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Limonene-enhanced botanical fungicides: A sustainable component of integrated tomato powdery mildew management in the tropical region

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ABSTRACT

Tomato powdery mildew, caused by *Oidium neolycopersici*, significantly reduces yields in humid tropical regions such as Tanzania. Overreliance on synthetic fungicides raises environmental and health concerns, creating a need for safer alternatives. This study evaluated the efficacy of ethanolic extracts from garlic (*Allium sativum*), custard apple (*Annona squamosa*), and fish bean (*Tephrosia vogelii*), applied individually and in combination with the adjuvant limonene, through two consecutive screen house experiments. The first experiment assessed individual extracts at different concentrations, identifying *A. sativum* at 0.4% as the most effective, reducing disease severity to 39.17%. In the second experiment, this optimal concentration was combined with limonene, which significantly improved efficacy, lowering disease severity to 21.33% - a 45.6% increase in disease suppression. The combined application of all three botanical extracts with limonene provided the highest level of control, reducing disease severity to 14.00%, closely matching the performance of the synthetic fungicide hexaconazole (3.50%). This ternary combination also produced the highest fruit yield among botanical treatments (1579 g per plant), comparable to hexaconazole-treated plants (1694 g per plant). The findings demonstrate that combining botanical extracts with limonene enhances disease control and yield, offering an effective, eco-friendly alternative to synthetic fungicides. Such locally available solutions present a sustainable approach to managing tomato powdery mildew in Tanzania and similar agro-ecological zones.

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important crop and source of human nutrition in Tanzania and many parts of the world. Tomato is one of the most widely cultivated vegetables globally, with annual production in 2019 exceeding 180 million tons (Panno *et al.*, 2021). Major producers include China, India, Turkey, the United States, and Egypt (Panno *et al.*, 2021). Tomatoes are widely recognized as a nutritious food, being low in calories and rich in essential vitamins and minerals such as vitamin C, potassium, folate, and vitamin K. (Sattar *et al.*, 2024). They also contain bioactive compounds, particularly lycopene, an antioxidant associated with various health benefits, including a reduced risk of chronic diseases such as cancer (Yousuf *et al.*, 2021). In Tanzania, tomatoes are widely grown by smallholder farmers and constitute the largest vegetable crop, with annual production in the hundreds of thousands of tonnes, supporting household nutrition and local markets (Tesha and Kongolo, 2023). However, its production is severely constrained by diseases, with tomato powdery mildew (TPM) caused by *Oidium neolycopersici* being a major threat. The disease can lead to yield losses of up to 50% or more if not properly managed, primarily through reduced photosynthetic area and premature defoliation (Abada *et al.*, 2018; Sattar *et al.*, 2024). In Tanzania, smallholder farmers heavily depend on synthetic fungicides application like hexaconazole for disease management (Kariathi *et al.*, 2016). The persistent and often improper use of these chemicals raises serious concerns regarding environmental pollution, toxic residues on food, and the development of fungicide-resistant pathogen strains (Behera *et al.*, 2024; Campos *et al.*, 2019; Manda *et al.*, 2020).

Botanical pesticides, derived from plants with inherent pesticidal properties, offer a promising, biodegradable, and less hazardous alternative disease management approach (Campos *et al.*, 2019; Deresa and Diriba, 2023). Plants such as garlic (*Allium sativum*), known for its antimicrobial organosulfur compounds (Aala *et al.*, 2014); custard apple (*Annona squamosa*), containing bioactive

acetogenins (Dang *et al.*, 2011; Kalidindi *et al.*, 2015), and fish bean (*Tephrosia vogelii*), a source of rotenoids and flavonoids (Utaji *et al.*, 2024), have all demonstrated antifungal potential. However, a significant challenge with botanical extracts is their variable field efficacy due to factors like rapid degradation and poor foliar adhesion (Barbu *et al.*, 2025; Deresa and Diriba, 2023; Isman, 2025).

To overcome these limitations, the use of adjuvants is crucial. Limonene, a natural monoterpene, acts as a potent adjuvant by enhancing the spreading, penetration, and stability of active ingredients on plant surfaces (Ammad *et al.*, 2018; Ibáñez *et al.*, 2020). It also possesses inherent antimicrobial properties, which can synergize with botanical extracts (Lin *et al.*, 2024). While the individual antifungal properties of *A. sativum*, *A. squamosa*, and *T. vogelii* have been reported to some extent, their combined efficacy and the role of limonene as an adjuvant in enhancing their fungicidal activity against TPM remain underexplored. Therefore, this study aimed to evaluate the baseline fungicidal activity of selected botanical extracts in managing TPM, determine the efficacy of limonene-enhanced botanical extracts, and assess the effects of these extracts on tomato fruits yield under screenhouse conditions.

MATERIALS AND METHODS

Description of the study site and experimental design

Two screenhouse experiments were conducted over two cropping seasons at the Tanzania Plant Health and Pesticide Authority (TPHPA). Experiment one was carried out from July to November 2024, while experiment two was conducted from January to April 2025. The TPHPA is located at an elevation of 1,491 m above sea level (3°19'53"S, 36°57'38"E). During the experimental periods, temperatures ranged from 20 to 26 °C, and relative humidity between 70% and 81%.

Pathogen identification was performed at NM-AIST, Arusha, using morphological and molecular techniques to confirm the identity of the pathogen

prior to experimental inoculation. Tomato leaves showing powdery mildew disease symptoms were collected from the TPHPA botanical garden. Samples were carefully collected, air-dried using sterile paper towels to remove surface moisture, and stored in airtight bags prior to pathogen identification. Identification of *O. neolycopersici* was carried out using morphological observation under a compound microscope (40 × magnifications) and molecular analysis using polymerase chain reaction (PCR).

All efficacy experiments were conducted under greenhouse conditions using potted tomato plants grown in plastic pots (27.2 cm diameter × 27 cm height). Each pot contained one plant.

The experiments were arranged in a completely randomized design (CRD), with a spacing of 1 m maintained between pots to reduce interference among treatments.

Preparation and concentration of plant extracts

Bioactive compounds were extracted using the maceration method. Powdered plant materials of *T. vogelii* and *A. squamosa*, along with freshly prepared paste of *A. sativum*, were separately macerated in 80% bioethanol at a ratio of 1:10 (w/v; plant material to solvent). The mixtures were kept at room temperature for 72 hours with periodic agitation to enhance extraction efficiency (da Gama *et al.*, 2025).

After extraction, the mixtures were filtered sequentially through a double layer of muslin cloth followed by Whatman No. 1 filter paper to obtain clear filtrates. The filtrates were then concentrated under reduced pressure using a rotary evaporator (Model KI-2198) operated at 120 rpm and a controlled temperature of 60 °C (Bitwell *et al.*, 2023). Evaporation was continued until complete removal of the solvent, yielding a concentrated, viscous crude extract. The crude extracts were stored in airtight amber-glass containers at 4 °C to prevent degradation of bioactive compounds until further use in bioassays.

Experiment one: Baseline screening of individual botanical extracts

This experiment was conducted to establish the baseline fungicidal activity of selected botanical extracts in managing TPM. Extracts of *A. sativum*, *A. squamosa*, and *T. vogelii* were evaluated individually at three concentrations (0.2%, 0.3%, and 0.4%). Treatments were compared with a negative control consisting of distilled water and a positive check (0.2% hexaconazole). A total of 66 tomato plants were used, with one plant per pot and six replicates per treatment.

Experiment two: Evaluation of limonene-enhanced botanical extracts

This experiment was conducted to build upon the baseline results obtained in the experiment one by evaluating the efficacy of limonene-enhanced botanical extracts in managing TPM. Based on the results of the experiment one, the 0.4% concentration was selected for all botanical extracts tested in experiment two. A total of nine treatments were evaluated: three individual botanicals extracts (*A. sativum*, *A. squamosa*, and *T. vogelii*), three binary combinations, and one ternary combination, all supplemented with 0.5% (v/v) limonene. These treatments were compared with the same negative control (distilled water) and positive check (0.2% hexaconazole). The experiment comprised of 54 tomato plants, with one plant per pot and six replicates per treatment.

Preparation and inoculation of *O. neolycopersici*

A conidial suspension of *O. neolycopersici* was prepared by gently scraping conidia from infected tomato leaves into sterile distilled water containing 0.01% Tween 20. The suspension was filtered through cheesecloth to remove leaf debris, and the concentration was adjusted to 10⁵ conidia/mL using a hemocytometer. Tomato plants at the 4-5 true leaf stage were then inoculated with this suspension (Sunarti *et al.*, 2022). Plants were evenly sprayed on both the adaxial and abaxial leaf surfaces using a hand-held sprayer, ensuring complete coverage (Xu *et al.*, 2023).

Disease symptoms appeared consistently seven days after inoculation, aligning with previous reports (Aumentado *et al.*, 2023). Following inoculation, the botanical fungicide was applied at 7-day intervals, and disease development was monitored over time (Lage *et al.*, 2019; Saleh *et al.*, 2024).

Disease assessment

Powdery mildew disease progression was assessed visually at 7, 14, 21, 28, 35, and 42 days post

inoculation (dpi). Disease severity was rated using a 0-5 ordinal scale, adapted from (Abada *et al.*, 2018) and (Behera *et al.*, 2024), based on the proportion of the leaf surface exhibiting fungal growth and the spread of symptoms to other plant parts (Table 1). A score of 0 indicated the absence of visible symptoms, whereas a score of 5 corresponded to severe infection, with fungal growth affecting more than 80% of the leaf surface (Abada *et al.*, 2018).

Table 1. Disease severity rating scale for powdery mildew disease on tomato plants

Scale	Severity level	Description
0	No infection	No visible symptoms.
1	Very low	Sparse, isolated colonies on 1-20% of leaf area; no distortion.
2	Low	White fungal growth on 21-40% of leaves; slight curling or chlorosis.
3	Moderate	Confluent colonies on 41-60% of leaves; curling, chlorosis, some necrosis; slight petiole infection.
4	High	Dense growth on 61-80% of leaves; severe curling, chlorosis, necrosis; petioles and stems infected.
5	Very High	81-100% leaf coverage; heavy fungal growth, stunting, defoliation; stems and petioles fully colonized.

Source: (Abada *et al.* 2018)

Yield assessment

Tomato fruit yield was assessed using the same plants as in powdery mildew disease assessment in experiment two. Yield measurements were taken at 7-day intervals from fruit set until the end of the experiment. For each plant, the total number of fruits and the fresh weight of harvested ripe fruits (in grams) were recorded. Fruits were harvested at a uniform ripeness stage (fully coloured and firm), and fresh weight was measured using a calibrated digital balance (iScale Mini). Cumulative fruit number and total yield per plant were calculated for each treatment.

Statistical analysis

Data on disease severity (expressed as %) and yield were subjected to a one-way Analysis of Variance (ANOVA). Where significant ($p < 0.05$) differences were found, means were separated using Fisher's Least Significant Difference (LSD) test. All analyses were performed using GenStat 18th Edition software.

RESULTS

Efficacy of individual botanical extracts in suppressing tomato powdery mildew

Disease severity in the negative control treatment increased steadily over time and reached 100 ± 0.00 at 42 dpi (Table 2). All treatments involving botanical extracts significantly ($p < 0.001$) reduced disease severity compared with the negative control from 14 dpi onward, with effectiveness increasing in a clear dose-dependent manner. The results indicate that *A. sativum* was the most effective botanical extract across all tested concentrations. At the highest concentration (0.4%), it resulted in the lowest final disease severity of 39.17 ± 1.54 at 42 dpi. The intermediate concentration (0.3%) of *A. sativum* also showed strong efficacy, with a final disease severity of 61.67 ± 3.80 .

On the other hand, *A. squamosa* and *T. vogelii* exhibited moderate but statistically significant ($p < 0.001$) reductions in disease severity (Table 2). At the 0.4% concentration, *A. squamosa* and *T. vogelii*

recorded the final disease severity values of 49.17 ± 2.01 and 50.00 ± 1.29 , respectively. The effects of *A. squamosa* and *T. vogelii* at lower concentrations (0.2% and 0.3%) were significantly less pronounced, further confirming a concentration-dependent

response. As expected, the positive check, which involved synthetic fungicide (0.2% hexaconazole), provided the highest level of disease suppression, recording a final disease severity value of 6.00 ± 0.45 at 42 dpi.

Table 2. Efficacy of individual botanical extracts in managing tomato powdery mildew: mean disease severity (%) (\pm SE)

Treatment	Conc. (%)	Disease severity values at each assessment duration (Number of days post-inoculation)					
		7	14	21	28	35	42
<i>A. sativum</i>	0.2	16.67 \pm 2.11 a	61.67 \pm 2.11 a	68.33 \pm 2.11 a	76.67 \pm 2.11 b	85.83 \pm 2.71 a	91.67 \pm 1.67 a
<i>A. squamosa</i>	0.2	15.83 \pm 2.01 a	58.33 \pm 3.07 a	68.33 \pm 3.07 a	77.50 \pm 2.50 b	86.67 \pm 2.11 a	95.00 \pm 1.83 a
<i>T. vogelii</i>	0.2	15.50 \pm 1.38 a	61.67 \pm 1.05 a	70.83 \pm 2.71 a	66.67 \pm 3.33 a	80.00 \pm 2.58 a	96.67 \pm 2.11 a
<i>A. sativum</i>	0.3	17.17 \pm 1.30 a	51.67 \pm 3.07 a	53.33 \pm 3.58 a	55.83 \pm 3.96 a	58.33 \pm 4.01 a	61.67 \pm 3.80 a
<i>A. squamosa</i>	0.3	18.33 \pm 1.05 a	51.67 \pm 3.07 a	55.83 \pm 2.01 a	59.17 \pm 2.01 a	62.50 \pm 1.71 a	65.00 \pm 2.24 a
<i>T. vogelii</i>	0.3	14.50 \pm 1.89 a	56.67 \pm 2.47 a	59.17 \pm 3.27 a	63.33 \pm 2.11 a	65.00 \pm 2.24 a	66.67 \pm 2.11 a
<i>A. sativum</i>	0.4	13.33 \pm 1.67 a	43.33 \pm 1.67 b	41.67 \pm 2.11 b	40.00 \pm 1.29 b	39.17 \pm 1.54 b	39.17 \pm 1.54 b
<i>A. squamosa</i>	0.4	14.17 \pm 2.01 a	52.50 \pm 2.50 c	50.00 \pm 2.58 c	49.00 \pm 2.31 c	49.00 \pm 2.77 c	49.17 \pm 2.01 c
<i>T. vogelii</i>	0.4	15.00 \pm 2.24 a	53.33 \pm 2.11 c	52.50 \pm 1.71 c	51.67 \pm 1.67 c	50.33 \pm 0.33 c	50.00 \pm 1.29 c
Hexaconazole (positive check)	0.2	15.00 \pm 1.83 a	6.50 \pm 0.81 a	6.00 \pm 0.37 a	6.00 \pm 0.44 a	5.33 \pm 0.33 a	6.00 \pm 0.45 a
No-fungicide (negative check)	0.0	13.83 \pm 2.01 a	65.00 \pm 2.24 d	75.00 \pm 1.83 d	84.17 \pm 2.01 d	93.33 \pm 2.11 d	100.00 \pm 0.00 d
F-statistic		0.14	131.19	179.58	279.68	336.73	688.57
p-value		0.964	<0.001	<0.001	<0.001	<0.001	<0.001

Means followed by different letters within a column differ significantly ($p < 0.05$) according to Fisher's LSD test. The negative check consistently recorded the highest disease severity, while hexaconazole (positive check) recorded the lowest disease severity values across the assessment period

Evaluation of the efficacy of limonene-enhanced botanical extract in suppressing tomato powdery mildew

The incorporation of 0.5% v/v limonene as an adjuvant significantly ($p < 0.001$) enhanced the antifungal efficacy of all tested botanical extracts from 14 dpi onward (Table 3). Among these, the limonene-enhanced triple combination (*A. sativum* + *A. squamosa* + *T. vogelii*) was the most effective, achieving a final disease severity score of 14.00 ± 1.53 a result that comes remarkably close to the treatment involving hexaconazole, a synthetic standard (positive check), which recorded score of 3.50 ± 1.12 .

The efficacy of individual botanical extracts was also markedly improved by mixing with limonene. The

garlic-based extract (*A. sativum* + limonene) showed the greatest improvement, reducing disease severity to 21.33 ± 0.88 from 39.17 ± 1.54 when used alone. This was followed by *A. squamosa*, which decreased disease severity from 49.17 ± 2.01 to 25.50 ± 2.43 , and *T. vogelii*, which reduced severity value from 50.00 ± 1.29 to 33.00 ± 3.87 . Overall, limonene acted as a potent additive, reliably boosting the efficacy of all tested botanical extracts.

Number of tomato fruits per plant

The assessment of number of fruits per plant was conducted from 21 to 42 dpi across all treatments. The number of tomato fruits per plant increased progressively from 21 dpi to 42 dpi in all treatments (Fig. 1), with significant ($p < 0.001$) differences

observed among treatments. Among the botanicals extracts used in this study, those with combinations performed significantly ($p \leq 0.05$) better than individual extracts. Ternary combination (*A. sativum* + *A. squamosa* + *T. vogelii*) resulted in to a significantly higher number of tomato fruits than other single- or two-way combination treatment. The number of tomato fruits in the ternary combination was significantly the same as in hexaconazole at 35 and 42 dpi. Additionally, the two-way combinations and individual botanical extracts produced significantly ($p < 0.001$) high number of tomato fruits

per plant compared with the negative check. The positive check consistently produced the highest numbers of tomato fruits throughout the assessment period, while the negative check recorded the lowest value of tomato fruits, confirming the suppressive impact of powdery mildew on the fruit yield when plants were left untreated. Overall, these findings demonstrate that the tested botanical extracts in this study, significantly enhanced tomato fruits production, with three-way botanical extracts combination performing comparably the same as synthetic pesticide.

Table 3. Tomato powdery mildew severity (%) recorded at different assessment duration in each tested limonene-enhanced botanical extract

Treatment	Conc. (%)	Disease severity values at each assessment duration (Number of days post-inoculation)					
		7	14	21	28	35	42
<i>A. sativum</i> + LMN	0.4	16.67 ± 1.67 a	35.83 ± 2.01 bc	29.17 ± 1.54 bc	25.33 ± 1.33 bc	21.50 ± 0.96 bc	21.33 ± 0.88 bc
<i>A. squamosa</i> + LMN	0.4	17.50 ± 1.71 a	37.50 ± 5.12 c	30.00 ± 2.24 c	26.67 ± 3.07 bc	25.83 ± 2.39 cd	25.50 ± 2.43 cd
<i>T. vogelii</i> + LMN	0.4	15.50 ± 1.89 a	45.83 ± 5.23 c	40.00 ± 4.28 c	35.83 ± 3.96 c	33.33 ± 3.80 d	33.00 ± 3.87 d
<i>A. sativum</i> + <i>A. squamosa</i> + LMN	0.4	17.83 ± 1.01 a	43.33 ± 2.47 c	36.67 ± 2.47 c	31.67 ± 2.11 c	25.83 ± 2.39 cd	25.50 ± 2.14 cd
<i>A. sativum</i> + <i>T. vogelii</i> + LMN	0.4	14.17 ± 1.54 a	38.33 ± 2.79 c	31.67 ± 2.11 c	27.50 ± 1.12 c	21.67 ± 1.05 bc	21.17 ± 1.30 bc
<i>A. squamosa</i> + <i>T. vogelii</i> + LMN	0.4	17.00 ± 1.41 a	44.17 ± 2.71 c	38.33 ± 2.11 c	33.33 ± 1.67 c	30.00 ± 2.24 cd	29.17 ± 1.54 cd
Triple blend + LMN	0.4	16.67 ± 1.67 a	22.50 ± 1.71 ab	18.33 ± 2.11 ab	16.67 ± 1.67 ab	14.17 ± 1.54 b	14.00 ± 1.53 b
Hexaconazole (positive check)	0.2	15.83 ± 1.54 a	10.83 ± 0.83 a	8.33 ± 1.05 a	6.67 ± 1.05 a	3.33 ± 1.05 a	3.50 ± 1.12 a
No fungicide (negative check)	0.0	17.50 ± 1.71 a	60.83 ± 2.39 d	68.33 ± 2.11 d	73.33 ± 3.07 d	81.67 ± 3.07 e	90.00 ± 2.89 e
F-statistic		0.54	20.81	48.53	61.92	93.41	126.65
p-value		0.818	<0.001	<0.001	<0.001	<0.001	<0.001

Values are mean disease severity (%) (±SE); means followed by different letters differ significantly at $p < 0.05$ according to Fisher's LSD test.

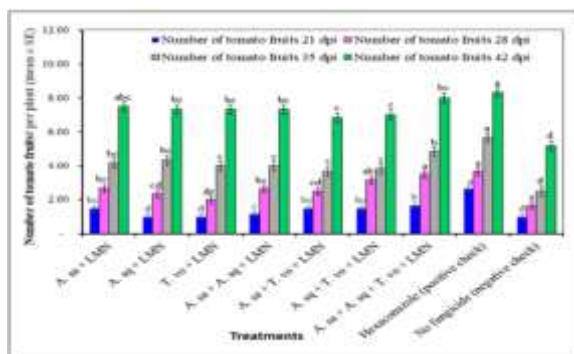


Fig. 1. Effects of Limonene-enhanced Botanical Extracts Treatments on the Number of Fruits per Plant at 42 days post-inoculation in Northern

Tanzania. Values represent mean ± standard error (SE) of the mean ($n = 6$).

Note: A. sa = *Allium sativum*, A. sq = *Annona squamosa*, and T. vo = *Tephrosia vogelii*.

Tomato fruit yield per plant

Data presented in Table 4 indicate significant ($p < 0.05$, LSD test) differences among the tested limonene-enhanced botanical extract treatments, establishing a clear performance hierarchy. The most effective botanical-based intervention was the ternary combination (*A. sativum* + *A. squamosa* + *T. vogelii*),

which produced the highest yield among all botanical treatments (1579 ± 62.15), ranking second only to the synthetic fungicide hexaconazole (1694 ± 62.48), used as the positive control. Treatments involving *A. squamosa* alone and the two-way combinations (*A. sativum* + *A.*

squamosa, *A. sativum* + *T. vogelii*, and *A. squamosa* + *T. vogelii*) formed a middle tier. The single limonene-enhanced botanical extracts (*A. sativum* and *T. vogelii*) were the least effective among the treatment groups, though still superior to the negative control.

Table 4. Effect of limonene-enhanced botanical fungicide treatments on cumulative tomato fruit yield per plant at 42 dpi

Treatment	Conc. (%)	Tomato fruit yield per plant (g/plant)
<i>Allium sativum</i> + LMN	0.4	1412 ± 40.33 b
<i>Annona squamosa</i> + LMN	0.4	1412 ± 51.02 b
<i>Tephrosia vogelii</i> + LMN	0.4	1398 ± 90.06 b
<i>A. sativum</i> + <i>A. squamosa</i> + LMN	0.4	1451 ± 44.98 bc
<i>A. sativum</i> + <i>T. vogelii</i> + LMN	0.4	1422 ± 22.99 b
<i>A. squamosa</i> + <i>T. vogelii</i> + LMN	0.4	1428 ± 31.14 bc
Triple blend + LMN	0.4	1579 ± 62.15 cd
Hexaconazole (positive check)	0.2	1694 ± 62.48 d
No fungicide (negative check)	0.0	1008 ± 51.02 a
F-statistic		11.64
p-value		<0.001

Means followed by different letters within the column differ significantly ($p < 0.05$) according to Fisher's LSD test. The negative control recorded the lowest fruit yield, hexaconazole the highest, and limonene-enhanced botanical treatments produced intermediate yields, with the triple blend showing the greatest increase.

This stratification highlights that while individual botanical-based interventions were less effective, the combined application, particularly the tripple blend, substantially mitigated disease pressure and resulted in a significant positive yield increase approaching that of the synthetic fungicide.

DISCUSSION

Fungicidal activity of the individual botanical plant extracts in suppressing tomato powdery mildew

The findings of this study demonstrated that the selected botanical extracts can be used as viable antifungal agents in managing tomato powdery mildew, with a clear, dose-dependent relationship observed across all the tested application concentrations. Among the botanicals tested, *A. sativum* extract emerged as the most effective at every concentration tier. However, the superior efficacy of *A. sativum* was most pronounced at the 0.4% concentration, achieving 39.17% disease severity at 42 dpi. The observed superior performance of *A. sativum* could be attributed to its rich content of organosulfur compounds, such as allicin, known to disrupt fungal membrane integrity and suppress

spore germination (Aala *et al.*, 2014; Li *et al.*, 2022). The intermediate concentration (0.3%) of *A. sativum* also showed substantial antifungal activity, with disease suppression of almost 50%, while the lowest concentration (0.2%) provided only minimal protection against tomato powdery mildew. This consistent performance hierarchy underscores the potency and reliability of *A. sativum*'s primary active compounds, allicin, which exerts a strong, multi-site antifungal effect (Adelusi *et al.*, 2023; Corbu *et al.*, 2021; Pereira *et al.*, 2021).

The extracts of *A. squamosa* and *T. vogelii* also exhibited significant, concentration-dependent fungicidal activity in suppressing tomato powdery mildew. At their highest concentration (0.4%), both achieved suppressed disease by almost 50% at 42 dpi. However, their efficacy was consistently surpassed by *A. sativum* at equivalent concentrations. On the contrary, their efficacy at the low concentrations (0.2% and 0.3%) was significantly less compared to *A. sativum*. This difference in performance can be traced to their distinct modes of action; *A. squamosa* derives its activity from acetogenins that inhibit mitochondrial function (Dzhemileva *et al.*, 2023;

Kumari *et al.*, 2022; Mukherjee *et al.*, 2006), while *T. vogelii* relies on rotenoids that interfere with cellular respiration (Garmier *et al.*, 2008; Said *et al.*, 2020; Siame *et al.*, 2019; Zhang *et al.*, 2020). These mechanisms, while effective, may require higher thresholds of concentration to manifest robust antifungal activity compared to the membrane-disrupting action of allicin.

Unsurprisingly, treatment involving hexaconazole (a standard positive check) showed the highest overall efficacy, with disease severity (6.00 ± 0.45) lower than all botanical extracts. The superior performance of hexaconazole could be linked to its established, single-site mode of action involving the potent inhibition of sterol biosynthesis in fungal membranes (Ashraf *et al.*, 2022; Tanwar *et al.*, 2024).

The performance of *A. sativum*, in particular, across multiple concentrations is noteworthy. This level of control, combined with their multi-site action and natural origin, positions *A. sativum*, especially when used at optimal concentrations, as crucial components for sustainable agriculture strategies, effectively balancing appreciable efficacy with practical and environmental considerations (Guleria and Tiku, 2009; Riyaz *et al.*, 2021).

Efficacy of limonene-enhanced botanical extracts in managing tomato powdery mildew

The enhanced efficacy observed in limonene-supplemented botanical extracts demonstrates that limonene is more than a minor constituent. It acts as a multifunctional enhancer, amplifying the antifungal potential of plant-derived extracts through synergistic physicochemical and biochemical mechanisms (Jian *et al.*, 2023; Tayeb *et al.*, 2025). As a natural adjuvant, limonene improves spray spreadability, droplet retention, and surface coverage on hydrophobic leaves, thereby minimizing run-off and promoting uniform film formation (Czarnota and Thomas, 2007; Ibáñez *et al.*, 2020; Lin *et al.*, 2024). Additionally, limonene serves as a permeation enhancer by disrupting the waxy cuticle and fungal cell wall, facilitating the uptake and translocation of

active compounds, such as, allicin, acetogenins, and rotenoids (Jian *et al.*, 2023). This enhanced penetration leads to high effective concentrations of bioactive constituents at infection sites, thereby improving disease suppression efficiency (Lin *et al.*, 2024). When combined with botanical extracts, these complementary actions of limonene generate a multi-site, synergistic antifungal effect that lowers the likelihood of resistance development (Dantas *et al.*, 2025; Jian *et al.*, 2023).

Limonene integration consistently lowered disease severity across all botanical extract treatments. The lowest disease severity value (14.00%) was achieved with the triple combination of *A. sativum*, *A. squamosa*, and *T. vogelii* + limonene, although this value remained higher than that recorded for the synthetic fungicide (3.50%). This high efficacy could be attributed to the cumulative antifungal mechanisms of the constituent botanicals and the synergistic activity of limonene, which disrupts fungal membranes and inhibits spore germination (Jian *et al.*, 2023; Tayeb *et al.*, 2025).

Among the binary combinations, *A. sativum* + *T. vogelii* achieved the disease severity of 21.17%, comparable to single *A. sativum* under limonene enhancement, suggesting a strong interaction between allicin and rotenoid compounds (Zainal *et al.*, 2021). The *A. squamosa* + *T. vogelii* and *A. sativum* + *A. squamosa* combinations recorded moderate inhibition levels of 29.17% and 25.50%, respectively, further confirming the amplifying effect of limonene. Among the single botanicals, *A. sativum* exhibited the lowest disease severity (21.33%), compared with *A. squamosa* (25.50%) and *T. vogelii* (33.00%). Its superior activity is attributed to allicin, a sulphur-containing compound known for its broad-spectrum antifungal properties (Li *et al.*, 2022; Rababah *et al.*, 2025). Notably, the efficacy of *A. sativum* improved markedly with the addition of limonene, reducing disease severity from 39.17% without limonene to 21.33% with limonene. This improvement highlights the role of volatile terpenes in enhancing the bioavailability and penetration of

active compounds (Chen *et al.*, 2016). The magnitude of improvement in disease suppression following limonene addition was quantified using percentage reduction relative to the non-limonene treatment. This was calculated as:

$$\text{Percentage increase in disease suppression} = \left(\frac{S_1 - S_2}{S_1} \right) \times 100$$

Where S₁ represents disease severity without limonene (39.17%) and S₂ represents disease severity with limonene (21.33%). Based on this calculation, limonene incorporation resulted in a 45.6% increase in disease suppression, demonstrating a substantial enhancement in antifungal efficacy. The comparatively higher disease severity observed in *A. squamosa* (25.50%) and *T. vogelii* (33.00%) can be explained by the instability and rapid degradation nature of acetogenins and rotenoids, which reduce residual activity and systemic protection (Zhang *et al.*, 2020).

Overall, limonene-enhanced botanical extracts combinations produced significantly lower disease severity than single extracts. The 14.00% disease severity recorded for the triple combination (*A. sativum*, *A. squamosa*, and *T. vogelii*) demonstrated a strong synergistic interaction between multiple phytochemicals and limonene, consistent with previous reports on essential oil - mediated pathogen suppression (Jian *et al.*, 2023; Tayeb *et al.*, 2025). These findings confirm that the integration of limonene with botanical extracts can yield eco-friendly, high-efficacy fungicidal formulations comparable to synthetic standards, supporting a viable pathway toward reduced chemical input in sustainable crop protection (Alimzhanova *et al.*, 2025).

Effects of limonene-enhanced botanical extracts on tomato fruit number

The study found that among the botanical treatments, the three-way combination of *A. sativum*, *A. squamosa*, and *T. vogelii* performed best in increasing the number of fruits per plant, ranking

second only to the synthetic fungicide standard. This enhanced performance suggests a synergistic interaction among the combined extracts, potentially improving antifungal persistence and broadening the spectrum of activity compared to single-plant formulations, as reported in previous studies (Dantas *et al.*, 2025; Yoon and Kim, 2023). The relatively strong performance of two-way combinations, particularly *A. sativum* + *A. squamosa*, further indicates that specific plant pairings may enhance disease suppression and reproductive success (Alimzhanova *et al.*, 2025).

At 21 dpi, fruit numbers were low across all treatments, reflecting early fruit initiation and limited separation among treatments (Alsudani, 2025; Sam *et al.*, 2024). This pattern is expected at early reproductive stages, where yield components have not yet fully responded to differences in disease suppression (Alsudani, 2025).

In contrast, individual limonene-enhanced extracts, especially *A. sativum* and *T. vogelii*, produced comparatively fewer fruits under powdery mildew pressure. This suggests that when applied alone, their antifungal activity may not be sufficient to fully protect flowers and developing fruits under high disease severity (Cho *et al.*, 2026; Sood *et al.*, 2025). These findings align with earlier reports showing that the efficacy of crude botanical extracts depends on plant source, formulation, and environmental conditions (Dantas *et al.*, 2025; Yoon and Kim, 2023).

Although hexaconazole recorded the highest fruit number, all limonene-enhanced botanical treatments significantly outperformed the negative control. This demonstrates their capacity to reduce disease impact and maintain reproductive performance, supporting their potential integration into sustainable disease management strategies.

Effects of limonene-enhanced botanical extracts on tomato fruit yield

The findings of this study demonstrated that significant differences in cumulative fruit yield were

observed among treatments at 42 dpi ($p < 0.001$). The three-way botanical combination (*A. sativum* + *A. squamosa* + *T. vogelii*) produced the highest yield among the botanical treatments (1579 ± 62.15 g/plant), ranking second only to hexaconazole (1694 ± 62.48 g/plant). This superior yield performance further supports the presence of synergistic interactions among the combined plant extracts, leading to improved disease suppression and enhanced productivity (Ghanbari Daryae *et al.*, 2025; Jan *et al.*, 2025).

Two-way combinations formed an intermediate performance tier, while single limonene-enhanced extracts produced comparatively lower yields, although still significantly higher than the negative control. The reduced performance of single extracts may reflect limited persistence or narrower antifungal activity when used alone (Jan *et al.*, 2025).

Despite hexaconazole remaining the most effective treatment, all botanical formulations significantly increased yield compared to the untreated control, demonstrating their practical potential. These findings support limonene-enhanced botanical blends particularly the three-way combination as promising tools for integrated pest management and reduced reliance on synthetic fungicides.

CONCLUSION

This study demonstrates that limonene-enhanced botanical fungicides provide an effective and eco-friendly strategy for managing tomato powdery mildew caused by *O. neolycopersici* in humid tropical conditions. In the initial screening, ethanolic extract of *A. sativum* at 0.4% produced the strongest suppression of disease severity, outperforming *A. squamosa* and *T. vogelii*. However, combining botanical extracts with limonene (0.5% v/v) markedly improved performance, starting from 14 dpi. The ternary formulation (*A. sativum* + *A. squamosa* + *T. vogelii*) with limonene achieved the lowest final disease severity (14.00%), although still higher than that recorded for the synthetic fungicide hexaconazole (3.50%).

Importantly, the same triple botanical-limonene blend also produced the greatest fruit yield among botanical treatments (1579 g per plant, highest under botanical options), approaching yields recorded for hexaconazole-treated plants. Overall, the findings support limonene-enhanced local botanical blends as promising alternatives to reduce reliance on synthetic fungicides.

RECOMMENDATIONS

We recommend further development and optimization of this ternary limonene-enhanced formulation for commercial use. Farmer-participatory validation is the essential next step to integrate this eco-friendly, locally sourced solution into mainstream agricultural practice in Tanzania and similar regions, reducing reliance on synthetic chemicals and supporting sustainable tomato production.

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