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Quercetin-infused Flax Seed Polymer: A Seed Quality Booster and It's Impact on Green Gram (*Vigna radiata* L.)

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ABSTRACT

Background: Guava leaves (*Psidium guajava* L.) are rich in quercetin, a flavonoid with antioxidant properties. Flaxseed polysaccharide, has a potential for organic polymer synthesis, is infused with quercetin to enhance the planting value of green gram seeds.

Methods: The study was carried out in the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore during the year of 2022-2024 to standardize the impact of flaxseed polymer infused with quercetin. The mucilage method was used for polymer extraction and treated over green gram (*Vigna radiata* L.) seeds to assess the physiological and biochemical traits. The degradability of flaxseed polymer was studied by assessing pH, EC and organic carbon content in the soil. The field trial was carried out, in order to evaluate the potential of flaxseed polymer infused with quercetin @ 1% over pod yield ha^{-1}.

Result: The flaxseed polymer infused with quercetin @ 1% resulted in increased speed of germination (32.99), germination per cent (98%), root length (16.6 cm), shoot length (19.1 cm), dry matter production (0.216 g 10 seedlings^{-1}) and vigour index (3499). The degradability studies indicated the changes in pH from 7.88 to 7.72, increased EC from 0.51 to 0.63 and improved soil organic carbon content 0.56 to 0.84. Increased plant height (36.6 cm), leaf area index (6.082), chlorophyll content (44.6 @ 40 DAS) and increased seed yield of 631.6 kg ha^{-1} was recorded. Hence, seeds coated with flaxseed polymer infused with quercetin @ 1% could be recommended as eco-friendly pre sowing seed treatment to enhance germination, root growth, seedling vigour and crop productivity.

Key words: Guava leaves, Flaxseed polymer, Quercetin, SEM, Seedling vigour, Yield.

INTRODUCTION

Green gram (*Vigna radiata* L.) seeds enhances soil fertility by fixing atmospheric nitrogen, which enriches the soil with essential nutrients and their deep roots help in preventing soil erosion and maintaining soil health. It is mainly grown in arid and semi-arid regions and plays important role towards achieving improved human nutrition and health conditions, reducing poverty through food security and enhancing ecosystem resilience as a source of human food, animal feed, soil nitrogen and soil health. Statistics showed that though average area under production has been growing since 1978, average production has been fluctuating and consumption increasing steadily upholding constant deficit which is catered for through imports. The country's average green gram yield ranges between 0.5-0.6 ton/ha compared to crop potential of 1.5 ton/ha and global average yield of 0.73 tons/ha by Muchomba *et al.* (2023).

Guava (*Psidium guajava* L.) is a widely cultivated fruit tree known for its nutritional and medicinal properties. Guava leaves contain various bioactive compounds, including quercetin (Magar *et al.,* 2020), which is a flavonoid with antioxidant properties. Several studies have reported the potential of guava leaf extracts in promoting plant growth and enhancing crop yield due to their allelopathic effect and nutrient content. According to Afzal *et al.* (2016), high-quality seed has the ability to boost food security and productivity. Plant uniformity is a sign of seed vigour. To guarantee consistent field establishment with highest yield, farmers and growers are always in want of for premium seeds (Ventura *et al.,* 2012). A number of agronomic and environmental factors such as effective fertilization, mother crop nutrition, edaphic and environmental conditions during development and maturation, harvesting at physiological maturity, appropriate drying and post-harvest handling procedures are having an impact on seed quality during seed production.

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Over the past 30 years, various techniques have been used to enhance seeds including film coating, encrusting, pelleting, magnetic stimulation, hardening, priming and pre-germination (Farooq *et al.,* 2021). Among those film coating with active chemicals with a quick-drying polymer, has gained popularity for its ease of application, minimal impact on seed size, shape and protection against pests and diseases. Seed coating involves applying nutrients, protective layers and other substances to improve seed performance under various conditions (Sohail *et al.,* 2022). However, long-term use of synthetic polymers can increase pollution, soil degradation and contamination, potentially affecting human health.

Mucilage-extracted flaxseed polymers, which are polysaccharides, have been studied for their potential as bioadhesives. They contain lignin polysaccharides contributing to fibre-rich characteristics and salts of polymerized aldotetronic acid including d-xylose, l-rhamnose, l-galactose and d-galacturonic acid. Their gel-forming ability after heating makes them useful as thickeners and stabilizers in the food industry. This study examines the degradation of flaxseed polymer and its effect on green gram seed quality focusing on polymers enriched with quercetin (Bhagat *et al*., 2019) to determine the best polymer for seedling survival.

MATERIALS AND METHODS

The laboratory and field experiments were carried out in the year 2023-2024 at Department of Seed Science and Technology, TNAU, Coimbatore by adapting completely randomized block design and randomized block design with five treatments and five replications (Fig 1).

Preparation of mucilage extracted polymer from flaxseeds

Mucilage extraction

Thirty grams of flaxseeds were soaked in 900 ml of distilled water overnight. The mucilage was then extracted by filtration and centrifuged for 15 minutes at 10,000 rpm. To isolate the polysaccharide, 30 ml of acetone was added and the beaker was submerged in a 90°C hot water bath for 30 minutes (Fig 2).

Polymer preparation

The extracted polysaccharide (2 g), agar (3 g), and turmeric powder (0.5 g) were added to a 50 ml beaker (Fig 2). The beaker was placed in a 90°C hot water bath for 20 minutes, then cooled in a desiccator.

Preparation of flaxseed polymer coating formulation

The seed coating polymer was formulated using flaxseed polymer (100 g), citric acid (2 ml) as a natural preservative, turmeric powder (5 g) as a natural colorant and quercetin (1 ml) (Fig 2). Citric acid was extracted from ripe lemons, centrifuged at 5000 rpm for 10 minutes and the supernatant was used. Turmeric powder, rich in curcumin, was sourced from Orgrain India Pvt. Ltd.

Treatment details

The green gram seeds were coated with flaxseed polymer and the treatment details are given below:

Treatment	Treatment details
T_0	Control
T_1	Hydropriming
T_2	Flaxseed polymer
T_3	Flaxseed polymer + Quercetin @ 1%
T_4	Flaxseed polymer + Quercetin @ 2%

Observations

Seed physical traits

Thousand seed weight and seed moisture content were estimated as per the protocol described in the seed testing manual. The germination test was conducted by following the procedure outlined in ISTA (2019). After 7 days, the root and shoot length was measured from the collar region to the tip of the primary root and tip of the shoot respectively. Also, dry weight was recorded and vigour index were computed as per Abdul-Baki and Anderson (1973).

Biochemical traits

Biochemical traits such as α-amylase, dehydrogenase, catalase and peroxidase activity were carried out by Simpson and Naylor (1962), Kittock and Law (1968), Sinha (1972) and Singh *et al.* (1982) respectively.

Scanning electron microscopy (SEM) analysis of the seed surface

The sample were examined by (Bal tec model SCD 050), with a scanning electron microscope (JEOL-JSM IT 300) at 15 kV.

Degradability of organic polymer

Degradability of flaxseed polymer was studied as per Kato *et al.* (2017).

The soil sample was analysed for its chemical properties before burial of polymer ball and after complete degradation of polymer balls.

Particulars	Procedure	References
Soil reaction (pH)	1:2.5 soil: water extract	Jackson (1973)
Electrical conductivity	1:2.5 soil: water extract	Jackson (1973)
Organic carbon	Chromic acid wet digestion	Walkley and Black (1934)

Physiological attributes under field condition

Plant height, days for 50% flowering and leaf area index was observed in 25 randomly tagged plants in each plot and chlorophyll content were assessed using chlorophyll meter (Model CCM 200 plus) under vegetative, flowering and harvesting stage in each treatment.

Yield attributes under field condition

Number of pods/plant/plot/ha and seed yield/plant/plot/ha were observed in each treatment and seed recovery percentage was calculated under different treatments by comparing with the seeds under control condition.

Data analysis

Variance analysis was conducted using Duncan's multiple range test (DMRT), with significance at $P<0.05$. Data were tabulated with the means of four replicates and standard errors. Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

This study evaluated the effect of flaxseed polymer and its combination with quercetin on green gram seeds by analysing physical, physiological and biochemical parameters. The aim was to enhance the planting value of green gram seeds using these treatments.

Physical parameters

The results showed no significant differences in seed physical parameters like thousand seed weight and moisture content among treatments. Flaxseed polymer formed a thin film around the seeds, maintaining their shape and weight (Table 1).



Fig 1: Experiment plot - field view on crop growth of green gram cv. CO 8.

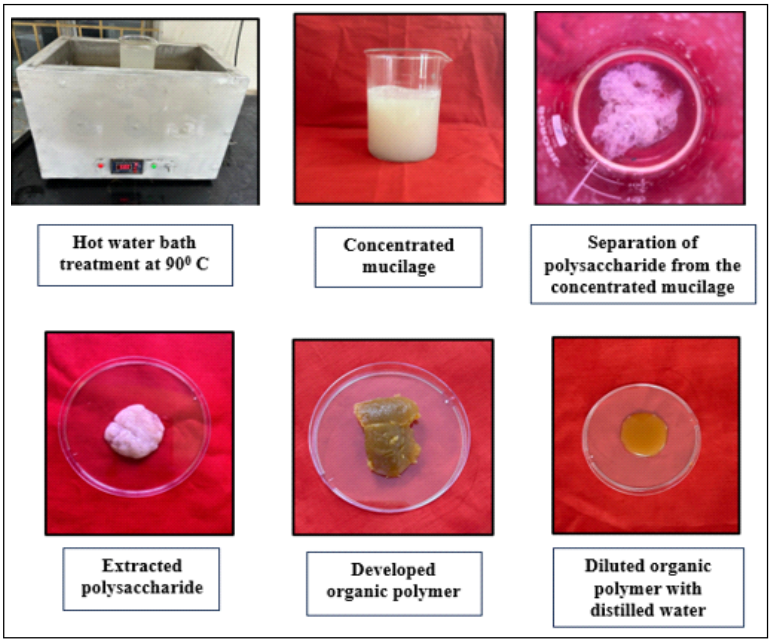

Fig 2: Preparation of mucilage extracted polymer from flaxseeds.

Physiological parameters

Flaxseed polymer infused with quercetin @ 1% significantly increased seed germination (98%) compared to control (82%) (Table 2). This treatment also reduced abnormal seedlings due to the presence of quercetin - a plant growth-promoting substances in the polymer, which enhances cell elongation (Wianowska *et al.,* 2022). Also, quercetin has strong antioxidant properties which was confirmed by HPLC study at a peak range of 10.38 and 10.34 as reported by Hem *et al.* (2024) that will indirectly promotes the root growth under laboratory conditions. Quercetin acts as a scavenger, improving germination by reducing abnormal seedlings and dead seeds (Yang *et al.,* 2021), Quercetin helps plants to tolerate stress (Singh *et al.,* 2024) by improving osmoregulation. The presence of quercetin @ 1% influences processes like photosynthesis, hormone production, antioxidant renewal and cell division Foyer *et al.* (2024). The flaxseed polymer with quercetin significantly increased speed of germination, shoot and root length, dry matter production and vigour index (32.99, 16.6 cm, 19.1 cm, 0.216 g and 3499, respectively) (Table 1 and 2). Quercetin also improved root fresh weight and length in various plants and enhanced root/shoot length by boosting chlorophyll content and photosynthesis (Yoon *et al.,* 2021).

Biochemical parameters

Seeds treated with flaxseed polymer infused with quercetin @ 1% showed a significant increase in antioxidant enzyme activity with peroxidase at 3.89ΔOD 430 mg^{-1} min^{-1} and catalase at 20.77 µg H_2 O_2/min/mg protein compared to control values (3.76 Δ OD 430 mg^{-1} min^{-1} and 20.32 µg H, O_2 /min/mg protein) (Table 3). Tewari *et al.* (2023) observed increased catalase and peroxidase activity in safflower under drought stress when treated with polysaccharide-based polymers. Also, increased enzyme activity was due to the presence of nutraceutical property in quercetin and was reported by Mundhe *et al.* (2016) in aonla juice and Richika *et al.* (2022) over value added products of grapefruit (*Citrus paradisi*).

Scanning electron microscopy (SEM) analysis of the seed surface

No significant differences were observed in the seed coat structure between flaxseed polymer-coated and control seeds. Coated seeds recorded a length and breadth of

Table 1: Effect of flaxseed polymer and it's formulation on stand establishment traits of green gram.

Treatments	1000 seed weight (g)	Speed of germination	Seed moisture content (%)
T_0	48.58±0.22^{NS}	32.26±1.31^{b}	9.24±0.11^{NS}
T_1	48.62±0.04^{NS}	32.15±0.89^{cd}	9.20±0.31^{NS}
T_2	49.15±0.88^{NS}	32.18±0.83^{cd}	9.35±0.05^{NS}
T_3	49.24±0.25^{NS}	32.99±0.16^{a}	9.39±0.01^{NS}
T_4	49.33±0.83^{NS}	31.93±0.84^{e}	9.41±0.16^{NS}

a, b, c is significantly different. Different letters (or) NS used in the column same is not significant.

Table 2: Effect of flaxseed polymer and it's formulation on seedling vigour parameters of green gram.

Treatments	Germination(%)	Root length (cm)	Shoot length (cm)	Dry matter production (g 10 seedlings^{-1})	Vigour index
T_0	82±1.31^{e}	15.8±0.03^{e}	17.3±0.13^{e}	0.137±0.0006^{c}	2714±48.42^{e}
T_1	86±1.74^{d}	16.3±0.01^{c}	18.1±0.06^{bc}	0.119±0.0016^{d}	2958±13.44^{d}
T_2	93±0.19^{c}	16.9±0.29^{a}	18.2±0.04^{bc}	0.201±0.0037^{b}	3264±28.17^{b}
T_3	98±1.35^{a}	16.6±0.26^{b}	19.1±0.08^{a}	0.216±0.001^{a}	3499±73.50^{a}
T_4	95±1.17^{b}	15.9±0.15^{d}	17.8±0.12^{d}	0.125±0.001^{e}	3202±9.26^{c}

a, b, c is significantly different. Different letters (or) NS used in the column same is not significant.

Table 3: Effect of flaxseed polymer and it's formulation on biochemical parameters of green gram.

Treatments	Peroxidase (Δ OD 430 $mg^{-1}min^{-1}$)	Catalase (µ g H_2O_2/min/mg protein)	α-amylase (mg maltose min^{-1})	Dehydrogenas (OD value)
T_0	3.76±0.03^{de}	20.32±0.06^{c}	1.67±0.01^{e}	1.14±0.00^{a}
T_1	3.77±0.12^{de}	20.22±0.36^{b}	1.89±0.00^{d}	1.12±0.01^{b}
T_2	3.97±0.02^{a}	20.56±0.33^{d}	2.01±0.07^{c}	1.09±0.03^{c}
T_3	3.89±0.01^{b}	20.77±0.33^{a}	2.22±0.08^{b}	0.99±0.01^{d}
T_4	3.83±0.01^{c}	19.68±0.31^{e}	2.46±0.07^{a}	0.67±0.04^{e}

a, b, c is significantly different. Different letters (or) NS used in the column same is not significant.

3.998 mm and 2.968 mm while control seeds measured 3.774 mm and 2.769 mm (Fig 3).

Degradability of organic polymer

Concerns about polymers arise from their lack of degradability, landfill closures and pollution emphasizing the need for biodegradable options. Studying flaxseed polymer's degradability is crucial. The degradability of polymer was studied by assessing the soil pH, EC and organic carbon content that will decide the period for complete disintegration in soil (Sonkaria *et al.,* 2022).

The biodegradability of flaxseed polymer was tested in soil under 60% moisture condition. Weight loss of polymer showed increased degradation over time and complete degradation were recorded in seven weeks for 1% quercetin and eight weeks for 2% concentration (Table 4). Slower degradation of polymer is due to preservative, colourant and active component, which are higher molecular weight compounds that degrade more slowly (Senesi *et al.,* 2007).

The soil properties, including pH, EC and organic carbon, were affected by flaxseed polymer. Soil pH decreased from 7.88 to 7.60 with flaxseed polymer, 7.72 with 1% quercetin and 7.53 with 2% quercetin. EC increased from 0.51 to 0.61 with flaxseed polymer, 0.63 with 1% quercetin and 0.59 with 2%. Organic carbon rose from 0.56 to 0.60, 0.84 and 0.74 with flaxseed polymer, 1% and 2% quercetin (Table 5), possibly due to the active compounds acting as biostimulant (Alagoz and Yilmaz, 2009). This shows flaxseed polymer with 1% quercetin is fully degradable without harming soil health.

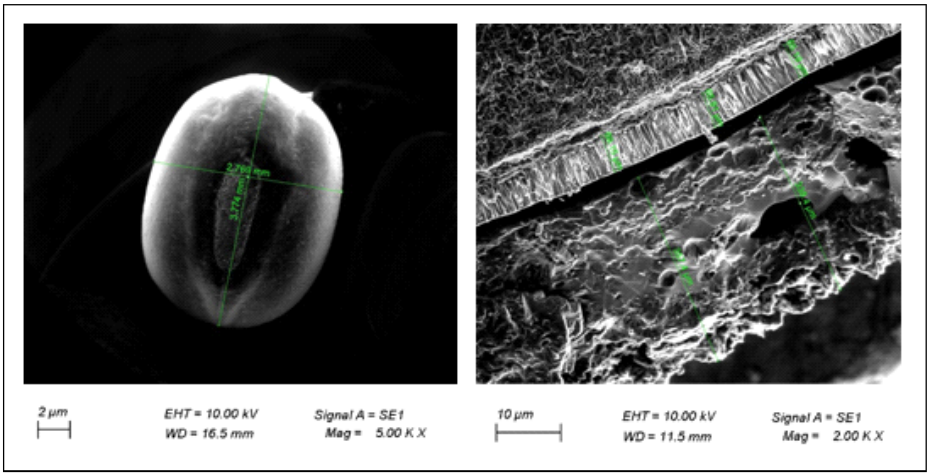

Fig 3: Structure of green gram seed coat and SEM image of flax seed polymer coated seeds.

Table 4: Effect on degradability of flaxseed polymer.

Period of burial (Weeks)	Weight of polymer (g)		
	Flaxseed polymer I	Flaxseed polymer II	Flaxseed polymer III
P_0	2.0	2.0	2.0
P_1	1.6	1.8	1.9
P_2	1.1	1.5	1.7
P_3	0.7	1.3	1.6
P_4	0.4	1.0	1.4
P_5	0.0	0.7	1.1
P_6	0.0	0.3	0.9
P_7	0.0	0.0	0.3
P_8	0.0	0.0	0.0

Table 5: Effect on soil properties before and after degradation of flaxseed polymer.

Parameter	Before burial	After burial		
		Flaxseed polymer I	Flaxseed polymer II	Flaxseed polymer III
pH	7.88	7.60	7.72	7.53
EC (dSm^{-1})	0.51	0.61	0.63	0.59
Organic carbon (%)	0.56	0.60	0.84	0.74

Physiological attributes under field condition

Plant height

Plants from treated seeds were observed with increased height at 20, 40 and 60 days after sowing compared to control. Notably, treatment with flaxseed polymer+Quercetin @ 1% showed significant height increases (11.7, 27.0 and 36.6 cm). Quercetin enhances root elongation and branching by interacting with auxins and cytokinin, crucial for root development and improves the availability of essential nutrient to the meristematic tissue of the plants even under stress condition as reported by Xu *et al.* (2020).

Days to 50% flowering

Treatments showed no significant effect on days to 50% flowering, but flaxseed polymer infused with quercetin @ 1% led to earlier flowering (58 DAS) (Fig 4). Quercetin likely improved root architecture, allowing better access to water and nutrients under stress. Agati *et al.* (2012) reported similar results with quercetin-treated maize showing improved root structure and drought tolerance.

Leaf area index

Results showed significant differences in treatments and their interaction with drought stress condition up to 20 days after sowing with flaxseed polymer infused with quercetin @ 1% (0.140) (Table 6). Quercetin aids in the synthesis of secondary metabolites, crucial for plant defence and resilience against pathogens (Santos *et al.,* 2019).

Chlorophyll content

Flaxseed polymer infused with quercetin @ 1% showed no significant differences in chlorophyll content at flowering stages of green gram under field conditions. However, at the vegetative stage (20 DAS), a significant increase in chlorophyll content (44.6) was observed (Table 6) due to antioxidant properties by Nirbhay *et al.* (2023).

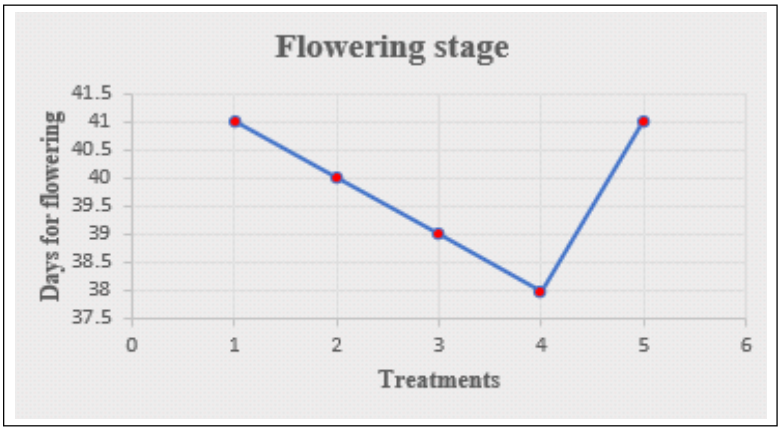

Fig 4: Influence of flax seed polymer on 50% flowering of green gram.

Table 6: Effect of flaxseed polymer on physiological attributes of green gram (CO 8) under field condition.

Treatments	Observation	Vegetative stage (20 DAS)	Flowering stage (40 DAS)	Harvesting stage (60 DAS)
T_0	Plant height (cm)	9.5±0.03^{e}	26.1±0.40^{e}	35.8±0.74^{e}
T_1		10.4±0.04^{d}	26.5±0.30^{bcd}	36.1±0.43^{c}
T_2		10.9±0.12^{c}	26.4±0.52^{bcd}	36.0±0.16^{d}
T_3		11.7±0.21^{ab}	27.0±0.23^{a}	36.6±0.26^{a}
T_4		11.2±0.10^{ab}	26.4±0.17b^{cd}	36.3±0.25^{b}
T_0	Leaf area index	0.059±0.00^{e}	1.329±0.01^{e}	5.853±0.06^{e}
T_1		0.108±0.00^{d}	1.359±0.02^{cd}	5.987±0.07^{d}
T_2		0.125±0.00^{c}	1.359±0.02^{cd}	6.015±0.05^{c}
T_3		0.140±0.01^{a}	1.390±0.04^{a}	6.082±0.09^{a}
T_4		0.132±0.00^{b}	1.382±0.03^{b}	6.037±0.08^{b}
T_0	Chlorophyll content	30.0±0.53 ^{NS}	42.7±0.51^{e}	29.9±0.26 ^{NS}
T_1		30.3±0.33 ^{NS}	43.1±0.60^{bc}	30.2±0.25 ^{NS}
T_2		30.5±0.42 ^{NS}	43.4±0.57^{d}	30.4±0.10 ^{NS}
T_3		30.7±0.47 ^{NS}	44.6±0.78^{a}	30.5±0.58 ^{NS}
T_4		30.4±0.09 ^{NS}	43.1±0.33^{bc}	30.2±0.29 ^{NS}

a, b, c is significantly different. Different letters (or) NS used in the column same is not significant.

Table 7: Effect of flaxseed polymer on pod yield attributes of green gram (CO 8) under field condition.

Treatments	No. of pods per plant	Pod yield per plant (g)	Pod yield per plot (kg)	Pod yield per ha (kg)
T_0	28±0.19^{de}	14.06±0.17^{e}	1.20±0.01^{e}	995.3± 6.87^{e}
T_1	31±0.13^{de}	15.19±0.26^{cd}	1.29±0.01^{d}	1075.2±8.43^{d}
T_2	32±0.35^{abc}	15.49±0.23^{cd}	1.32±0.02^{c}	1097.0±2.93^{c}
T_3	32±0. 58^{abc}	15.91±0.01^{ab}	1.36±0.01^{ab}	1126.1±2.33^{a}
T_4	32±0.48^{abc}	15.76±0.25^{ab}	1.35±0.02^{ab}	1115.1±11.91^{b}

a, b, c is significantly different. Different letters (or) NS used in the column same is not significant.

Table 8: Effect of flaxseed polymer on seed yield attributes of green gram (CO 8) under field condition.

Treatments	Seed yield per plant (g)	Seed yield per plot (kg)	Seed yield per ha (kg)	Seed recovery (%)
T_0	7.79±0.04^{e}	0.671±0.01^{e}	558.3±5.02^{e}	56.10±0.01^{e}
T_1	8.66±0.14^{d}	0.736±0.01^{d}	613.2±4.05^{d}	56.27±0.08^{d}
T_2	8.89±0.08^{c}	0.741±0.01^{c}	617.4±5.22^{c}	56.29±0.55^{c}
T_3	8.92±0.14^{a}	0.758±0.01^{a}	631.6±4.01^{a}	56.54±0.40^{a}
T_4	8.90±0.06^{b}	0.756±0.00^{b}	629.6±2.52^{b}	56.28±0.53^{b}

a, b, c is significantly different. Different letters (or) NS used in the column same is not significant.

Yield attributes under field condition

Pod yield

Significant differences in pod yield (15.91 g/1.36 kg/1126 kg/ha) were observed in the treatment with flaxseed polymer infused with quercetin @ 1% (Table 7). Quercetin's antioxidant properties likely protected root cells from oxidative damage Zhang *et al.* (2020) and the percentage of quercetin will be varied based on the nature of soil and that will influence over the final pod yield by Patil *et al.* (1995).

Seed yield

Seed yield significantly increased in flaxseed polymer infused with quercetin @ 1%, with yields of 8.92 g/0.758 kg/ 631.6 kg/ha and a seed recovery rate of 56.54% (Table 8). Quercetin modulates root growth by interacting with auxins and cytokinin essential for root development by Tariq *et al.* (2023).

CONCLUSION

This study demonstrate that flaxseed polymer infused with quercetin @ 1% is an efficient seed coating method for enhancing the physical, physiological and biochemical traits of green gram seeds. The finding highlights flaxseed polymer as a natural bio-stimulant for sustainable agriculture and their potential applications in crop management practices.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions.

Conflict of interest

All authors declared that there is no conflict of interest

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