



RESEARCH ARTICLE

Synergistic effects of cincturing and biofertilisers on reproductive growth and yield of litchi

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Abstract

The present experiment was conducted during 2022–23 and 2023–24 at Horticulture Research Centre, School of Agricultural Sciences, Shri Guru Ram Rai University, Dehradun, Uttarakhand to study the synergistic effects of cincturing and biofertilisers on reproductive growth and yield of litchi on 15-year-old litchi trees. The Experiment was laid out in factorial randomised block design (RBD) with 3 replications comprising 24 treatments. The result obtained from the investigation revealed that among different cincturing levels, C₂ (Cincturing level 4 mm width) took fewest days to panicle initiation, days taken to flower initiation and maximum number of fruits/panicles as compared to uncinctured plants whereas C₁ (Cincturing level 2 mm width) showed positive response in taking minimum days to bloom. In continuation, the maximum number of fruits/trees was noticed in treatment C₃ (Cincturing level 6 mm width). In contrast of this the application of biofertilisers also influenced the flowering and yield parameters of litchi. Biofertilisers treatment B₁ (at 150 g/tree) recorded best treatment as it took the minimum days to panicle emergence. Treatment B₃ (phosphate-solubilising bacteria (PSB) at 150 g/tree) took minimum days to flower initiation, duration of blooming, number of fruits/panicle whereas, the maximum number of fruits/trees was recorded in treatment B₂ (vesicular-arbuscular mycorrhiza (VAM) at 150 g/tree). There was a positive interaction effect was also observed in combined application of cincturing and biofertilisers in which treatment combination C₁B₁ (CL₁ + *Azotobacter* at 150 g/tree) took the minimum time to panicle initiation in both the years of investigation C₁B₃(CL₁ + PSB at 150 g/tree) and C₄B₀(CL₄ + control) took minimum time to flower initiation. The C₁B₀(CL₁ + Control) took minimum time for the duration of flowering while C₃B₃ (CL₃ + PSB at 150 g/tree) recorded with maximum fruits/panicle.

Keywords: azotobacter; biofertilisers; cincturing; flowering; litchi; yield

Introduction

Litchi was introduced to India in 18th century. Litchi needs temperatures between 15 °C and 27 °C for fruit set and a certain level of water stress is necessary for flower initiation (1). Low humidity appears to be harmful to fruit set, while high temperatures (above 38 °C) and relative humidity below 60 % can cause significant skin cracking in litchi fruits. Both low and high temperatures, along with improper nutrient levels and irrigation, can lead to undesirable yield losses or a decline in fruit quality (1, 2).

Despite the wide variety of fruits available, the demand for fresh litchi remains consistently high because of its distinct taste, flavour and colour. Generally, the reddening of the epicarp and the flattening of the tubercles are considered indicators of fruit maturity (3). India has seen substantial growth in both the area and production of litchis and currently ranks second, just after China, in terms of both area and production. Bihar is the top state in India for litchi cultivation and production. Litchi cultivation is confined and restricted to some states of India because; many litchi orchards experience irregular flowering,

excessive vegetative growth, poor fruit set and inconsistent fruit bearing (4, 5). Vegetative flushing occurring just before floral induction leads to poor or no floral initiation, often resulting in the growth of vegetative shoots instead. In litchi (*Litchi chinensis* Sonn.), poor fruit set is typically caused by a failure in pollination and fertilisation, a lack of carbohydrates due to overcast weather, or competition between fruit and vegetative growth (6). A portion of the carbohydrates produced through photosynthesis is used for the growth of new tissues, while another part is stored as reserves during times of surplus production. To overcome the problem of alternate bearing, excessive and irregular vegetative growth girdling can be effectively used to suppress the vegetative growth before flowering (7).

Cincturing, also known as girdling, is a horticultural practice that involves the removal of a narrow strip of bark from around the entire circumference of a branch or trunk. This technique is widely utilised in litchi cultivation to regulate vegetative growth and enhance flowering and fruiting. In litchi, excessive vegetative growth, particularly under high humidity and nutrient-rich conditions, often hampers flowering and subsequently fruit set. Cincturing disrupts the downward flow of photosynthates and growth regulators through the phloem, thereby increasing the accumulation of carbohydrates and hormones like auxins and cytokinins above the girdled zone (8–10). This physiological change promotes floral initiation and improves fruit retention and size. The practice is typically performed during the post-monsoon or early winter months, aligning with the natural floral induction period of litchi. It is especially beneficial in subtropical regions, where temperature fluctuations and vegetative vigour pose challenges to flowering (11). When properly applied, cincturing can significantly improve productivity and fruit quality in litchi orchards, making it a valuable tool in sustainable fruit production. The girdling technique is effective in reducing vegetative growth, enhancing fruit size, weight, yield and promoting fruit maturity in various fruit crops such as peach (12, 13).

The continue and indiscriminate uses of chemical fertilisers have become the common practice in modern litchi orchards that adversely affect the soil fertility and health by disturbing the soil properties and plant mycorrhizal association, decline the humus, quality and yield of produce and increased the environmental hazards and resistant power against biotic and abiotic factors (14). Phosphorus (P) availability in Indian soils ranges from poor to medium but applying the right amount of P has been shown to promote the formation of healthy nodules (15). The application of biofertilisers is one of the important techniques and sustainable approach to increase and maintain the soil health by providing the essential nutrients to the soil. Biofertilisers viz., phosphate-solubilising bacteria (PSB), vesicular-arbuscular mycorrhiza (VAM) etc., converts in soluble soil P into a form that plants can readily absorb. The VAM fungi enhance plant growth by improving P nutrition. Unlike phosphate solubilisers, VAM fungi do not convert unavailable or insoluble P into accessible forms. Instead, they assimilate P, zinc and other nutrients, transporting them into the host root to meet both their own needs and those of the plant (16, 17). The mode of action of *Azotobacter* on plant growth is linked with the production of biologically active compounds, including auxin, Vitamin B complex and cytokinin (18).

Materials and Methods

The present field experiment was conducted during 2022–23 and 2023–24 for two consecutive years at Horticulture Research Block of Department of Horticulture, School of Agricultural Sciences, Shri Guru Ram Rai University, Dehradun, Uttarakhand to assess synergistic effects of cincturing and biofertilisers on reproductive growth and yield of litchi. The centre is in the foothills of the Himalayas, experiencing a humid subtropical climate. It is positioned at latitude of 30.31° N and longitude of 78.03° E, with an elevation of 640 m above sea level. The region experiences a subtropical climate with moderate seasonal variation. During the investigation period, the mean maximum temperature ranged from approximately 18 °C in winter months to about 34 °C in summer, while the mean minimum temperature varied between 50 °C and 22°C. Relative humidity fluctuated between 50 % and 85 %, with higher values recorded during the monsoon season and lower values during summer months. The area received an average annual rainfall of about 2000–2200 mm, the majority of which occurred during the monsoon period June–September. Occasional winter showers were also observed. The variation in winter temperature and pre-flowering climatic conditions during 2023–24 was particularly important, as litchi flowering is highly sensitive to cool temperature exposure and relative humidity levels. Therefore, the recorded meteorological data were considered essential for interpreting treatment responses related to growth, flowering, yield and fruit quality under subtropical conditions at Pathribagh campus, Dehradun. 15-year-old litchi tree with 10 × 10 m planting distance and uniform age were selected for the study. The trees were chosen based on uniformity in vigour, canopy size and general growth performance to minimise experimental variation. Only healthy trees free from visible pest and disease infestation, mechanical damage, or physiological disorders were included. Efforts were made to ensure that the selected trees had a comparable previous bearing history, avoiding extremes of regular heavy bearing or alternate bearing tendencies. All selected trees were grown under similar cultural and management practices, including irrigation, fertilisation and pruning history, to maintain uniformity in growing conditions. The trees were spaced uniformly within the orchard and received identical environmental exposure. To reduce experimental error arising from tree-to-tree variability, the selected trees were grouped into homogeneous sets as per randomised block design (RBD), where each treatment was replicated across comparable trees within each block. This ensured that variation due to soil heterogeneity, microclimate differences and inherent tree variability was statistically controlled. The soil of the experimental field was a sandy loam texture, having a pH range between 6.5–7 and had a medium fertility level. The experiment was conducted in a factorial randomised block design (FRBD) comprising two factors, namely cincturing (Factor A) with six levels and biofertilisers application (Factor B) with four levels. The combination of these factors resulted in a total of 24 treatment combinations ($6 \times 4 = 24$). All the combinations are illustrated in Table 1. Each treatment combination was replicated three times, thereby constituting three experimental trees per treatment combination. In total, 72 uniform litchi trees ($24 \times 3 = 72$) were included in the experiment. Each treatment combination was randomly allotted to one tree per replication within each block to ensure uniform distribution and to minimise environmental

Table 1. Treatment symbol with their combination

Treatment symbol	Treatment combinations
C ₀ B ₀	Control (No cincturing and no biofertiliser)
C ₀ B ₁	No cincturing + <i>Azotobacter</i> at 150 g/tree
C ₀ B ₂	No cincturing + VAM at 150 g/tree
C ₀ B ₃	No cincturing + PSB at 150 g/tree
C ₁ B ₀	Cincturing level 2 mm width + No biofertilisers
C ₁ B ₁	Cincturing level 2 mm width + <i>Azotobacter</i> at 150 g/tree
C ₁ B ₂	Cincturing level 2 mm width + VAM at 150 g/tree
C ₁ B ₃	Cincturing level 2 mm width + PSB at 150 g/tree
C ₂ B ₀	Cincturing level 4 mm width + No biofertilisers
C ₂ B ₁	Cincturing level 4 mm width + <i>Azotobacter</i> at 150 g/tree
C ₂ B ₂	Cincturing level 4 mm width + VAM at 150 g/tree
C ₂ B ₃	Cincturing level 4 mm width + PSB at 150 g/tree
C ₃ B ₀	Cincturing level 6 mm width + No biofertilisers
C ₃ B ₁	Cincturing level 6 mm width + <i>Azotobacter</i> at 150 g/tree
C ₃ B ₂	Cincturing level 6 mm width + VAM at 150 g/tree
C ₃ B ₃	Cincturing level 6 mm width + PSB at 150 g/tree
C ₄ B ₀	Cincturing level 8 mm width + No biofertilisers
C ₄ B ₁	Cincturing level 8 mm width + <i>Azotobacter</i> at 150 g/tree
C ₄ B ₂	Cincturing level 8 mm width + VAM at 150 g/tree
C ₄ B ₃	Cincturing level 8 mm width + PSB at 150 g/tree
C ₅ B ₀	Cincturing level 10 mm width + No biofertilisers
C ₅ B ₁	Cincturing level 10 mm width + <i>Azotobacter</i> at 150 g/tree
C ₅ B ₂	Cincturing level 10 mm width + VAM at 150 g/tree
C ₅ B ₃	Cincturing level 10 mm width + PSB at 150 g/tree

PSB: phosphate-solubilising bacteria; VAM: vesicular-arbuscular mycorrhiza.

variation. The experiment was analysed following standard FRBD procedures to account for variability among blocks and treatment interactions. For recording observations, a systematic within-tree sampling approach was followed. From each experimental tree, five primary branches were selected in each of the four cardinal directions (north, south, east and west), ensuring uniform canopy representation. Thus, a total of 20 primary branches per tree were used for recording observations related to vegetative growth, flowering, fruit set, yield and fruit quality parameters. The selected branches were of similar age, vigour and exposure to sunlight to minimise intra-tree variability and ensure consistency in data collection.

The cincturing was performed in the last week of September after the completion of vegetative growth of litchi trees in both years (2022 and 2023). A strip of bark was removed in a circular pattern around the chosen primary branches as part of the treatment in both years. The biofertilisers, namely PSB, VAM and *Azotobacter* at 150 g/replication were applied in the tree basin during the month of December in litchi plants through soil application. The required quantity of each biofertilisers was mixed thoroughly with well-decomposed farmyard manure (FYM)/compost as a carrier material to ensure uniform distribution and better survival of microbial inoculants. The application was done in a circular basin around the tree, approximately extending up to the drip line. The mixture was incorporated into the soil at a depth of about 10–15 cm in the active root zone to facilitate effective colonisation and nutrient interaction. Care was taken to avoid injury to major roots during soil incorporation. Immediately after application of biofertilisers, light irrigation was provided to maintain adequate soil moisture and to enhance microbial activity and establishment in the rhizosphere. The data were recorded as flowering attributes in litchi total number of flowers per panicle, number of male flowers per panicle, number of female flowers per panicle, percentage of male flowers per panicle and percentage of female flowers per panicle.

Statistical analysis

The observations collected during the field experiment, along with the data obtained, were analysed statistically using SPSS software. The experiment was conducted in a FRBD and analysis of variance (ANOVA) was performed to test the significance of treatment effects and their interactions. After ensuring that the assumptions of ANOVA were adequately satisfied, the significance of differences among treatment means was tested at the 5 % probability level ($p = 0.05$). The critical difference (CD) was used for comparing treatment means and interaction effects between factors were interpreted accordingly. After ensuring that the assumptions of ANOVA were adequately satisfied, the significance of differences among treatment means was tested at the 5 % probability level ($p = 0.05$). The CD was used for comparing treatment means and interaction effects between factors were interpreted accordingly.

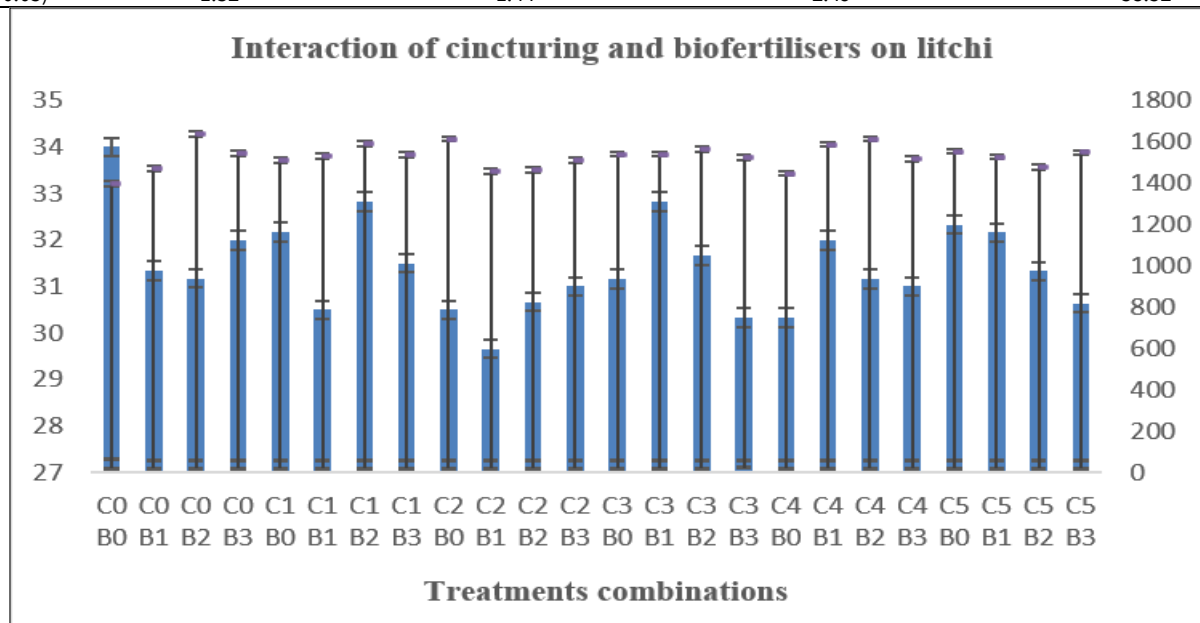
Results and Discussion

Days taken to panicle initiation

As data presented in Table 2 and Fig. 1, regarding the days taken to panicle initiation, notable variation was recorded among both the applied factors. Cincturing treatment C₂ (30.91 days and 30.00 days) took minimum time to panicle emergence as compared to C₀ (32.16 days and 32.08 days) in both the year of investigation. Earlier panicle initiation was observed in cincturing treatments this may be because of high carbon (C) : nitrogen (N) ratio, high cytokinin : auxin ratio and carbohydrate availability in cinctured shoots that induces early panicle initiation than non-treated plants. The girdling done in first fortnight of September and removal of new shoots result in early panicle emergence as compared to control and advance the early harvest of crop (19). All the girdled treatments induced early panicle emergence of about 28–42 days after girdling as compared to control (20). On the other hand, considering the different levels of biofertilisers treatment B₁ (31.16 and 31.50) showed best response maximum mean value

Table 2. Interaction of cincturing and biofertilisers treatments on flowering and fruiting in litchi

Treatment	Days to panicle initiation	Days to flower initiation	Number of fruits per panicle	Number of fruits per tree
C0 B0	34.00a	64.17a	20.66bc	1399.2d
C0 B1	31.34cd	63.00ab	21e	1470.5bc
C0 B2	31.17d	60.16d	18b	1639.9a
C0 B3	32.00bc	62.33bc	21.83b	1543b
C1 B0	32.17bc	62.33bc	22.33e	1512.6b
C1 B1	30.50e	62.16bc	18.33bc	1530.5b
C1 B2	32.83ab	60.67cd	21.33b	1592.1a
C1 B3	31.5 °Cd	61.0 °Cd	21.66a	1536.5b
C2 B0	30.50e	61.17cd	22.83a	1613.8a
C2 B1	29.66f	62.66bc	23.33b	1458c
C2 B2	30.67e	61.17cd	21.67ab	1464.3c
C2 B3	31.00de	61.83cd	22.5c	1510.6b
C3 B0	31.16d	62.00bc	20.83b	1540.5b
C3 B1	32.83ab	61.16cd	22.33bc	1541.1b
C3 B2	31.66cd	61.17cd	21.17a	1566.3ab
C3 B3	30.33e	61.16cd	23.83b	1525.7b
C4 B0	30.33e	63.50ab	21.83bc	1446.8c
C4 B1	31.99bc	61.66cd	21.33b	1586.8a
C4 B2	31.16d	61.5 °Cd	21.83bc	1614.1a
C4 B3	31.00de	61.5 °Cd	21.33bc	1516.3b
C5 B0	32.33b	61.83cd	21b	1554.6b
C5 B1	32.16bc	62.33bc	21.67b	1527.1b
C5 B2	31.33cd	60.67cd	22.33cd	1479.2c
C5 B3	30.64e	61.83cd	19.66bc	1549.3b
SE(m) ±	0.46	0.50	0.87	20.49
CD (p = 0.05)	1.32	1.44	2.49	58.52

**Fig. 1.** Interaction of cincturing and biofertilisers on litchi.

was found in C₀(32.00 and 31.66) in 2023–24.

This trend of results may be due to the *Azotobacter* effect on soil nutrient status. *Azotobacter* is mainly responsible for the fixation of atmospheric N, nutrient mineralisation and increases soil microbial activity that results in higher carbohydrate accumulation and advances the time of panicle initiation. Application of *Azotobacter* increase the N metabolism and growth regulators production which is mainly responsible for profuse floral initiation (21, 22). The interaction between cincturing and biofertilisers was found significant in which the maximum time was taken by control C₀B₀ (34.00 and 34.01) in both the years of study trail and minimum time was taken by C₁B₁ (30.33).

Days taken to flower initiation

According to the findings summarised in Table 2 and Fig. 1, days taken to flower initiation was greatly influenced by the various treatments of cincturing and biofertilisers. In cincturing treatment

C₂(61.16 days) took minimum days to flower initiation as compared to C₀ (65.00 days) in 2023 and in 2024 cincturing treatment C₃(61.25 days) took minimum time to flower initiation as maximum time taken to flower initiation was recorded in C₀ (62.48 days). The recorded trends from the present investigation may be associated with the fact that cincturing increases the auxin: cytokinin ratio and reduce the biosynthesis of gibberellins which is considered as a flowering inhibitor because of this the level of cytokinins like growth promoting enhances the early flowering in litchi tree. Girdling improved earliness of flowering in mango (23). Among the different treatment of biofertilisers treatment B₃(60.77 and 61.00 days) recorded the most significant results in terms of taking the minimum time to flower initiation as compared to B₀ (62.83 and 62.72 days) in 2023–24. These observed results may be associated with the fact that improved nutrient availability from the application of PSB and others biofertilisers encourages vegetative growth and support carbohydrate accumulation that

imparts early flowering. The obtained results are also confirmatory with the results of many scientists who concluded that the treatment PSB (6 kg/ha) + GA3 (100 ppm) registered an improved plant growth with least time to produce first flower (57 days) compared to control treatment (24, 25). The combined application of N : P : potassium (K) + vermicompost + biofertilisers (*Azotobacter*, *Azospirillum* and PSB) had a significant effect on guard flowering also as the applied treatment took minimum days taken to appearance of first male as well as female flower anthesis that appeared at earliest node (26). The positive interaction between the cincturing and biofertilisers was also observed during the period of experimental study in which minimum value was found in C₁B₃ (59.66 days) which was found statistically at par with C₀B₂ (59.66 days) however, the maximum value of interaction was recorded in C₀B₀ (65.00) in first year of investigation 2023 and in 2024 the highest value of interaction was noticed in C₄B₀ (64.66 days) and minimum value was noticed in C₃B₀ (60.33 days). When cincturing combines with biofertilisers, the effect becomes more pronounced, both the factor shows their individual effect on flowering attributes of litchi tree cv. Calcuttia.

Duration of blooming

The results presented in Table 2 and Fig. 1 demonstrated that duration of blooming was also influenced by various treatments of cincturing. Among the various cincturing treatment applied C₁ (12.66 and 13.75 days) showed the best results in terms of duration of blooming, as it took the minimum days to blooming as compared to C₀ (14.16 and 15.41 days) as it took maximum time to bloom in the consecutive year of experimental trail. This might be due to the adequate availability of stored photosynthates in primary branches and at blooming time the utilisation of stored photosynthates become easy that results in early flowering and blooming. Cincturing treatment took less time to bloom as compared to control (27). Biofertilisers application in year 2023 was found non-significant on duration of blooming however, in year of 2024 the biofertiliser treatment B₃ (14.22) was proved to be highly effective and showed significant response towards the duration of blooming with maximum mean value as compared to B₀ (15.41).

The earliest flowering in strawberry plant provided with application of PSB, vermicompost and 50 % recommended dose of fertiliser (RDF) (28). The minimum number of days taken to first flower initiation and days to 50 % flowering was recorded in strawberry plants supplied with 100 % NPK + FYM + *Azotobacter* + PSB inoculation (29). The significant effect of interaction between cincturing and biofertilisers were also noticed in 2023 the minimum value was obtained in treatment of C₁B₀ (12.33 and 12.66) in both the years of study and maximum value was recorded in treatment C₂B₀ (15.66) respectively, in 2024 the maximum value was observed in treatment C₁B₀ (12.66).

Number of fruits/panicles

An examination of Table 2 and Fig. 1 shows that number of fruits/panicles observed noticeable variation among the different treatments. Among the cincturing, treatment C₂ (22.75) was found most effective in terms of maximum number of fruits/panicle as compared to C₀ (20.33) with in 2023 however, in 2024 the maximum mean value was observed in treatment C₃ (22.58) and the minimum number of fruits/panicle was found in C₀ (20.41). The increased number of fruits/panicles in cinctured treatments might

be associated with the fact that the branches bear a greater number of fruits/panicles that contain a greater number of leaves there is a direct relationship between number of leaves per panicle and total number of fruits/panicles, more leaves determined more assimilates, sugar and greater fruit load per panicle. There was a direct relation between girdling and number of leaves, the maximum fruits at pea stage were recorded in 150 leaf treatments, followed by 100 and 50 leaf treatments (30). The highest number of fruits per raceme was found in girdled and pruned trees as compared to control (31). On the other hand, biofertilisers application in 2023 was found non-significant whereas, the maximum mean value was noticed in treatment B₂ (21.72) and minimum mean value was recorded in treatment B₁ (*Azotobacter* at 150 g/tree) in 2023.

In second year of experimental trail biofertilisers application found to be effective in which the maximum number of fruits/ Panicle was observed in treatment B₃ (22.61) and minimum mean value in B₀ (20.38). This might be because of the application of biofertilisers like PSB which convert the insoluble form of P to the available form that increased the root growth, energy metabolism and carbohydrate content in the litchi tree that favours more fruits/panicle. The maximum number of fruits per pillar was observed in treatment treated with vermicompost (0.5 kg/plant), *Azospirillum* (50 g/plant) and PSB (50 g/plant) in treatment (T₁₁) followed by T₁₀ treatment respectively (32). Fruit yield parameters like number of fruits/plants, average fruit weight, fruit yield, fruit length and pulp weight/fruit were found maximum in treatment RDF + *Azospirillum* at 10 g/ plant + PSB at 10 g/ plant (33). There was a positive interaction between cincturing and biofertilisers were recorded in which the maximum value was recorded in treatment C₃B₃ (24.00) and minimum value was recorded in C₀ (18.66) in 2023 and in 2024 maximum value of interaction was found in C₃B₃ (23.66) and minimum value was obtained in C₀B₂ (17.00). This might be due the synergistic effect of both the factors.

Number of fruits/tree

The data presented in Table 2 and Fig. 1, on number of fruits/trees clearly indicate that both the factors viz, cincturing and biofertilisers had an impactful influence on fruiting behaviour. Among the different treatments of cincturing C₃ (1589.1) showed best response in terms of maximum number of fruits/tree and minimum number of fruits/tree was recorded in C₀ (1523.0). This might be due to that cincturing decreased the leaf/fruit ratio resulting increased in number of fruits/trees. Number of fruits and yield of Kinnow mandarin was significantly increased by pruning along with girdling in Kinnow mandarin as compared to ungirdled trees (34). The C-shaped girdling technique significantly increase the total number of fruits per plant (35). Biofertilisers Application was found significant in 2023–24 in which the best observation was noticed in treatment B₂ (1540.7 and 1578.0) and the lowest mean value was observed in B₀ (1479.6 and 1542.9).

The obtained results from the present investigation agree with previous studies, which concluded that the application of biofertilisers in combination was highly effective in improving yield -contributing attributes in strawberry. The highest number of fruits per plant and highest fruit weight was observed in treatment RDF + *Azospirillum* at 7 kg/ha + PSB at 6 kg/ha + VAM at 10 kg/ha respectively. In 2023 the maximum value of interaction was noticed in treatment C₀B₂ (1746.6) and minimum in C₀B₀ (1306.3)

which was found non-significant and in 2024 the maximum value was noticed in C₄B₂ (1689.0) and minimum in C₄B₀ (1441.6) (36).

Conclusion

The present study demonstrates that the combined application of cincturing and biofertilisers exerts a synergistic effect on the reproductive growth and yield of litchi. Cincturing, by regulating carbohydrate translocation and enhancing flowering intensity, complements the role of biofertilisers, which improve soil fertility, nutrient availability and microbial activity. Together, these practices significantly increase key reproductive parameters such as flowering percentage, fruit set and fruit retention. This positive interaction ultimately leads to a substantial improvement in yield attributes, including number of fruits per tree and overall fruit productivity. The integrated use of cincturing and biofertilisers therefore represents an effective and sustainable strategy for enhancing litchi production, offering both physiological and ecological benefits over the use of either practice alone.

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Authors' contributions

AB executed the research work, designed the methodology and drafted the initial manuscript. SSB, AS and KKC analysed the data. RPJ, VD, GB and PK interpreted the data. AKR, AKS, DD, DKV and R reviewed the manuscript and helped with the final corrections. All authors have read and approved the final manuscript.

Compliance with ethical standards

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

Ethical issues: None

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