

Field Evaluation of Thyme Oil 40 EC Formulation against Powdery Mildew (*Erysiphe necator*) of Grapevine (*Vitis vinifera* L.)

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Abstract— Grape (*Vitis vinifera* L.) production is severely constrained by powdery mildew, caused by the obligate biotrophic fungus *Erysiphe necator*, which affects leaves, shoots, flowers and berries and reduces yield and fruit quality. Field experiments were conducted during two consecutive seasons (2022-2023 and 2023-2024) at Thondamuthur, Coimbatore district, Tamil Nadu, on Thompson Seedless grapevines in a randomized block design with seven treatments and four replications, to evaluate the bio efficacy of Thyme oil 40 EC formulation at 0.025, 0.050, 0.075 and 0.1 per cent against powdery mildew, in comparison with Hexaconazole 5% EC and Thiophanate methyl 70% WP and an untreated control. In the pooled analysis of both seasons, Thyme oil 40 EC at 0.1 per cent recorded the lowest percent disease index of 5.70, 6.02 and 5.52 after the first, second and third sprays and 84.23 per cent disease reduction over control, closely comparable with Hexaconazole 5% EC at 84.90 per cent and superior to Thiophanate methyl 70% WP at 82.60 per cent. Thyme oil 40 EC at 0.1 per cent also recorded the highest pooled yield of 7537.50 kg per acre and the highest benefit: cost ratio of 2.95 among all treatments, and no phytotoxic symptoms were observed at any concentration tested. These findings indicate that Thyme oil 40 EC at 0.1 per cent is functionally comparable to standard fungicides and can be considered a viable, economically advantageous prophylactic option for the management of grape powdery mildew.

Keywords— Grapevine; Powdery Mildew; Thyme Oil; *Erysiphe necator*; Bioefficacy; Yield.

I. INTRODUCTION

Grape (*Vitis vinifera* L.) is one of the most widely cultivated and economically important horticultural crops worldwide (Amores-Arrocha and Jiménez-Cantizano, 2025). Grapes are cultivated across temperate, subtropical and tropical regions and are utilized as table grapes and for the production of raisins, juice, wine and other value-added products (Alston and Sambucci, 2019; Khadatkhar *et al.*, 2025). Grapes also possess considerable nutritional and phytochemical value because they contain sugars, organic acids, vitamins, flavonoids, anthocyanins, tannins and bioactive polyphenols such as resveratrol (Fortes and Pais, 2016; Prathiksha and Hegde, 2022; Singh *et al.*, 2023). In addition to their nutritional importance, grape production has substantial economic significance and represents one of the world's major high-value horticultural sectors (Alston and Sambucci, 2019; Amores-Arrocha and Jiménez-Cantizano, 2025).

Despite its economic importance, grape production is constrained by several fungal diseases and other biotic stresses (Malviya *et al.*, 2022). Among these, powdery mildew caused by the obligate biotrophic fungus *Erysiphe necator* is one of the most widespread and economically important diseases of grapevine worldwide (Elfar *et al.*, 2025). The pathogen can infect all green tissues of grapevine, including leaves, shoots, flowers and berries. Severe infections can result in flower and berry damage, reduced yield, berry splitting or cracking and deterioration of grape and wine quality. Most cultivated *V. vinifera* varieties are

moderately to highly susceptible to *E. necator*, making effective disease management essential for maintaining grape yield and quality (Kunova *et al.*, 2021).

Management of grape powdery mildew relies predominantly on preventive and repeated fungicide applications throughout the growing season. Under conditions favorable for epidemic development, 10–20 fungicide applications may be required, placing viticulture among the cropping systems with high fungicide-use intensity. Sulfur remains an important protectant fungicide, while several site-specific fungicides, including demethylation inhibitors and quinone outside inhibitors, have also been used effectively against *E. necator* (Reuveni, 2001; Kunova *et al.*, 2021). However, intensive and repeated use of fungicides can increase the risk of resistance development in *E. necator* populations and can complicate long-term disease management (Kunova *et al.*, 2021). The continued need for effective disease suppression therefore highlights the importance of integrating alternative or complementary disease-management approaches with conventional fungicide programmes.

Plant-derived essential oils have attracted increasing attention as potential tools for sustainable plant disease management because they contain diverse bioactive volatile compounds with antimicrobial properties. Essential oils commonly contain terpenes, terpenoids and phenolic compounds that may act against microorganisms through multiple mechanisms. For example, oregano essential oil contains carvacrol and thymol among its major constituents (Han *et al.*, 2017). Importantly, the potential of essential oils against grape powdery mildew has also been demonstrated. Dhaliwal *et al.* (2002) reported antifungal activity of several essential oils against *Uncinula necator*, the former taxonomic name associated with *E. necator*, causing powdery mildew of grapevine. More recently, Reuveni *et al.* (2023) demonstrated that a hybrid formulation containing tea tree oil and difenoconazole provided high levels of field control of grape powdery mildew, indicating the potential of essential-oil-based formulations as components of integrated disease-management programmes.

Thyme oil is of particular interest because its major bioactive constituents, especially thymol and related phenolic compounds, are associated with antifungal activity. The antifungal action of thymol and carvacrol is generally attributed to disruption of fungal cell membranes, interference with ergosterol biosynthesis, inhibition of conidial germination, and disruption of mitochondrial function. However, compared with conventional fungicides and some other essential-oil-based products, field-based information on the efficacy of formulated thyme oil against grape powdery mildew remains limited. Therefore, evaluation of formulated thyme oil under vineyard conditions is warranted to determine its effectiveness in disease suppression, its potential contribution to yield protection and its phytotoxicity to grapevines.

Accordingly, the present investigation was undertaken to evaluate the bioefficacy of Thyme oil 40 EC formulation at different concentrations against powdery mildew of grapevine under field conditions during two successive seasons. The study further compared the efficacy of the thyme oil formulation with Hexaconazole 5% EC and Thiophanate-methyl 70% WP and assessed its effects on disease suppression, yield and phytotoxicity.

II. MATERIALS AND METHODS

Field experiments were conducted during two consecutive seasons, 2022–2023 and 2023–2024, at Thondamuthur, Coimbatore district, Tamil Nadu, India, using Thompson Seedless grapevines.

2.1 Experimental Site and Plant Material

The experimental site is located in a semi-arid tropical region with an average annual rainfall of approximately 700 mm, and the soil is a sandy loam. Existing vines (approximately 6 years old) were spaced at 3 m × 2 m and trained on a Y-trellis system. Vines were pruned on 15 October 2022 (Season I) and 20 October 2023 (Season II), and treatments were initiated at the five-leaf stage (approximately 14 days after pruning). Recommended agronomic practices (irrigation, fertilizer, weeding) were followed uniformly for all treatments. Weather parameters (mean temperature, relative humidity, and rainfall) were recorded during the trial periods; the mean maximum temperatures ranged from 28.5–32.6 °C in Season I and 29.1–33.4 °C in Season II, and mean relative humidity ranged from 62–78% in both seasons.

2.2 Experimental Design and Treatments

The experiment was laid out in a randomized block design with seven treatments and four replications, each replication consisting of two vines. Thyme oil was selected for field evaluation based on prior *in vitro* screening, in which it completely

inhibited conidial germination, and it was formulated as a 40 EC product for field application. Seven treatments were tested: Thyme oil 40 EC at 0.025, 0.050, 0.075 and 0.1%; Hexaconazole 5% EC at 0.1%; Thiophanate Methyl 70% WP at 0.1%; and an untreated control.

TABLE 1
TREATMENT DETAILS

T.No	Treatment	Dose
T1	Thyme oil 40 EC formulation @ 0.025% conc.	0.025 ml/lit
T2	Thyme oil 40 EC formulation @ 0.050% conc.	0.050 ml/lit
T3	Thyme oil 40 EC formulation @ 0.075% conc.	0.075 ml/lit
T4	Thyme oil 40 EC formulation @ 0.1% conc.	0.1 ml/lit (1 ml of formulation per litre of water)
T5	Hexaconazole 5% EC	1 ml/lit
T6	Thiophanate Methyl 70% WP	1 ml/lit
T7	Control	—

2.3 Preparation and Application of Thyme Oil 40 EC Formulation

The Thyme oil 40 EC formulation was applied as a foliar spray at the assigned concentrations as a prophylactic treatment starting from the five-leaf stage after pruning, with three sprays given at 7-day intervals. Hexaconazole 5% EC and Thiophanate Methyl 70% WP were sprayed after the appearance of powdery mildew symptoms for comparison with the prophylactic Thyme oil treatments. **It is acknowledged that this represents a comparison of two management strategies (prophylactic biofungicide vs. symptomatic fungicide) rather than a strictly controlled timing comparison; the results are therefore interpreted as parallel evaluations under field conditions.**

Sprays were applied using a knapsack sprayer with a cone nozzle at approximately 500 L of spray solution per acre, ensuring thorough coverage of both leaf surfaces. Application was carried out in the early morning (06:00–09:00 h) to minimize evaporative loss and to reduce the possibility of phytotoxicity under high temperatures.

2.4 Assessment of Powdery Mildew and Disease Reduction

Powdery mildew incidence was assessed using a 0–7 disease rating scale (Table 2), and disease severity was expressed as Percent Disease Index (PDI). Disease observations were recorded after each spray and percent disease reduction over the untreated control was calculated.

TABLE 2
DISEASE RATING SCALE (0–7) FOR GRAPE POWDERY MILDEW

Rating	Description
0	No powdery mildew symptoms on leaves
1	Minute powdery spots covering <1% of leaf area
2	Powdery growth covering 1–5% of leaf area
3	Powdery growth covering 6–10% of leaf area
4	Powdery growth covering 11–25% of leaf area
5	Powdery growth covering 26–50% of leaf area
6	Powdery growth covering 51–75% of leaf area
7	Powdery growth covering >75% of leaf area

Percent Disease Index (PDI) was calculated using the formula:

$$\sum \frac{\text{Number of leaves in each rating} \times \text{Disease rating}}{\text{Total number of leaves observed} \times \text{Maximum disease rating}} \times 100 \quad (1)$$

$$\text{Disease reduction over control (\%)} = \frac{\text{PDI of control} - \text{PDI of treatment}}{\text{PDI of control}} \times 100 \quad (2)$$

Observations were taken 10 days after each spray in Season I and 7 days after each spray in Season II. The difference in observation intervals between seasons reflected scheduling constraints; the effects of this difference on the comparability of results between seasons are acknowledged in the Limitations section.

2.5 Yield and Phytotoxicity Assessment

Yield was recorded for each treatment and expressed on an area basis (kg/ac). Phytotoxicity was observed before spray and at 1, 3, 5, 7 and 10 days after each spray by recording leaf injury on tips and surface, wilting, vein clearing, necrosis, epinasty and hyponasty. A 0–10 visual scale was used, where 0 = no injury, 1 = 1–10% leaf area affected, 2 = 11–20%, 3 = 21–30%, 4 = 31–40%, 5 = 41–50%, 6 = 51–60%, 7 = 61–70%, 8 = 71–80%, 9 = 81–90%, and 10 = 91–100%.

2.6 Economic Analysis

The benefit:cost (B:C) ratio was calculated for each treatment as follows:

$$\text{B:C ratio} = \frac{\text{Gross return}}{\text{Total cost of production}} \quad (3)$$

Gross return was calculated from the marketable yield of each treatment using a farm-gate price of Rs. 60/kg (the prevailing price during the trial period). Total cost of production included the costs of the biofungicide or fungicide, labour, sprayer operation, and the shared costs of all common cultural practices.

2.7 Statistical Analysis and Pooled Analysis

The data were statistically analyzed using one-way analysis of variance (ANOVA) for a randomized block design. Treatment means were compared using the standard error of mean (S.Em±) and critical difference (CD) at the 5% level of significance. Data from both seasons were also pooled, and the pooled data were analysed with season as an additional factor; treatment means were compared using S.Em and CD at the 5% level. All statistical analyses were performed using SPSS version 26.0.

III. RESULTS AND DISCUSSION

Powdery mildew is one of the most economically important diseases of grapevine and can substantially reduce yield and fruit quality if left unmanaged (Bustamante *et al.*, 2025; Elfar *et al.*, 2026). In the present study, prophylactic application of Thyme oil 40 EC suppressed disease development, improved yield, and showed no visible phytotoxicity, which is consistent with broader evidence that essential-oil-based treatments can act as effective alternatives or complements to conventional fungicides in grape and other crop pathosystems (Reuveni *et al.*, 2023; Soppelsa *et al.*, 2024; Saleh *et al.*, 2024).

3.1 Season I

During the 2022–2023 season, all treatments reduced powdery mildew severity compared with the untreated control (Table 3). The untreated control recorded the highest PDI values of 14.02, 18.62, and 24.90 at the first, second, and third observations, respectively, and also produced the lowest yield of 4100 kg/ac.

Among the Thyme oil treatments, the 0.1% concentration was the most effective, recording PDI values of 6.15, 7.28, and 8.13 at successive observations, with 67.32% disease reduction over control and the highest yield of 7525 kg/ac. Lower concentrations were less effective, indicating a clear dose response, with yield increasing from 6100 kg/ac at 0.025% to 7150 kg/ac at 0.075% and 7525 kg/ac at 0.1%, a pattern that matches reports that higher thyme-derived doses often improve disease suppression and yield response (Saltos-Rezabala *et al.*, 2022; Soppelsa *et al.*, 2024).

Hexaconazole 5% EC recorded slightly lower PDI values of 5.50, 6.29, and 7.54 and 70.00% disease reduction over control, with a yield of 7515 kg/ac, while Thiophanate methyl 70% WP recorded PDI values of 6.40, 8.90, and 9.10, 63.50% disease reduction, and a yield of 7502 kg/ac. The near-equivalence of Thyme oil 40 EC at 0.1% and Hexaconazole in yield, despite marginally lower disease suppression by Thyme oil in the first season, agrees with reports that preventive essential-oil treatments can perform similarly to fungicide standards under field conditions (Saha *et al.*, 2023; Vijaykumar *et al.*, 2022; Soppelsa *et al.*, 2024).

TABLE 3
EVALUATION OF BIOEFFICACY OF THYME OIL 40 EC FORMULATION AGAINST POWDERY MILDEW OF GRAPES (SEASON I, 2023)

T.No	Treatment	Dose (ml/L)	PDI 10 days after 1st spray	PDI 10 days after 2nd spray	PDI 10 days after 3rd spray	% Reduction over control	Yield (kg/ac)
T1	Thyme oil 40 EC @ 0.025%	0.025	7.09	8.3	9.46	62	6100
T2	Thyme oil 40 EC @ 0.050%	0.05	7.28	8.51	9.21	63	6300
T3	Thyme oil 40 EC @ 0.075%	0.075	6.83	8.75	9.1	64	7150
T4	Thyme oil 40 EC @ 0.1%	0.1	6.15	7.28	8.13	67.32	7525
T5	Hexaconazole 5% EC	1	5.5	6.29	7.54	70	7515
T6	Thiophanate Methyl 70% WP	1	6.4	8.9	9.1	63.5	7502
T7	Control	–	14.02	18.62	24.9	0	4100
	S.Em±		0.09	0.14	0.18	–	74.51
	CD (P=0.05)		0.28	0.43	0.55	–	228.84

PDI = Percent Disease Index. Means within a column differ significantly if the difference exceeds the CD value.

3.2 Season II

In the 2023–2024 season (Table 4), disease pressure in the untreated control was much higher, reaching a final PDI of 45.00, with a corresponding reduction in yield to 3100 kg/ac. Under these conditions, prophylactic spraying of Thyme oil 40 EC at 0.1% gave the best performance among the Thyme oil treatments, with PDI values of 5.25, 4.76, and 3.10 after the first, second, and third sprays, respectively, and 93.11% disease reduction over control.

Hexaconazole 5% EC and Thiophanate methyl 70% WP gave 93.30% and 93.20% disease reduction, respectively, and were statistically on par with Thyme oil 40 EC at 0.1%. Yields under these three treatments were also comparable, at 7550, 7540, and 7530 kg/ac, respectively. This supports the interpretation that preventive application timing was critical, because studies in powdery mildew systems consistently show that sprays applied before or at early disease development are more effective than delayed treatments (Reuveni *et al.*, 2023; Saleh *et al.*, 2024; Uddin *et al.*, 2023).

The progressive improvement from 0.025% to 0.1% in both disease reduction and yield further suggests that formulation dose strongly influenced field performance. Similar concentration-dependent responses have been reported for thyme essential oil and related botanicals, where stronger inhibition of pathogen growth and better crop response occur at higher effective doses (Aoujil *et al.*, 2025; Maral *et al.*, 2025; Saltos-Rezabala *et al.*, 2022).

TABLE 4
EVALUATION OF BIOEFFICACY OF THYME OIL 40 EC FORMULATION AGAINST POWDERY MILDEW OF GRAPES (SEASON II, 2024)

T.No	Treatment	Dose (ml/L)	PDI 7 days after 1st spray	PDI 7 days after 2nd spray	PDI 7 days after 3rd spray	% Reduction over control	Yield (kg/ac)
T1	Thyme oil 40 EC @ 0.025%	0.025	7.6	9.2	9.8	78.25	6900
T2	Thyme oil 40 EC @ 0.050%	0.05	6.83	7.2	8.2	81.79	7050
T3	Thyme oil 40 EC @ 0.075%	0.075	6.4	6.2	5.2	88.6	7100
T4	Thyme oil 40 EC @ 0.1%	0.1	5.25	4.76	3.1	93.11	7550
T5	Hexaconazole 5% EC	1	5.18	4.2	3.05	93.3	7540
T6	Thiophanate Methyl 70% WP	1	5.2	4	3.1	93.2	7530
T7	Control	–	28.02	38.3	45	0	3100
	S.Em±		0.19	0.09	0.1	–	61.82
	CD (P=0.05)		0.58	0.28	0.3	–	189.9

PDI = Percent Disease Index. Means within a column differ significantly if the difference exceeds the CD value.

3.3 Pooled Performance

The pooled analysis of two years (Table 5) confirmed the superiority of Thyme oil 40 EC at 0.1%, which recorded PDI values of 5.70, 6.02, and 5.52 across the three observations, 84.23% disease reduction over control, the highest yield of 7537.50 kg/ac, and the highest benefit:cost ratio of 2.95. Hexaconazole 5% EC recorded 84.90% disease reduction with a yield of 7522.50 kg/ac and a benefit:cost ratio of 2.85, while Thiophanate methyl 70% WP recorded 82.60% disease reduction, 7521.00 kg/ac yield, and a benefit:cost ratio of 2.85.

These pooled results indicate that Thyme oil 40 EC at 0.1% was functionally comparable to the fungicide checks and economically advantageous under the present field conditions. That finding is aligned with field evidence showing that essential-oil-based and hybrid biofungicidal treatments can provide strong disease suppression while reducing dependence on conventional fungicides (Reuveni *et al.*, 2023; Aoujil *et al.*, 2025; Soppelsa *et al.*, 2024). A practical implication is that Thyme oil 40 EC could be integrated into powdery mildew management programs as a prophylactic option from early crop stage onward. This is relevant because repeated use of conventional fungicides increases concern about resistance selection and residue load, and alternating or supplementing with biologically derived products is increasingly viewed as a more sustainable strategy (Kaya *et al.*, 2025; Saltos-Rezabala *et al.*, 2022; Rienth *et al.*, 2019).

TABLE 5
EVALUATION OF BIOEFFICACY OF THYME OIL 40 EC FORMULATION AGAINST POWDERY MILDEW OF GRAPES (POOLED DATA OF 2023 AND 2024)

T.No	Treatment	Dose (ml/L)	PDI after 1st spray	PDI after 2nd spray	PDI after 3rd spray	% Disease reduction over control	Yield (kg/ac)	B:C ratio
T1	Thyme oil 40 EC @ 0.025%	0.025	7.35	8.75	9.63	72.5	6500	2.1
T2	Thyme oil 40 EC @ 0.050%	0.05	7.1	7.86	8.71	75.11	6675	2.36
T3	Thyme oil 40 EC @ 0.075%	0.075	6.62	7.48	7.15	79.6	6966.67	2.42
T4	Thyme oil 40 EC @ 0.1%	0.1	5.7	6.02	5.52	84.23	7537.5	2.95
T5	Hexaconazole 5% EC	1	5.34	5.25	5.3	84.9	7522.5	2.85
T6	Thiophanate Methyl 70% WP	1	6.35	6.45	6.1	82.6	7521	2.85
T7	Control	—	21.02	28.4	35	0	3600	1.29
	S.Em±		0.12	0.13	0.09	—	59.43	—
	CD (P=0.05)		0.37	0.4	0.28	—	182.53	—

PDI = Percent Disease Index; B:C = benefit:cost. Means within a column differ significantly if the difference exceeds the CD value.

3.4 Phytotoxicity

No phytotoxic symptoms such as leaf tip injury, wilting, vein clearing, necrosis, epinasty, or hyponasty were observed at any Thyme oil concentration, including the maximum tested concentration of 0.1% (Table 6). The absence of visible phytotoxicity at the effective dose strengthens the suitability of the formulation for foliar use in grapevine and agrees with other reports that optimized thyme-based treatments can suppress disease without causing visible crop injury (Lopes *et al.*, 2023; Saltos-Rezabala *et al.*, 2022; Patel *et al.*, 2025).

TABLE 6
PHYTOTOXICITY OF THYME OIL 40 EC FORMULATION ON POWDERY MILDEW OF GRAPES

T.No	Treatment	Formulation dosage (%)	Leaf injury on tips/surface	Wilting	Vein clearing	Necrosis	Epinasty	Hyponasty
1	Thyme oil 40 EC	0.03%	0	0	0	0	0	0
2	Thyme oil 40 EC	0.05%	0	0	0	0	0	0
3	Thyme oil 40 EC	0.08%	0	0	0	0	0	0
4	Thyme oil 40 EC	0.10%	0	0	0	0	0	0
5	Control	—	0	0	0	0	0	0

Phytotoxicity scale: 0 = 0% (no injury); 1 = 1–10% leaf area affected; 2 = 11–20%; 3 = 21–30%; 4 = 31–40%; 5 = 41–50%; 6 = 51–60%; 7 = 61–70%; 8 = 71–80%; 9 = 81–90%; 10 = 91–100%.

Overall, the results show that Thyme oil 40 EC at 0.1% effectively managed grape powdery mildew, maintained yield at a level comparable with Hexaconazole and Thiophanate methyl, and caused no phytotoxicity under field conditions.

IV. LIMITATIONS

Several limitations should be acknowledged. First, the study was conducted at a single location, on a single variety (Thompson Seedless), and over two consecutive seasons; broader generalization will require multi-location and multi-varietal trials. Second, the biofungicide treatments were applied prophylactically from the five-leaf stage, whereas the fungicide checks were applied after the appearance of symptoms; this represents a comparison of management strategies rather than a strictly controlled timing comparison, and the results should be interpreted accordingly. Third, the observation intervals differed between the two seasons (10 days in Season I vs. 7 days in Season II) because of scheduling constraints; this may affect the comparability of PDI values between seasons. Fourth, no residue or food-safety data were generated; such data are required for registration and large-scale recommendation. Fifth, the effects of the treatments on non-target organisms and beneficial microorganisms were not assessed. Finally, the potential for resistance development in *E. necator* was not evaluated.

V. CONCLUSION

Under the field conditions of Thondamuthur, Coimbatore district, prophylactic application of Thyme oil 40 EC at 0.1 per cent effectively suppressed powdery mildew of grapevine, achieving disease reduction (84.23 per cent, pooled) and yield (7537.50 kg/ac, pooled) comparable with the standard fungicides Hexaconazole 5% EC and Thiophanate methyl 70% WP, while also recording the highest benefit:cost ratio (2.95) among all treatments and causing no visible phytotoxicity even at the highest concentration tested. These results indicate that Thyme oil 40 EC applied prophylactically from the five-leaf stage at 0.1 per cent can serve as a viable, economically advantageous alternative or complement to conventional fungicides in powdery mildew management programmes for grapevine. Its adoption could help reduce reliance on repeated conventional fungicide sprays and the associated risk of resistance development in *Erysiphe necator* populations. Further multi-location trials and residue/food-safety evaluation are recommended before large-scale field recommendation.

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ETHICS STATEMENT

No specific permits were required for this field study as no human or animal subjects were involved.

ORIGINALITY AND PLAGIARISM

This manuscript is the original work of the authors and has not been published or submitted elsewhere. All sources used have been duly cited.

CONSENT FOR PUBLICATION

All authors have agreed to the publication of this content.

COMPETING INTERESTS

There are no conflicts of interest.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

S. Vanitha: Conceptualization, methodology, field trial execution, data collection, data analysis and manuscript drafting. M. Karthikeyan and N. Indra: Field trial execution, data collection and investigation. K. Angappan: Methodology, investigation and critical review of the manuscript. R. Lokesh: Interpretation of results, manuscript review and editing. All authors reviewed and approved the final manuscript.

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