

**RESEARCH ARTICLE** 



## Exogenous applications of salicylic acid, ethrel and cycocel: Synergistic effects on yield, quality and storability in Garlic (*Allium sativum* L.) cv. Ooty 2

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#### **ARTICLE HISTORY**

Received: 04 September 2024 Accepted: 09 October 2024 Available online Version 1.0: 30 October 2024

() Check for updates

#### **Additional information**

**Peer review**: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc See https://horizonepublishing.com/journals/ index.php/PST/indexing\_abstracting

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#### **CITE THIS ARTICLE**

Indhumathi S, Irene Vethamoni P, Senthil A, Vanitha S, Thamaraiselvi SP. Exogenous applications of salicylic acid, ethrel and cycocel: Synergistic effects on yield, quality and storability in Garlic (*Allium sativum* L.) cv. Ooty 2. Plant Science Today (Early Access). https://doi.org/10.14719/pst.4947

## Abstract

Garlic (Allium sativum L.) is a valuable bulb crop, known for its rich bioactive compounds and health benefits. This study aimed to evaluate the impact of various foliar spray treatments on the growth, yield and quality of garlic cv. Ooty 2. The treatments included a control, 100 ppm, 200 ppm and 300 ppm of salicylic acid, cycocel at 1000 ppm and ethrel 200, 300 ppm sprayed at 45 and 60 days after planting. The experiment was conducted using a randomized block design with 7 treatments and 3 replications. Results revealed that salicylic acid 300 ppm increased growth traits followed by ethrel 300 ppm over the control. Cycocel 1000 ppm showed that high bulb yield and less reduction in quality characters viz., TSS (39.2 °Brix), acidity (0.38 %), ascorbic acid  $(11.25 \text{ mg } 100 \text{ g}^{-1})$ , total carbohydrate  $(39.82 \text{ g } 100 \text{ g}^{-1})$ and phenolics (4.10 mg 100 g<sup>-1</sup>) content during 3 months storage period, followed by ethrel 300 ppm. Additionally lowest levels of physiological loss in weight (17.47 %), rubbering (5.13 %), rotting (5.88 %) and total loss (23.64 %) was observed compared to all other treatments during the storage period. The results indicate that cycocel at 1000 ppm had the most beneficial effects and can be effectively used to enhance the harvest, postharvest traits and quality and storability of garlic bulbs.

## **Keywords**

garlic; cycocel; ethrel; pre harvest spray; post-harvest; salicylic acid

## Introduction

Garlic (Allium sativum L.) is a bulb belonging to the family Alliaceae, a diploid species (2n = 2x = 16) and it is the second most widely cultivated Alliaceae crop globally, following onion. Originating from Central Asia, garlic spread to other parts of the world through trade and colonization, a monocotyledonous modified underground stem reproduces vegetatively (1). The bulb, composed of few to many cloves, is the main economic organ of *A. sativum* however, fresh leaves, bulbils and pseudostems are also utilized for different purposes (2). Pre-harvest factors play a significant role in controlling the post-harvest deterioration of garlic to a great extent (3). Plant growth regulators have been used to modify various plant characteristics such as plant height, number of leaves per plant, number

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and size of cloves per bulbs, biomass yield, net bulb yield, etc., by influencing the physiological processes within the plant. These modifications were responsible for an increase in garlic yield and quality in recent years (4). Important among them is the pre-harvest application of growth regulators such as maleic hydrazide, ethrel, cycocel and carbendazim, etc., which are known to enhance the post-harvest life of garlic (3, 5). A naturally occurring phytohormone called salicylic acid (SA) is essential for fostering healthy plant growth and improving resistance to a range of biotic and abiotic stresses (6, 7). It is also essential for the general development of plants, ion uptake and transport, water interactions and plant growth. In particular, it improved the garlic endogenous salicylic acid level, which in turn improved the development of garlic bulbs (8). Chloro choline chloride (CCC), is chemically known as 2-chloro ethyl trimethyl ammonium chloride also referred as chlormequat, cyclocel helps to regulate vegetative growth and also increases the yield of certain horticultural crops (5). Ethrel has a beneficial effect on the yield and quality of bulbs. External application of ethrel has increased the development of bulbous plants in an array of ways, including the number of leaves, leaf weight, plant height, bulb length, bulb diameter and weight of bulb (9). By reducing post-harvest losses viz., sprouting, rotting, physiological weight loss, etc., it has significantly improved the yield, quality and shelf life of garlic bulbs. Therefore, the objective of the study was to examine the effects of pre-harvest spray of growth regulators viz., salicylic acid, cycocel and ethrel on yield, quality and shelf life of garlic (Allium sativum L.) cv. Ooty 2.

## **Materials and Methods**

#### **Experimental site**

A field experiment was conducted at the Wood House Farm, Horticultural Research Station (HRS), Ooty, Tamil Nadu, India during the rabi season of the year 2023-2024 located at 11° 42' N latitude, 76° 72' E longitude and 2500 m altitude above mean sea level. The region experiences a temperate climate with moderate temperature ranging between 10 °C to 20 °C, making it suitable for garlic cultivation. In addition loamy soil with pH in the range 6.0 to 6.5, is also ideal for garlic cultivation. The soil was prepared by ploughing and incorporating organic matter like farmyard manure at a rate of 25 tons per ha to enhance soil fertility.

## **Experimental setup**

This experiment was laid out in a randomized block design with seven treatments and 3 replications with a plot size of  $3.0 \text{ m} \times 1.5 \text{ m}$  and a spacing of  $15 \text{ cm} \times 10 \text{ cm}$ , accommodating 300 plants block <sup>-1</sup>. Ooty 2 garlic cultivar was selected as the study crop for the present work. Different treatments analyzed in the present study include: T<sub>1</sub>: Control; T<sub>2</sub>: Salicylic Acid at 100 ppm; T<sub>3</sub>: Salicylic Acid at 200 ppm; T<sub>4</sub>: Salicylic Acid at 300 ppm; T<sub>5</sub>: Cycocel at 1000 ppm; T<sub>6</sub>: Ethrel at 200 ppm and T<sub>7</sub>: Ethrel at 300 ppm. The required quantity of the plant growth regulator was measured, dissolved in distilled water and the final volume was adjusted to make the stock solution. After preparation, the stock solution was diluted with distilled water to achieve the desired concentration for foliar application. The first spray was given 45 days after planting and second spray was given at 60 days after planting. The spraying of chemical was done by hand sprayer (Fig. 1). The plots were irrigated as per the requirement and the weeds were managed by hand weeding at interval of 15 days. The plants were not sprayed with any insecticide and fungicides.



Fig. 1. Spraying of plant growth regulators on 45 and 60 days after planting.

## **Measurement of plant growth characters**

The plant height (cm), number of leaves per plant, leaf length (cm), leaf width (cm) of garlic was recorded at 30,60 and 90 DAP, the chlorophyll content of the plant was calculated by a portable SPAD meter and neck thickness (cm) was measured in 15 randomly selected plants from each treatment.

## Evaluation of morphological traits on garlic bulb

After harvest (half of the leaves are turned yellowish green and pended after 125 days of sowing), 15 garlic bulbs were randomly selected from each treatment for study of yield parameters, i.e., bulb diameter (cm), number of cloves per bulb, clove width (cm), clove length (cm), average bulb fresh weight (g) and total yield (kg/plot). The weights of the bulbs were recorded before and after drying and the moisture content was calculated based on the differences in bulb weight. After a week of shade curing, the leaves were removed, leaving 2-3 cm of neck space from the bulb and stored in ambient condition for storage study.

## Assessment biochemical traits of garlic

The quality and post-harvest losses of bulbs were assessed at 30, 60 and 90 days after storage. 15 bulbs were selected from each treatment and were divided into 3 groups. The ascorbic acid content (mg/100 g) in garlic bulb was analyzed using the titrimetric method with 2,6dichlorophenol indophenol dye (10). The titratable acidity was measured following the method (11). The total soluble solids were determined using an ERMA hand Refractometer with a range of 28-62 °Brix m, previously calibrated with distilled water the following method (12). The total carbohydrate content (g/ 100 g) in the garlic was

Table 1. Effect of pre harvest spray on growth character of garlic.

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estimated using the anthrone reagent method (13). The total phenol content (mg/g) in the garlic was estimated using the Folin-Ciocalteu reagent (FCR) method (14). The amount of pyruvic acid ( $\mu$  mol g<sup>-1</sup>) in the sample was calculated using the method (15). Post-harvest losses, including physiological loss in weight (PLW), rotting and rubbering of garlic bulbs was recorded over the storage of three month.

## **Statistical analysis**

An analysis of variance for a randomized design was performed using the statistical software packages in KAU GRAPES to evaluate the significance of the application of different pre harvest spray on garlic growth, yield, quality and shelf life. The means were compared using an LSD test with a level of significance of P<0.05.

## Results

## Effect of pre harvest spray on growth traits on garlic

The foliar application of 300 ppm salicylic acid and 300 ppm ethrel had a significant impact on all vegetative growth parameters of garlic plants compared to the control (Table 1). The distinct effect was noticed with the highest concentrations of both salicylic acid and ethrel. The highest mean values of plant height (61.34 cm), number of leaves plant<sup>-1</sup> (9.4), leaf length (35.94 cm), leaf width (1.91 cm), neck thickness (4.66 cm) and total chrolophyll content (66.89 SPAD) were recorded after 90 days of planting when salicylic acid was sprayed at the concentration of 300 ppm, followed by ethrel 300 ppm (Fig. 3).

observed	PH (cm)			NOL (No.)			NT (cm)				LL (cm)		LW (cm)		
Treatment /DAP	30	60	90	30	60	90	30	60	90	30	60	90	30	60	90
T1: Control	16.56 **	34.06*	45.78 **	2.80	4.73 *	8.20	1.20 **	1.52 **	3.44 **	13.72*	24.26*	29.10 *	0.64*	0.98**	1.12 **
T2: Salicylic acid at 100 ppm	18.51 **	35.02*	50.16 **	3.40 **	4.86 *	8.46 *	1.14 **	1.70 **	3.96 **	15.15*	25.02*	29.30 *	0.87* *	1.06**	1.25*
T3: Salicylic acid at 200 ppm	17.56 **	39.60*	57.16*	2.80 *	5.26 **	8.66 **	1.38 *	1.84 **	4.28 **	15.18*	29.34*	33.03 **	0.62*	1.32**	1.48*
T4: Salicylic acid at 300 ppm	18.35*	45.20* *	61.34*	3.10 *	6.20 **	9.40 **	1.59 *	2.40 **	4.66 **	15.84* *	34.60* *	35.94 **	0.71*	1.66**	1.91 **
T5: Cycocel at 1000 ppm	17.82*	40.80*	57.28 **	3.13 *	5.33 **	8.73 **	1.64 *	2.04 *	4.48 *	14.98* *	29.42*	33.56 **	0.72*	1.40**	1.54*
T6: Ethrel at 200 ppm	16.84 **	37.57* *	52.96 **	2.60 **	5.00 **	8.53 *	1.22 **	1.76 **	4.06 **	13.32*	26.78* *	29.67 *	0.70*	1.15**	1.31*
T7: Ethrel at 300 ppm	18.12*	41.02*	58.78*	3.00 *	5.60 **	9.06 **	1.44 *	2.10 *	4.50 *	15.29*	30.20*	34.90 **	0.69* *	1.50**	1.74 **
SEd	0.43	0.91	1.08	0.08	0.13	0.17	0.03	0.05	0.11	0.33	0.74	0.71	0.01	0.03	0.03
CD (P<0.05)	0.93	1.99	2.36	0.18	0.27	0.37	0.07	0.10	0.25	0.71	1.62	1.55	0.03	0.06	0.07

PH-plant height, NOL- number of leaves, NT- Neck thickness, LL- Leaf length, LW- Leaf width (\*\* Significant, \* non-significant).

## Effect of pre harvest spray on yield traits of garlic

All preharvest treatments clearly improved total yield, fresh bulb weight and bulb diameter compared to the control (Table 2). The application of cycocel 1000 ppm had the most pronounced effect on total yield (3.75 kg/ plot), followed by salicylic acid 300 ppm (3.57 kg/plot) and Ethrel 300 ppm (3.47 kg/plot). The lowest yield (2.87 kg/plot) was obtained from the control. The cycocel 1000 ppm treatment resulted in increased fresh bulb weight (50.27 g), bulb diameter (17.75 cm), no. of cloves per bulb (22.60), clove length (3.60 cm), clove width (1.76 cm), clove weigth (8.26 g) and moisture content (66.56 %) at harvested stage significantly over the control (Fig. 2). Followed by cycocel, salicylic acid (300 ppm) exhibited second best impact on growth and productivity with fresh bulb weight (43.15 g), bulb diameter (16.20 cm), no. of cloves per bulb (22.00), clove length (3.22 cm), clove width (1.62 cm), clove weight (6.99 g) and moisture content (65.15 %) which was again better than control.

# Effect of pre harvest spray on biochemical traits of garlic

The ascorbic acid, acidity, phenolic content and pyruvic acid levels decreased progressively as the storage duration



Fig. 2. Cycocel 1000 ppm sprayed in garlic bulbs (Allium sativum L.) cv. Ooty 2.

increased from 30 to 90 days (Fig. 4 and 5). Cycocel at 1000 ppm consistently recorded the highest values across all 3 parameters, with ascorbic acid content at 20.00, 15.40 and 11.25 mg 100 g<sup>1</sup>, acidity at 0.58 %, 0.46 % and 0.38 %, pyruvic acid at 44.66, 41.83 and 38.99 µ mol g<sup>1</sup> and total phenolics at 18.05, 10.85 and 4.10 mg g<sup>1</sup> at 30, 60 and 90 days after storage respectively. Followed by the treatment with ethrel 300 ppm. In contrast, the control showed the lowest values, with ascorbic acid content decreasing to 10.00, 7.10 and 5.00 mg 100 g<sup>1</sup>, acidity dropping to 0.38 %, 0.25 % and 0.19 % and phenolic content reducing to 7.00, 4.35 and 2.15 mg 100 g<sup>1</sup> at 30, 60 and 90 DAS respectively. In addition, the bar chart depicts (Fig. 5) total soluble solids (TSS) and total carbohydrate content increased with the prolonged storage period, with cycocel at 1000 ppm exhibiting the highest TSS values of 37.0, 38.0 and 39.2 ° Brix, total carbohydrate content of 34.10, 36.96 and 39.82 g 100 g<sup>1</sup> at 30, 60 and 90 DAS respectively, followed by ethrel at 300 ppm. The lowest TSS and total carbohydrate content was recorded in the control treatment, with TSS of 30.5, 31.05 and 31.8 °Brix, total carbohydrate content of 23.12, 25.98 and 28.84 g 100  $g^1$  at 30, 60 and 90 DAS respectively.





Fig. 3. Effect of pre harvest spray on total chlorophyll content (SPAD) of garlic cv. Ooty 2.

Table 2. Effect of pre harvest spray on yield character of garlic bulbs.

Yield Traits	YP (kg)	BW (g)	BD (cm)	NOC	CW (g)	CL (cm)	WC (cm)	MC (%)
T1: Control	2.87**	30.8 5**	13.80**	14.50**	2.66**	2.77**	1.38*	62.00**
T2: Salicylic acid at 100 ppm	3.02**	35.88**	14.50**	17.66**	3.54**	3.30*	1.41*	63.54*
T3: Salicylic acid at 200 ppm	3.28**	39.55**	15.40**	21.00**	5.87**	3.33*	1.43*	64.36**
T4: Salicylic acid at 300 ppm	3.57*	43.15**	16.20**	22.00**	6.99**	3.22*	1.62*	65.15*
T5: Cycocel at 1000 ppm	3.75**	50.27**	17.75**	22.60**	8.27**	3.60**	1.76**	66.56**
T6: Ethrel at 200 ppm	3.11**	37.74**	14.93**	18.33**	4.57**	3.21*	1.42*	64.01*
T7: Ethrel at 300 ppm	3.47*	40.59**	15.86**	21.50**	6.16**	3.26*	1.60*	65.35*
SEd	0.08	0.99	0.31	0.27	0.08	0.06	0.04	1.11
CD (P<0.05)	0.18	2.15	0.67	0.60	0.18	0.14	0.08	2.43

YP- Yield per plot, BW- Bulb weight, BD- Bulb diameter, NOC- Number of cloves per bulb, CW- Clove weight, CL- Clove length, WC- Width of clove, MC- Moisture content (\*\* Significant, \* non-significant).







Fig. 4. Effect of pre harvest spray on ascorbic acid (mg 100 g<sup>-1</sup>), acidity (%) and total phenols content (mg g<sup>-1</sup>) of garlic cv. Ooty 2.

Fig. 5. Effect of pre harvest spray on TSS (°Brix), total carbohydrate content (g 100 g -1) and pyruvic acid (µ mol g<sup>-1</sup>) on garlic cv. Ooty 2.

## Effect of pre harvest spray on storability of garlic

The physiological loss in weight, rotting, rubbering and total loss of garlic bulbs increased with the duration of storage from 30 to 90 days (Table 3). The highest physiological loss in weight was observed in the control treatment with values of 4.13 %, 10.73 % and 17.47 % after 30, 60 and 90 days after storage (DAS) respectively. In contrast, the lowest physiological loss was recorded in garlic bulbs which were treated with pre harvest spray of cycocel at 1000 ppm recorded 3.63 %, 8.17 % and 12.63 % at 30, 60 and 90 DAS respectively, followed by ethrel at 300 ppm. Similarly, rotting and rubbering losses were only observed at 90 DAS. Cycocel at 1000 ppm exhibited the least rotting (5.88 %) and rubbering (5.13 %) compared to the control, which showed the highest rotting (8.57 %) and rubbering (8.12 %) at 90 DAS. Consequently, the total loss was highest in the control treatment, with 4.13 %, 10.73 % and 34.15 % at 30, 60 and 90 DAS respectively, while Cycocel at 1000 ppm recorded the lowest total loss, with values of 3.63 %, 8.17 % and 23.64 % over the same periods. Notably, no sprouting loss was observed at 30, 60 and 90 days after storage under ambient condition in Ooty 2 variety of garlic.

## Discussion

The results revealed that the plant growth regulators had a significant impact on plant growth, yield and quality traits of garlic cv. Ooty 2. Application of both growth regulators salicylic acid (300 ppm) and ethrel (300 ppm) resulted in improved growth characters (Table 1). Salicylic acid and ethrel are known to be important photosynthesis regulators because they have an impact on stomatal closure, leaf and chloroplast structure, chlorophyll and carotenoid contents and the activity of enzymes like 5bisphosphate RuBisCO (ribulose-1, carboxylase/ oxygenase) (16). This could lead to a greater transfer of photoassimilates to the bulb and an increase in the number of leaves per plant. An increase in plant growth characteristics may be largely attributed to the enhanced effect on cell division in the sub apical meristems and cell enlargement growth regulator on cell division in the sub

<b>Fable 3.</b> Effect of pre harvest spray on storability of garlic bulb	S.

apical meristems and cell enlargement (17, 18). Exogenous application of salicylic acid results in increased photosynthetic activity which improves the growth characters like plant height, number of leaves and chlorophyll content, etc. (18). Similar reports on positive impact of SA on vegetative growth in garlic, such as (8, 9, 19-22). Cycocel is an anti-gibberellin dwarfing chemical that causes a gibberellin deficiency in plants and slows down development by preventing the initial step in the production of gibberellin, geranyl pyrophosphate, from being converted to copalyl pyrophosphate and also increase the cytokinin biosynthesis. By preventing cell proliferation and elongation in the shoot tissues, growth retardants such as CCC control plant height without changing the overall structure of the plant. Cycocel reduces the height of the plant and promotes the vertical growth of its branches, allowing the plant to absorb more light for photosynthesis and increasing seed yield (23). Cycocel at 1000 ppm, a growth retardant, resulted in a highest yield compared to other growth regulators (Table 2). Similar reports regarding the accelerate yield of cycocel have also been noticed (24, 25) in garlic and (26) in redgram.

Decrease in ascorbic acid during storage is due to the enzyme ascorbinase oxidizing L-ascorbic acid to dehydro ascorbic acid (27). Acidity and phenolic content also decreased during prolonged storage of 3 months. The finding of the present investigation is in accordance to earlier reported studies (3, 28, 29). The increase of TSS could be attributed to growth promoting substances that potentially accelerate the synthesis of vitamins, carbohydrates and other qualitative traits (30). Pyruvic acid is inversely proportional to TSS and carbohydrate content of garlic (Fig. 5).

To extend the storage life of garlic bulbs, it is essential to minimize weight loss of the bulb during storage. Cycocel functions as an inhibitory chemical that reduces respiration rates, thereby decreasing moisture loss from the bulbs (5). When compared to the control, the total loss was lowest in bulbs treated with cycocel at 1000 ppm. This could be the result of anti-gibberellin activity,

Trait observed	PLW (%)			Rubbering (%)			Rotting (%)			Total loss (%)		
Treatment/DAP	30	60	90	30	60	90	30	60	90	30	60	90
T1: Control	4.13*	10.73**	17.47**	0.0 0	0.0 0	8.11**	0.0 0	0.0 0	8.57**	4.13*	10.73**	34.15**
T2: Salicylic acid at 100 ppm	4.08*	10.38**	16.74**	0.0 0	0.0 0	7.41**	0.0 0	0.0 0	7.41**	4.08*	10.38**	31.56*
T3: Salicylic acid at 200 ppm	3.81**	9.13**	14.38**	0.0 0	0.0 0	6.25*	0.0 0	0.0 0	6.25**	3.81**	9.13**	26.88*
T4: Salicylic acid at 300 ppm	3.95**	9.59**	15.27**	0.0 0	0.0 0	5.88*	0.0 0	0.0 0	6.90**	3.95**	9.59**	28.05*
T5: Cycocel at 1000 ppm	3.63*	8.17**	12.63**	0.0 0	0.0 0	5.13**	0.0 0	0.0 0	5.88**	3.63*	8.17**	23.64**
T6: Ethrel at 200 ppm	4.02*	10.01**	16.01**	0.0 0	0.0 0	7.14**	0.0 0	0.0 0	7.14**	4.02*	10.01**	30.30**
T7: Ethrel at 300 ppm	3.72*	8.65**	13.52**	0.0 0	0.0 0	6.90**	0.0 0	0.0 0	6.67**	3.72*	8.65**	27.08*
SEd	0.09	0.19	0.47	-	-	0.18	-	-	0.13	0.09	0.19	0.62
CD (P<0.05)	0.19	0.41	1.02	-	-	0.39	-	-	0.29	0.19	0.41	1.34

PLW- Physiological loss in weight (\*\* Significant, \* non-significant).

which may have made it easier to preserve the quality of bulbs during storage by inhibiting sprouting, which reduced moisture and caused a physiological weight loss (31). These findings align with the results of previous studies (29, 32, 33). The lack of sprouting loss could be related to the influence of growth inhibitors like endogenous abscisic acid (ABA), which is recognized as a component of the growth inhibitor complex present in bulbs. As storage time increases, the levels of endogenous ABA decreases, potentially preventing sprouting, as observed in onion bulbs during prolonged storage (34).

This knowledge about relationships between plant growth regulators and garlic bulb can be particularly important for garlic growing farmer to increase the yield as well as storability of bulb. However, it is important to recognize that the precise interactions between leaf and bulb anatomy and their quantitative and qualitative characteristics remains to be fully defined.

## Conclusion

This study investigated the significant impact of pre harvest spray on garlic Ooty 2 cultivar for enhancing the yield, quality and shelf life. The plant growth regulators were significantly affecting plant growth, yield and storability of bulb compare to control. The pre-harvest application of salicylic acid 300 ppm after 45 and 60 days of planting resulted in superior growth characters, followed by ethrel (300 ppm) but for yield and quality attributes were maximum by pre-harvest spray with CCC 1000 ppm compared to the control. The pre-harvest spray with CCC 1000 ppm also led to a minimum physiological loss in weight, rubbering, rotting and total loss which was found to be superior when compared to all other treatments utilized during the storage period. A preharvest double spray of Cycocel 1000 ppm minimized the quality loss and storage loss over the control after 3 months storage presents a novel approach that can substantially benefit garlic farmers. By implementing these pre-harvest spray strategies, garlic cultivators can achieve higher yields and improved product quality, translating to enhanced market value and economic gain. This research fills a critical gap in sustainable agricultural practices, providing actionable insights for local stakeholders to optimize their cultivation methods and ultimately improve their livelihoods. Future investigations should focus on scaling these practices and examining their long-term benefits to further support for garlic production.

## Acknowledgements

We would like to thank Horticultural Research Station Ooty for providing field for investigated in this study. We also thank all members of the Horticultural Research Station, Wood house farm located at Ooty, Tamil Nadu, India for their help with entire crop production specially sowing, irrigation, weeding, spraying, harvesting and also storage studies.

## **Authors' contributions**

IS Conceptualization of the research, methodology design, significantly to writing the original draft and editing the manuscript. IP Conducted the literature search, organized the data, ensured the integrity of the research and wrote sections of the original draft and overall supervision. SA contributed to data visualization and formatting of the manuscript. VS played a key role in writing parts of the original draft and in the review and editing process. TSP contributed to the formal analysis and validation of the collected data and also participated in writing the manuscript.

## **Compliance with ethical standards**

**Conflict of interest:** Authors do not have any conflict of interests to declare.

Ethical issues: None

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