DBMARK 30 EC, bioinsektisid komersial, piawaian kimia (imidacloprid + minyak putih) dan kawalan tanpa rawatan. Kadar kematian koya putih, kepadatan populasi hidup, serta kejadian kulapuk jelaga yang dapat dilihat dinilai sehingga 21 hari selepas aplikasi (HSA). DBMARK 30 EC menunjukkan kesan sinergistik yang kuat dalam ujian makmal dengan mencapai kematian penuh pada 96 jam dan menunjukkan prestasi yang jauh lebih baik berbanding rawatan komponen tunggal ($P < 0.05$). Dalam ujian lapangan, DBMARK 30 EC berjaya mengurangkan populasi koya putih hidup sehingga 86.2% pada 21 HSA, setanding dengan piawaian kimia, di samping mengurangkan liputan kulapuk jelaga dengan ketara pada pelepah yang dirawat. Dapatan ini membuktikan bahawa DBMARK 30 EC merupakan bioinsektisid yang berkesan dan mesra alam, serta berpotensi tinggi untuk diintegrasikan ke dalam program Pengurusan Perosak Bersepadu (IPM) di ladang kelapa sawit.

Kata kunci: Kelapa sawit; *Nipaecoccus nipae*; azadiraktin; insektisid botani; pengurusan perosak bersepadu

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is a major economic crop in Malaysia and other tropical regions, contributing significantly to global vegetable oil production. However, productivity is increasingly threatened by emerging pest outbreaks, including sap-sucking insects such as mealybugs (Hassaan et al. 2025). In recent years, *Nipaecoccus nipae* has been reported as an important pest in oil palm plantations in Sabah, where severe infestations have resulted in widespread sooty mould development and measurable yield losses (Zin et al. 2023).

Mealybugs feed by inserting their stylets into phloem tissues, extracting plant sap and excreting excess sugars in the form of honeydew (Othman et al. 2025). This honeydew serves as a substrate for sooty mould fungi (*Capnodium* spp.), which form a black coating on leaf surfaces and reduce photosynthetically active radiation (PAR) absorption (Si et al. 2025). Previous studies have reported reductions of 58–78% in photosynthetic activity and Fresh Fruit Bunch (FFB) yield losses ranging from 5–10% in heavily infested palms ((Zin et al. 2023). In addition, mutualistic relationships with ants where ants tend mealybugs for honeydew provide the pests with protection from natural enemies, further exacerbating infestations (Styrsky & Eubanks 2006).

Various control strategies have been investigated to manage mealybug infestations in oil palm, including the use of synthetic insecticides such as neonicotinoids (e.g., imidacloprid), organophosphates, and pyrethroids. While these compounds have demonstrated effectiveness

in reducing pest populations, their repeated application has led to several limitations, including the development of insecticide resistance, resurgence of secondary pests, and negative impacts on beneficial arthropods and environmental health (Mwanauta et al. 2023). Furthermore, increasing regulatory restrictions and sustainability requirements, particularly under Malaysian Sustainable Palm Oil (MSPO) standards, have emphasized the need for safer and environmentally compatible pest control alternatives.

Botanical insecticides, particularly those derived from neem (*Azadirachta indica*), have gained attention as viable alternatives. Azadirachtin, the primary bioactive compound in neem, has been extensively studied for its multiple modes of action, including antifeedant activity, inhibition of molting, disruption of hormonal regulation, and suppression of reproduction in various insect pests (Isman 2020). Previous studies have demonstrated the efficacy of azadirachtin-based formulations against a range of sap-sucking insects, including aphids, whiteflies, and mealybugs, with relatively low toxicity to non-target organisms and natural enemies. However, variability in efficacy due to formulation stability, environmental conditions, and pest biology remains a challenge.

Chemical insecticides, particularly neonicotinoids such as imidacloprid, are commonly used for mealybug control. However, concerns regarding insecticide resistance, environmental contamination, and adverse effects on non-target organisms have increased interest in botanical and bio-based alternatives (Mwanauta et al. 2023). Although *Nipaecoccus nipae* infestation in oil palm is relatively recent, resistance to commonly used insecticides has been widely reported in other sap-sucking insect species, suggesting the potential risk of resistance development under continued chemical use (Mwanauta et al. 2023).

Azadirachtin, a limonoid derived from the neem tree (*Azadirachta indica*), is widely recognized for its antifeedant, growth-regulating, and reproductive inhibitory effects on insects, primarily through disruption of hormonal regulation and molting processes (Isman 2020). An azadirachtin-based formulation combined with a plant-derived extract was evaluated in this study. The formulation is intended to improve insecticidal performance while maintaining lower environmental impact compared to conventional synthetic insecticides. This study aimed to evaluate the laboratory and field efficacy of the formulation against *N. nipae* and to assess its potential suitability for integration into Integrated Pest Management (IPM) programs in oil palm plantations, in line with Malaysian Sustainable Palm Oil (MSPO) standards (Zhou et al. 2024).

MATERIALS AND METHODS

Location of Study

Field trials were conducted at Segaria Oil Palm Estate (4.4790° N, 118.6150° E), where active infestations of *Nipaecoccus nipae* were observed. Selected oil palm trees exhibited visible infestation symptoms, including honeydew accumulation and extensive sooty mould development on fronds. Laboratory bioassays were conducted at the Toxicology Laboratory, Department of Protection, Faculty of Agriculture, Universiti Putra Malaysia.

Preparation of *N. nipae* Rearing for Insecticidal Testing

Nipaecoccus nipae individuals were collected from naturally infested oil palm plantations located in Terengganu. Additional field observations confirmed the presence of infestations in nearby pineapple cropping areas, indicating a broader host association within the sampling

region. Collected specimens were transported to the laboratory and reared prior to bioassay experiments to ensure uniform physiological condition.

In the laboratory, the mealybugs were maintained under ambient room temperature conditions of $27\pm 2^{\circ}\text{C}$, which is suitable for normal development of tropical sap-sucking insects. Rearing was conducted inside a plastic aquarium measuring $40\text{ cm} \times 26\text{ cm} \times 22\text{ cm}$, following standard procedures for sap-sucking insects to minimize handling stress and variability (Isman 2020). Fresh oil palm leaf material was provided as the primary food source and replaced regularly to maintain nutritional quality and prevent desiccation. The leaves were carefully cleaned and inspected before use to avoid contamination by predators, pathogens, or other arthropods. The rearing setup was monitored daily to ensure adequate humidity, ventilation, and food availability, allowing the insects to develop under stable and controlled conditions prior to treatment exposure.

Laboratory Evaluation of Azadirachtin and Botanical Extract Formulations Against *Nipaecoccus nipae*

Laboratory bioassays were performed using a leaf-spray method following Food and Agriculture Organization guidelines (Zeni et al. 2021) to evaluate contact and ingestion toxicity of botanical insecticides. Oil palm leaf discs were sprayed with test solutions at a concentration of 5 mL formulation per liter of water using a hand-held sprayer until runoff and allowed to air-dry at $25\pm 2^{\circ}\text{C}$. Four treatments were evaluated: untreated control (distilled water), azadirachtin formulation (based on manufacturer-recommended active ingredient concentration), plant extract formulation, and a combination formulation of azadirachtin with plant extract B (DBMARK 30 EC; emulsifiable concentrate). The experiment was arranged in a completely randomized design (CRD), where each treatment consisted of five replicates, and each replicate consisted of one Petri dish containing 10 mealybugs (mixed adults and nymphs). After drying, treated leaf discs were placed in sterile Petri dishes, and insects were carefully introduced onto the leaf surface. Mortality was recorded at 24, 48, 72, and 96 hours after treatment (HAT), and data were corrected using Abbott's formula when control mortality occurred (Abbott 1925).

Field Evaluation of Azadirachtin, Botanical Extract Formulation, and Imidacloprid Against *Nipaecoccus nipae* in Oil Palm Plantation

The field experiment was conducted in Semporna using a completely randomized design (CRD) comprising four treatments with four replicates. Each replicate consisted of 35 oil palm trees, resulting in a total of 140 palms per treatment. The treatments evaluated were: (i) untreated control, (ii) DBMARK 30 EC (azadirachtin + plant extract formulation), (iii) a commercial bioinsecticide based on neem oil (azadirachtin), and (iv) a combination of imidacloprid (18.3%) with white oil (72%).

All treatments were applied using a mist blower fitted with a No. 4 nozzle. Spraying was directed beneath the oil palm fronds to ensure thorough coverage of the canopy, particularly targeting the infestation sites of *Nipaecoccus nipae*. Applications were carried out at the manufacturer-recommended rate of 750 mL ha^{-1} to ensure consistency and uniform distribution across treatments. Sampling was conducted following a standardized protocol. Three fronds per palm were randomly selected from the middle canopy at each sampling interval. Mealybug populations were assessed within a fixed observation area on each frond to maintain consistency across all observations.

Population assessments were carried out prior to treatment application (pre-census) and subsequently at 7, 14, and 21 days after application (DAA). The number of live mealybugs was recorded, and percentage mortality was calculated to evaluate treatment efficacy. The primary parameter measured in this study was the mortality of *Nipaecoccus nipae* populations following treatment application.

Data Analysis

All data were analyzed using SAS version 9.4. Laboratory mortality data were subjected to one-way analysis of variance (ANOVA) under a completely randomized design, while field data were analyzed using repeated measures ANOVA. Treatment means were separated using Tukey's Honest Significant Difference (HSD) test at a significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Bioassay Mortality Assessment

The laboratory bioassay demonstrated a clear time-dependent increase in *Nipaecoccus nipae* mortality across all treated groups. Among the treatments, the combined formulation (DBMARK 30 EC) consistently resulted in higher mortality compared to azadirachtin or plant extract B applied individually. At 24 hours after treatment, DBMARK 30 EC recorded a mean mortality of 3.33 ± 0.88 , which was significantly higher than azadirachtin (1.00 ± 0.00) and plant extract B (0.67 ± 0.33) ($P < 0.05$). This trend continued at 48 and 72 hours, with DBMARK 30 EC maintaining the highest mortality values across observation periods.

By 96 hours after treatment, DBMARK 30 EC achieved complete mortality (10.00 ± 0.00), whereas azadirachtin and plant extract B reached 5.00 ± 0.00 and 4.33 ± 1.45 , respectively. Mortality in the untreated control remained minimal throughout the experiment, indicating that the observed effects were attributable to the treatments rather than natural causes. The higher mortality observed in the combined formulation suggests that the interaction between azadirachtin and plant extract B may contribute to improved insecticidal performance compared to individual applications. However, as no formal synergistic analysis was conducted, this effect is interpreted as a complementary or additive response rather than confirmed synergism.

Azadirachtin is well-documented as an insect growth regulator (IGR) and antifeedant that disrupts hormonal regulation and molting processes (Isman 2020; Mordue & Nisbet 2000). Previous studies indicate that azadirachtin-based biopesticides predominantly affect the prepupal and pupal stages by interfering with endocrine signaling and ecdysone synthesis (Isman 2020; Mordue & Nisbet 2000).

When combined with plant extract B, which likely facilitates cuticular disruption, the bioavailability and penetration of azadirachtin are substantially enhanced. This interaction is supported by the chronological morphological degeneration observed in Figure 3, progressing from waxy filament disruption to complete melanization of *N. nipae*. These observations suggest that DBMARK 30 EC effectively penetrates and compromises the protective waxy layer of the mealybug. Such effects are consistent with reports that oil or lipophilic botanical formulations disrupt the waxy coatings of soft-bodied insects such as *Maconellicoccus hirsutus* and *Phenacoccus solenopsis*, leading to rapid desiccation and mortality (Tong et al. 2022).

Table 1. Mean mortality of *N. nipae* in laboratory bioassay following different treatments

Treatment	24 Hours	48 Hours	72 Hours	96 Hours
Azadiractin	1.00±0.00b	2.67±0.33b	4.67±0.88b	5.00±0.00b
Plant extract B	0.67±0.3b	1.33±0.33c	2.33±0.88c	4.33±1.45b
Azadiractin+ B (DBMARK 30 EC)	3.33±0.88 a	5.00±1.54a	7.70±0.33a	10.00±0.00a
Control	0.33±0.33c	0.67±0.33d	1.00±0.00d	1.33±0.33c

Note: Means followed by the same letter in the same column are not significantly different according to the Turkey ($P \geq 0.05$). The *P*-value is for the analysis of variance (ANOVA). ± is standard error.

Comparative Efficacy of Insecticidal Treatments on Mealybug Mortality at the Field

At 21 days after application (DAA), differences in treatment efficacy were evident under field conditions. The imidacloprid + white oil treatment produced the highest mortality (73.7%), followed by DBMARK 30 EC (65.6%). Statistical analysis indicated that both treatments were within a similar performance group, suggesting comparable levels of effectiveness within the context of this study.

The commercial organic insecticide showed moderate efficacy, with a mortality rate of 42.6%, while the untreated control remained low at 10.9%. These results confirm that all active treatments contributed to population reduction relative to the control. Although DBMARK 30 EC did not exceed the efficacy of the synthetic standard, its performance under field conditions indicates that botanical-based formulations can provide substantial suppression of *N. nipae*. The differences observed between treatments may be influenced by variations in mode of action, persistence, and environmental factors such as rainfall and temperature.

The results may be relevant in the context of sustainable oil palm management, particularly where reduced reliance on synthetic insecticides is encouraged. Botanical pesticides are generally recognized for their biodegradability and lower toxicity to non-target organisms (Saikumar et al. 2025)). However, further studies are required to confirm long-term effectiveness and consistency under different field conditions.

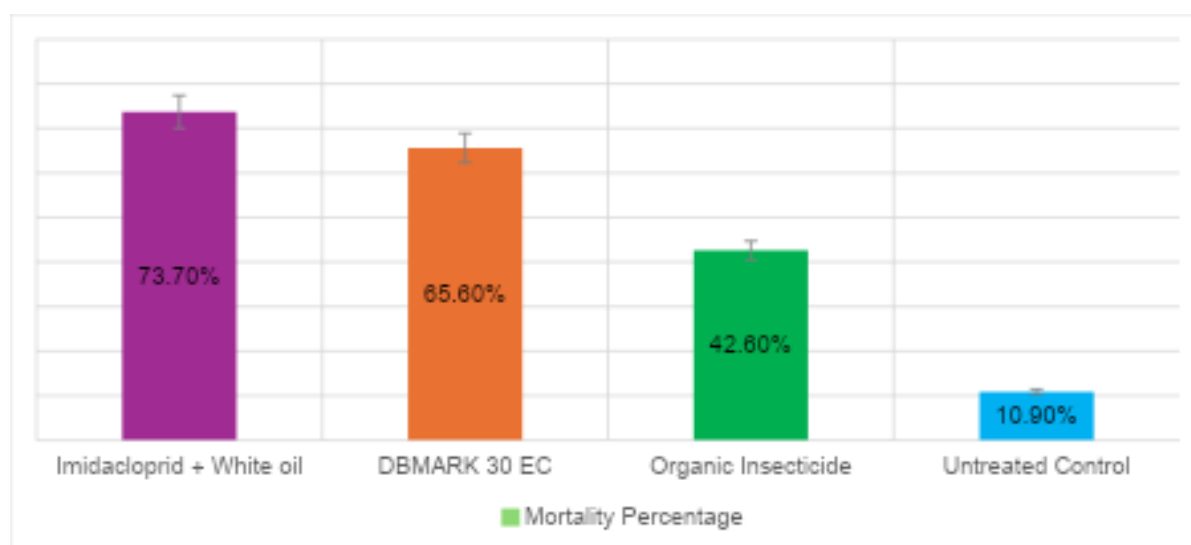


Figure 1. Comparative efficacy of selected insecticidal formulations on mealybug mortality at 21 days after application (DAA)

Live Population Dynamics of *N. nipae* at Field

Field population assessments showed a consistent decline in *N. nipae* populations following treatment application. DBMARK 30 EC reduced the mean population from 1,329 individuals at pre-treatment to 183 individuals at 21 DAA, corresponding to an 86.2% reduction. This reduction was comparable to that observed for the imidacloprid + white oil treatment, which achieved an 83.3% reduction over the same period.

Temporal trends indicated that DBMARK 30 EC maintained a steady decline in population throughout the observation period, suggesting sustained activity over time. In contrast, the commercial organic insecticide showed a slower and less consistent reduction, with indications of population stabilization after 14 DAA. The untreated control maintained relatively stable population levels, supporting the conclusion that reductions in treated plots were due to treatment effects.

These findings indicate that DBMARK 30 EC can effectively reduce *N. nipae* populations under field conditions, with performance comparable to a conventional insecticide within the timeframe of this study. However, variability in initial population densities and environmental conditions should be considered when interpreting these results. Within the Malaysian oil palm context, *N. nipae* infestation is associated with sooty mould development that can affect photosynthetic efficiency and yield (Zin et al. 2023).

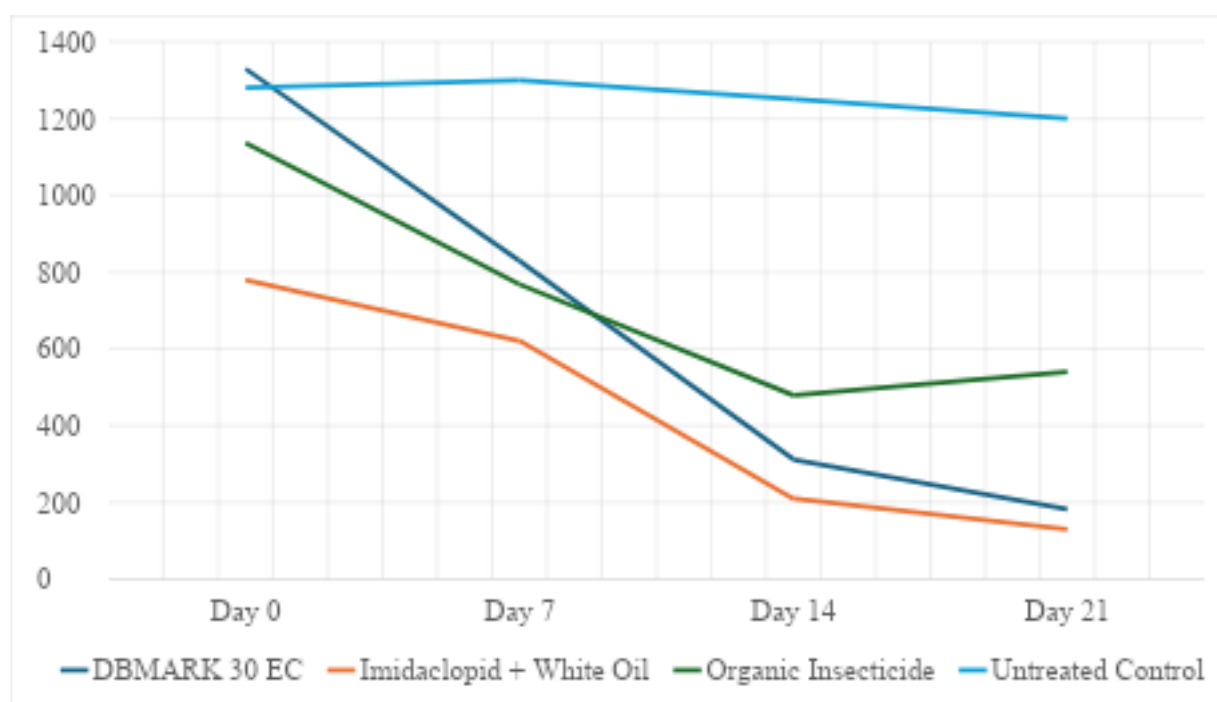


Figure 2. Comparative efficacy of DBMARK 30 EC on live mealybug (*N. nipae*) population dynamics over a 21-day evaluation period

Morphological Degeneration and Necrosis of *N. nipae* post-DBMARK 30 EC Exposure

Visual observations (Figure 3) showed progressive morphological changes in *N. nipae* following treatment with DBMARK 30 EC. Initially, insects exhibited intact waxy coatings typical of healthy individuals. Following exposure, gradual deterioration was observed, including disruption of wax structures, discoloration, and eventual desiccation. By Day 7,

treated insects showed complete structural collapse. These observations provide qualitative support for the recorded mortality data, although they do not confirm specific physiological mechanisms.



Figure 3. Chronological morphological degeneration of *N. nipae* before spray and during 7 days after treatment with DBMARK 30 EC

Efficacy of DBMARK 30 EC in Controlling Sooty Mould on Palm Leaflet

Treatment with DBMARK 30 EC was associated with a visible reduction in sooty mould on oil palm fronds between 14 and 21 DAA. The reduction in fungal coverage corresponded with decreased mealybug populations, suggesting that suppression of honeydew-producing insects contributed to the observed improvement in leaf condition. In contrast, untreated palms remained heavily covered with sooty mould, indicating continued pest activity. The relationship between mealybug infestation and sooty mould development is well established, as honeydew excretion supports fungal growth

The observed improvement in leaf condition may have implications for photosynthetic efficiency and plant performance, as heavy sooty mould coverage can interfere with normal physiological processes (Zin et al. 2023; Pinnamaneni & Potineni 2023). However, direct measurements were not conducted in this study, and further investigation is required.

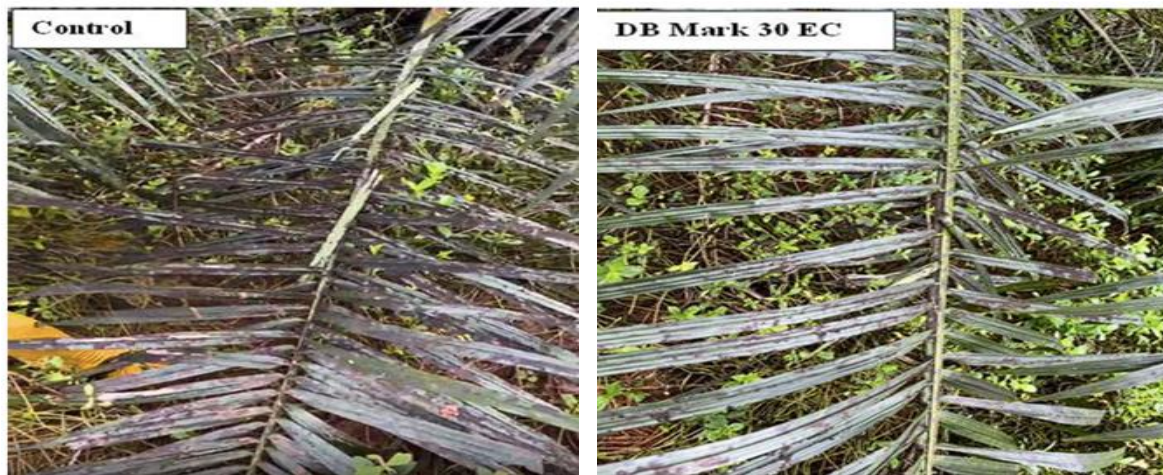


Figure 4. Comparison of sooty mould infestation on palm fronds between control and treated groups. The DB Mark 30 EC treated frond shows a significant reduction in black fungal coating and recovery of green leaf tissue at 14–21 DAA, while the Control displays persistent, heavy sooty mould coverage

CONCLUSION

The findings of this study indicate that DBMARK 30 EC was effective in reducing *Nipaecoccus nipae* populations under both laboratory and field conditions. The combined formulation demonstrated higher efficacy than individual components in laboratory bioassays, while in field trials its performance was comparable to that of the imidacloprid-based treatment within the duration of the study. The reduction in mealybug populations was associated with a visible decrease in sooty mould coverage on oil palm fronds, suggesting a link between pest suppression and improvement in leaf condition. However, the effects on photosynthetic efficiency and yield were not directly measured and therefore cannot be conclusively determined. These results suggest that DBMARK 30 EC has potential for use as part of pest management strategies in oil palm plantations. Nevertheless, further studies are required to evaluate its long-term effectiveness, impact on non-target organisms, and performance under different environmental and field conditions.

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AUTHORS DECLARATION

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Conflict of Interest

No conflict of interest declared by authors.

Data Availability Statement

All data generated or analyzed during this study are included in this published article.

Authors' Contributions

The authors' contributions are as follows: MFA: Data curation, investigation, methodology, formal analysis and writing original draft; MFA: Writing original draft and writing review; NA: Conceptualization, data curation, formal analysis, funding acquisition, project administration, resources, supervision, validation and writing review; RNR: Funding acquisition, project administration, resources, supervision and validation

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