Original Research Article

Evaluating the Effectiveness of Organic Seed Priming Formulations on Germination and Seedling Growth in Acid Lime (*Citrus aurantifolia*Swingle) cv. Kagzi

Abstract

The primary objective of this research study was to evaluating the effectiveness of organic seed priming formulations on germination and seedling growth in acid lime cv. Kagzi at CCS Haryana Agricultural University, Hisar, during the period 2020-2021. The study comprised six distinct treatments of organic formulations, including beejamrit, cow urine (50% and 100%), cow dung, cow dung slurry, and a control (without soaking). The seeds were soaked for a duration of 24 hours. The experiment was conducted using a completely randomized design with three replications.The study findings revealed that the control (without soaking) exhibited the highest germination percentage (45.17) and germination rate (2.38), followed closely by the beejamrit. Furthermore, the beejamrit treatment demonstrated remarkable seedling growth at 180 days after sowing, with the highest values recorded for seedling diameter (3.23 mm), seedling shoot length (36.90 cm), fresh shoot weight (3.26 g), fresh root weight (1.26 g), tap root length (37.57 cm), and survival percentage (91.50). Cow dung slurry also exhibited notable performance in terms of seedling growth parameters at 180 days after sowing with the beejamrit. Cow dung slurry showcased the highest number of secondary roots (50.00), number of leaves per seedling (31.67), leaf chlorophyll content (33.80 SPAD), and nitrogen content (1.52%), closely followed by the beejamrit treatment. Accelerating the rate of germination and growth of lime seedlings has become a top concern for nurserymen and scientists on a national level to reduce expenditures and time for seedling maintenance. For overcoming such types of problems at the nursery stage,contributing to more resilient and sustainable farming practices usingorganic seed priming is a good alternative against chemical seed priming.

KEYWORDS: Beejamrit, Cowdung slurry, Cowdung, Cowurine, Kagzi lime, Organic formulations, Seed priming

Introduction

Citrus is considered world's most common, popular, and favorite fruit. In tropical and sub-tropical regions, citrus fruit is widely grown and enjoys a prominent place among the people. It possesses greater adaptability to different climatic conditions. Internationally citrus plantation is confined to 0-40˚ latitude covering different regions having different soil and climatic condition (Ghosh, 2000). Globally citrus is grown in 114 countries. The world citrus is dominated by sweet orange (71%) followed by mandarin (13%), lime & lemon (10%), grapefruit and other species (6%) (FAO, 2020-21). India is the sixth-largest producer of citrus in the world. In India, citrus is grown in an area of about 1104.900thousand hectareswith a production of 14535.900 thousand metric tons with productivity of 13.2 metric tons per hectare (Indiastat, 2022-23) and the third most important fruit crop after mango and banana.In Haryana, citrus is grown in an area of about 24,400 ha with a production of 570.88 thousand metric tons and productivity of 23.40 metric tons per hectare (Indiastat, 2022-23). Among various citrus species, acid lime (*Citrus aurantifolia* Swingle) originated in India and is commercially grown in tropical and subtropical regions.Acid lime belongs to the family *Rutaceae*and is native to India and South-Eastern China. The lime is called Kagzi in India, which owes its origin to the Indian word kagaj, meaning paper, with which the rind of this fruit is always compared for its thinness. Nutritional and medicinal value are all highly regarded benefits of citrus fruits. It is a rich source of vitamin C and has good antioxidant properties. Fruits being acidic, they are largely used for garnishing and flavoring several vegetarian and non-vegetarian dishes. Besides its value-added products like pickles, juice,

squash, cordials, syrups, lime peel oil, peel powder, etc. are also in great demand in the soap and cosmetic industry (Debaje*et al.*, 2011). It also makes delicious and refreshing cold drinks. Kagzi lime is one of the important fruit crop grown throughout the world. The major kagzi lime-producing countries are China, Mexico, Brazil, and India. India ranks first among major lime-producing countries in the world (Indiastat, 2022-23). The total area of lime& lemon in India is 311.9 thousand hectareswith a production of 3586.5thousand metric tons with average productivity of 12.1 metric tons per hectare. (Indiastat, 2022-23).

Priming refers to pre-sowing seed soaking treatment with water, chemicals, bio-stimulants, organic formulations etc. It is receiving considerable attention by the scientific community in the last two decades (Yakhin *et al.,* 2017). Very little information has been reported on the effects of organic formulations seed priming in fruit crops and there are no reports on appropriate natural or organic seed priming techniques and the subsequent seedling development in acid lime. With the increasing demand for organic fruits, there is a need to evaluate organic seed priming methods. In conventional systems, seed priming is widely used for many crops to accelerate the rate of germination and to improve uniformity in the establishment of the seedlings (Taylor and Harman 1990; Halmer 2003). This main aim of this experiment was to evaluate organic formulations seed priming effect on germination and seedling growth of acid lime, to meet the requirements of organic nursery stock production and organic fruit production. The use of organic formulationsforabating the seed dormancy and enhance germination & seedling growth of young seedling in various fruit crops has been reported by Kumar*et al.* (2020) observed significant increase in germination, root length, root diameter, number of secondary roots, fresh root weight, dry root weight in ber with treatment of water and cowurine; Yadav *et al*. (2018) reported that water and cowurine has substantial increase in fresh & dry shoot weight, fresh & dry root weight at 150 DAS in custard apple; panchgavya was found to be most beneficial for improving germination percent and took minimum days for germination, height of seedling, number of leaves, stem girth, leaf area, length of tap root, number of roots per seedling, root/shoot ratio, fresh weight of seedling, dry weight of seedling, survival percentage and minimized mortality percentage Bagul*et al.* (2013)& Desai *et al*. (2017)cow urine 10% dipping for 18 hours noted significantly the maximum height of seedling, fresh weight of root, stem diameter, highest fresh shoot weight,highest leaf area at 45 DAS. in papaya; Vachhani*et al.* (2014) observed substantial increase in germination percentage, seedling length, primary roots, secondary roots, number of leaves, leaf area, & mortality percentage in Khirnee with treatment cowdung slurry; Jadhav and Deshmukh (2019) observed noteworthy increase in number of seedlings per seed, primary root length, secondary root length and seedling diameter at 180 DAS in Rangpur lime with cowurine 50 & 100% ; a substantial increase in sprout height, number of leaves, rootstock diameter & height and germination index was observed in mango with amritpani and panchagavya by Kumar*et al.* (2008); Patel *et al*. (2017)observed significant increase in shoot and root parameter with treatment of beejamruth (3%) in mango. similar results were obtained by Reshma and Simi (2019) in mango with cowdung slurry & Pavithra *et al.* (2018) in Suriname cherry with vermiwash (10%); Tandon *et al.* (2019) reported substantial increase in germination and survival percentage in tamarind with cowurine and cowdung slurry.

The farmer of the state show very high enthusiasm for the cultivation of acid lime. Hence, there is a scope for an increase in the area under this fruit crop. In kagzi lime low germination, unbalanced seedling growth, high mortality, and slow growth of seedling in the nursery are among the major problems faced by farmers (Shant and Rao, 1973 & Gupta, 1989). So, the major problem is the lack of sufficient and healthy seedlings. Its germination percentage is low and it varies between 27-58 percent (Anandam and Singh, 1969) and takes about 3 weeks

to germinate (Naik, 1949 and Cheema *et al.*, 1954). However, kagzi lime is commercially propagated through seeds in India as it comes true to type, because of the high degree (39-60%) of nucellar embryony and self-pollination of the hermaphrodite flower rarely results in genetically variable seeds (Salih & Idris, 2018). In plants, propagated through seed, better and quicker germination of seeds and production of maximum number of seedlings are highly essential to meet the increasing demand of the cultivars in the shortest possible time. Therefore, accelerating the rate of germination and growth of lime seedlings has become a top concern for farmers, nurserymen and scientists on a national level to reduce expenditures and time for seedling maintenance.

Materials and methods

1)**Location:**The present experiment was carried out on freshly extracted seeds of acid lime cv. Kagzi in the screen house, Department of Horticulture, College of Agriculture, CCS Haryana Agricultural University, Hisar, India during the year 2020-2021,

2) Climate: Hisar is situated at 29º10′ N latitude and 75º46′ E longitude, with the experimental location situated at 215.2 m above mean sea level. The climate in Hisar is semiarid with hot and dry summers and extremely cold winters. Both maximum and minimum monthly temperatures exhibit a wide range of variations both during summer and winter. During the season of May to June, there is a maximum temperature of around 45°C and a minimum temperature of freezing point accompanied by occasional frost in winter. The total rainfall as well as its distribution in the region is affected by weather patterns. Rainfall is erratic with 20-30% annual variation and 30-50% seasonal variation. Around 80 percent of the annual rainfall (about 450 mm) occurs from July to September. A few showers can also be expected from December to February due to westerly disturbances.

3) Seed extraction: The required seed material of acid lime (*Citrus aurantifolia* Swingle) cv. Kagzi for the experiment was collected from experimental orchard, Department of horticulture, CCS Haryana Agricultural University, Hisar. Fully ripened healthy fruits with even size were selected for seed extraction from a single tree. The seeds were properly extracted from the fruits and washed in running water. The seeds were also rinsed in a strainer and gently pushed off with a soft towel to remove the jelly-like coating from the seeds Then, all extracted seeds were placed in water. Floating seeds were discarded and seeds sink to the bottom of water used for the experiment purpose.

4) **Preparations of organic formulations:**

a) Beejamrit: 5kg desi cowdung + 5 ltr cow urine + 50 gm chuna + 50 gm field soil. All these constituents are mixed well in 20 litre of water and allowed for 24 hours; 2 times clockwise rotation was given in a day.

b) Cow urine (50%): 50 ml cowurine of desi cow was added to 50 ml distilled water

c) Cow urine (100%): 100 ml cowurine (pure)

d) Fresh cow-dung (desi cow): Cowdung and water was mixed in the ratio of 9:1

e) Cowdung slurry: Cowdung, cowurine& water were mixed in the ratio of (200 g: 200 ml: 2000 ml) and allowed for the fermentation process for 12 hours. After that 24 g jaggery was added and further allowed for fermentation (12 hours).

f) Control (withoutsoaking)

5) Treatments: The experiment was laid out in completely randomized design (CRD), with three replications, with 40 seeds per replication. There were six treatments. The treatments were as follows: T1– beejamrit,T2– cowurine 50%, T3– cowurine 100%,T4– cowdung, T5– cowdung slurry, T6– control (no soaking at all- freshly extracted seeds were sown).

6) Seed treatments: Seeds soaked for 24 hours for each treatment andsown in polybags containing media: sandy soil, FYM and coco-peat (2:1:1) (v/v), which were properly filled,

labeled with tags and placed as per layout. Seeds were sown by using dibbling method at a depth of 3-4 cm.

7) **Data collection:**The observations on height, diameter and no. of leaves were recorded at monthly interval starting from 90 days after sowing up to 180 days after sowing. The observations as number of seedling emerged per seed, days taken for first & fifty percent seed germination, germination rate and survival percentage were recorded upto 45 days after sowing. The data on number of branches per seedling, leaf area, shoot fresh and dry weight, chlorophyll content, leaf nitrogen content, root length & root diameter, root fresh & dry weight, number of secondary & fibrous roots, dry shoot to root ratio were measured at the end of the experiment 180 days after sowing of seeds at randomly selected five plants in each treatment. The germination rate provides a measure of the time course of seed germination. The number of seedlings emerging daily were counted from day of sowing the seeds in growing media till the germination is complete (upto 45 DAS). Thereafter, germination index (G.I.) was computed by using the formula (Abdul-Baki and Anderson, 1973):

$$G.I. = n/D$$

Where, n = number of seedlings emerging on day'd'

d = day after sowing

The seedling having greater germination index was considered to have more germination rate.The leaf area of five representative plants was measured for each replication in each treatment at 180 days after sowing by laser leaf area meter CI-202 and average value was computed & expressed in cm^2 per leaf. The chlorophyll content of sixth & seventh leaf from tip of each selected plant was measured by SPAD meter and the average value of each replication was expressed as SPAD value.

8) **Statistical Analysis**:Analysis of variance (ANOVA) for completely randomized design was done and significant ($P \leq 0.05$) differences between treatments were determined using the least significant difference ($LSD_{0.05}$). The software program used for the analysis was statistical software package for agricultural research workers (Sheoran *et al.*, 1998).

Results and discussion

A. Germination parameters

The germination parameters *i.e.*germination percentage and germination rate were significantly influenced by different organic formulations priming treatments. All the treatments found low values of germination parameters over control. The control treatment has substantially more pronounced effect on germination percentage (45.17) and germination rate (2.38). After control, cowdung slurry and beejamrit were at par with each other for all germination parameters.However, the lowest values were obtained under cowurine 100% followed by cowurine 50% (Figure I and II). These results were contrary to earlier findings Pal *et al.* (2019) who reported significant increase in germination as compared to control in karonda when treated with cow-urine 25, 50 and100 percent;Prajapati *et al.* (2014) recorded significant upsurge in germination and survival percentage with treatment of cowdung and cowurine;Vacchani*et al.* (2014) using priming treatment cowdung slurry & the significantly early and highest germination percentage were recorded by soaking in cattle urine followed by in cowdung slurry for twelve hours by Shinde and Malshe (2015) in Khirni; Parmar *et al.* (2018) reported significantly higher germination and survival percentage with treatment of cowdung, cowurine and beejamrit in jack fruit; Desai *et al.* (2017) noted significant increase in germination in papaya with application of cowurine; parallel results reported by Chandel *et al.* (2013) in mango;Significant higher germination percentage was observed with priming treatment of cowurine and cowdung paste in Rangpur lime by Jadhav and Deshmukh (2019); Patel *et al.* (2017) discovered noteworthy improvement in survival percentage with beejamrit 2% & 3% in mango. The delayed and low germination percentage by the organic formulations was might be due to unknown qualitative and

quantitative constituents which causes delayed and poor germination in acid lime. It might be because of unavailability of constituents of organic formulations instantly and becomes available at the later stages of seedling growth.

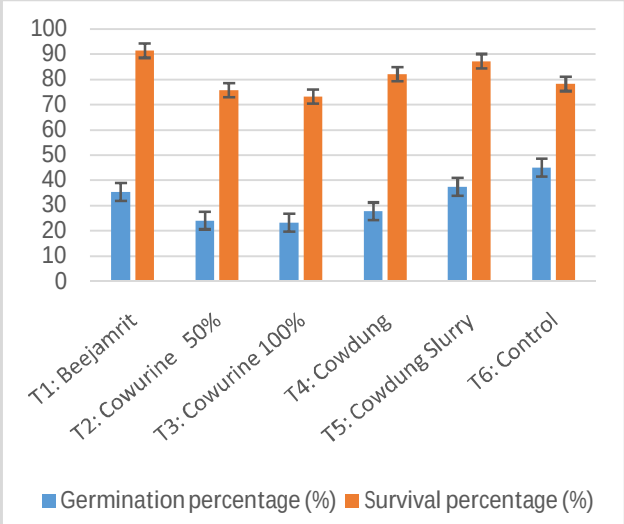

Figure I Effect of seed priming with organic formulations on germination and survival percentage of acid lime (*Citrus aurantifolia* Swingle)cv. Kagzi

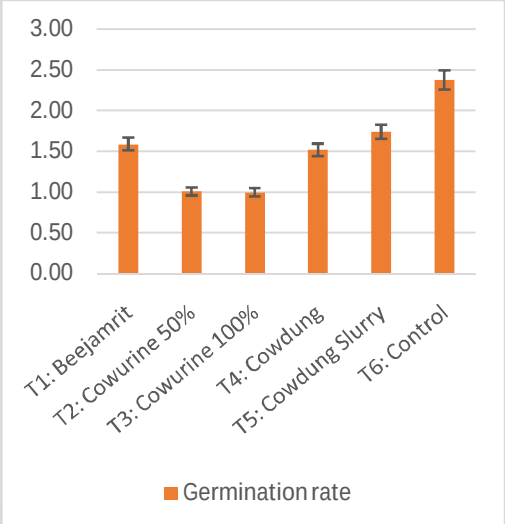

Figure II Effect of seed priming with organic formulations on germination rate of acid lime (*Citrus aurantifolia* Swingle) cv. Kagzi

B. Shoot growth parameters

The shoot growth parameters were significantly influenced by different organic formulations priming treatments. All the treatments found higher values of shoot growth parameters over control, among various treatments highest shoot growth viz. seedling diameter (3.23 mm), seedling height (36.90 cm), leaf area (8.89 cm^2) was observed in beejamrit followed by cowdung slurry after 180 days after sowing. The highest number of leaves per seedling (31.67), leaf chlorophyll (33.80 SPAD) & nitrogen content (1.52%) was observed in cowdung slurry followed by beejamrit. beejamrit and cowdungwere at par with each other for all shoot parameters (Figure III, IV& VI).

This might be due to build-up of microbial consortia supported by Patel (1996) in custard apple and Chaudhari (2010) in mango. Seedling growth parameters like number of branches per seedling could not be affected significantly. This might be also possible that increase in colony of microbial consortia, more availability of micronutrients in beejamrit&cowdung slurry due to fermentation process, which supports catalytic, metabolic and hormonal processes. Parmar *et al.* (2018) reported significantly higher shoot growth, seedling height, seedlingdiamete, number of leaves per plant and leaf area with treatment of cowdung slurry and beejamrit in jack fruit; Pal *et al.* (2019) reported significant increase in shoot growth parameters viz. seedling height, seedling diameter, number of leaves per seedling, leaf area, fresh and dry shoot weight in karonda when treated with cow-urine 50 & 100% in karonda; Analogousresults were obtained by Patel (1996) in custard apple and in mango by Chandel *et al.* (2013); Jadhav and Deshmukh (2019) noted significant higher number of seedling per seed, seedling height and diameter with cowurine 50% and cowdung paste in Rangpur lime; in suriname cherry significant higher seedling height, number of leaves, seedling girth was reported by Pavithra *et al.* (2018); Prajapati *et al*. (2014) discovered significant increase in seedling height, seedling diameter, number of leaves per seedling, fresh and dry shoot weight in treatment cowdung and cowurine; Vacchani*et al*. (2014) recorded significant higher values for shoot growth parameters in cowdung slurry seed priming in

Khirnee; All shoot growth parameters were significantly improved with cowurine seed priming by Desai *et al.* (2017) in papaya and also Bagul *et al.* (2018) reported significant higher values for number of leaves per seedling, seedling height, girth of seedling , leaf area, fresh shoot and dry shoot weight in papaya with beejamrit, cowdung slurry anscowurine 50%.

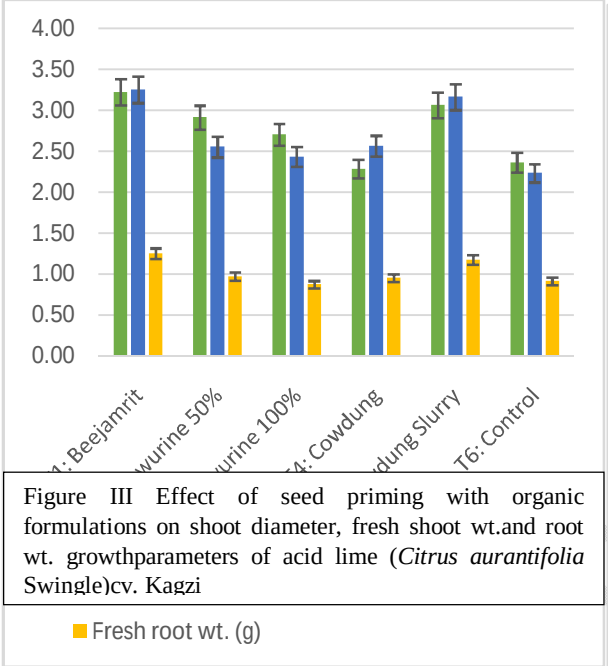

Figure III Effect of seed priming with organic formulations on shoot diameter, fresh shoot wt.and root wt. growthparameters of acid lime (*Citrus aurantifolia* Swingle)cv. Kagzi

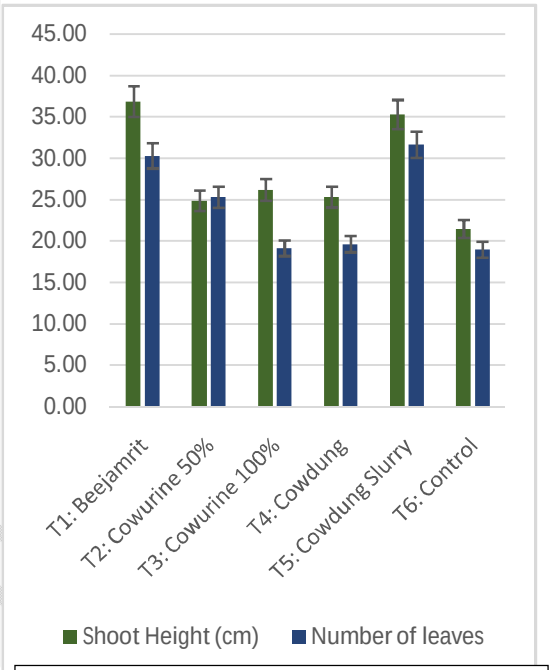

Figure IV Effect of seed priming with organic formulations on shoot height and numberof leavesof acid lime (*Citrus aurantifolia* Swingle)cv. Kagzi

C. Root growth parameters

The root growth parameters were significantly influenced by different organic formulations priming treatments. All the treatments found significantly higher values of root growth parameters over control, among various treatments beejamrit found highest root length (37.57 cm), number of secondary roots (50.00), root fresh weight (1.26 g)& dry shootto root ratio was observed in beejamrit followed by cowdung slurry at 180 days after sowing. Beejamrit and cowdung slurry were statistically at par with each other (Figure III, VI).Improved seedlingroot growth was might be due to increased microbial population and more availability of micronutrients in organic formulations due to fermentation processin beejamrit and cowdung slurry whichsupports catalytic, metabolic and hormonal processes, which helped in re-distribution of photosynthates within the seedling parts and improved root growth. These results are in correspondence with Vaccani*et al.* (2014) who observed significantly effective results in respect of root growth parameters viz. primary root length and no. of secondary roots when seeds were treated with cowdung slurry in khirnee rootstocks; Also Prajapati *et al.* (2014) reported similar results by treating Khirnee rootstock seeds with cowdung, cowurine; Significantly increased root parameters of mango seedling was observed when sown by treating with cowdung slurry by Reshma and Mini (2019); Also analogous results was reported by Kumar *et al.* (2008) & Chandel *et al.* (2013) by treating seed with cowdung, cowurinein mango; In Rangpur lime, Jadhav and Deshmukh (2019) recorded significantly higher primary and secondary root length parameters with cowurine and cowdung paste; Parmar *et al.* (2018) noted maximum number of roots in jack

fruit seed primed with cowdung slurry; In papaya Desai *et al*. (2017) &Bagul*et al*. (2018) recorded significant effective results in case of rooting parameters viz. root length, fresh and dry weight of root with cowurine, cowdung, beejamrit and cowdung slurry primed seeds; In tamarind, seed primed with cowurine and cowdung slurry found an effective increase in roots parameters compared to control reported by Tandon *et al*. (2019).

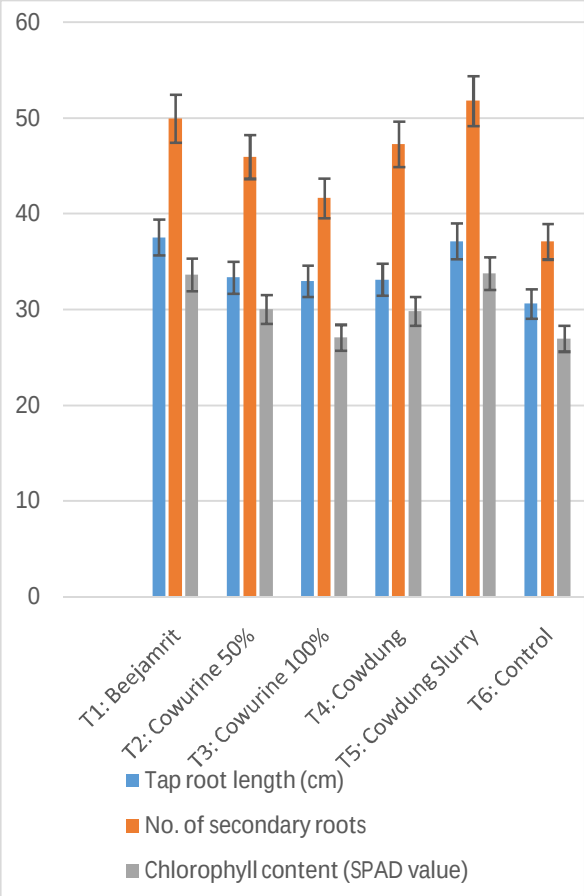

Figure V Effect of seed priming with organic formulations on rootlength, secondary roots and chlorophyll content of acid lime (*Citrus aurantifolia* Swingle)cv. Kagzi

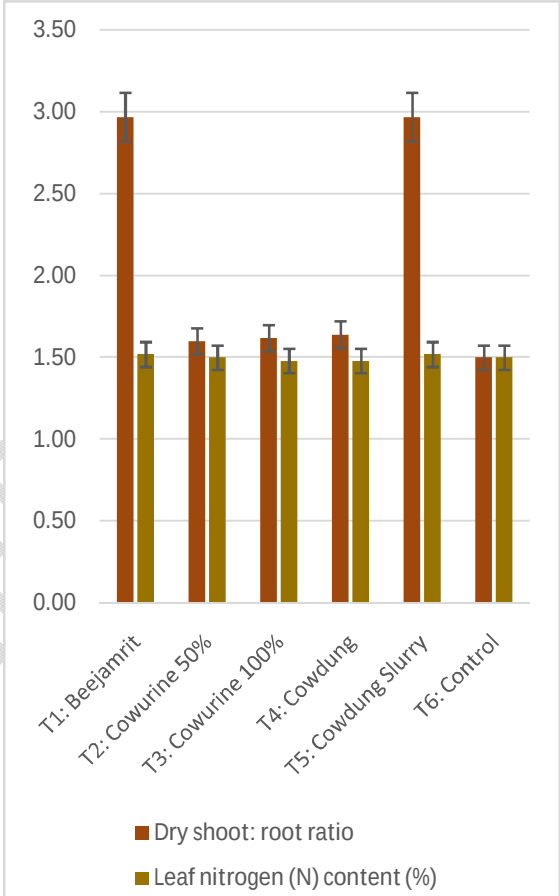

Figure VI Effect of seed priming with organic formulations on dry shoot to root ratio & leaf nitrogen content of acid lime (*Citrus aurantifolia* Swingle) cv. Kagzi

D.Leaf biochemicalparameters

Chlorophyll content and nitrogen content in leaf was found highest values in cowdung slurry and beejamrit. This might be because of increased microbial population and more availability of micronutrients in organic formulations during fermentation process in beejamritand cowdung slurry due to fermentation process which supports catalytic, metabolic and hormonal processes, which helped in re-distribution of photosynthates within the seedling parts ultimately improved root growth. These results are in confirmation with Vacchani*et al*. (2014) who recorded significant higher values for shoot

growth parameters in cowdung slurry seed priming in Khirnee; All shoot growth parameters were significantly improved with cowurine seed priming reported by Desai *et al.* (2017) in papaya and also Bagul *et al.* (2018) reported significant higher values for number of leaves per seedling, seedling height, girth of seedling , leaf area, fresh shoot and dry shoot weight in papaya with beejamrit andcowdung slurry.The organic formulations seed priming analysis revealed that beejamrit and cowdung slurry have found numerically higher values for the leaf nitrogen content. This might be attributed to the increase in colonies of microbial consortia & better availability of micronutrient, hormones and organic matter content in beejamrit and cowdung slurry.

Conclusion

The appraisal of organicseed priming formulations revealed that sowing of seeds without any organic formulations priming treatment was the most effective method compared to soaking seeds in organic formulations, but soaking of acid lime seeds in beejamrit and cowdung slurry for twenty-four hours was found to be the most successful in promotingshoot and root growth,resulting in higher seedling quality other treatments.The application of beejamrit and cow dung slurry as a seed treatment showed promising results in enhancing shoot and root growth in the organic production of acid lime seedlings.Organic priming formulations offer a promising alternative to chemical treatments, potentially improving seedling vigor, contributing to more resilient and sustainable farming practices.Further investigation into the chemical, physical and microbiological properties of the seed priming treatments is necessary to better understand the underlying causes of these effects. There is potential to further improve acid lime germination by exploring alternative pre-sowing seed treatments.

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