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Optimization and characterization of quercetin to enhance seedling vigour in greengram (*Vigna radiata*) under abiotic stress conditions

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ABSTRACT

A field experiment was conducted during the *zaid* seasons of 2022–23 and 2023–24 at Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu to study the effect of guava (*Psidium guajava* L.) leaf extract-treated greengram [*Vigna radiata* (L.) Wilczek] seeds under abiotic stress. The active ingredient, quercetin, was confirmed using High Performance Thin Layer Chromatography (HPTLC) with a peak area of 0.00039. The experiment was laid out in a split plot design (SPD) comprised of 3 treatments, viz. control (T_0); Seeds coated with organic flax seed polymer (T_1); Seeds coated with organic flax seed polymer + quercetin @1% (T_2) with six replications. Subplots had different irrigation schedules, including weekly irrigation (control) (S_0) and withholding irrigation for 15 days at different growth stages [25 DAS (days after sowing) (S_1); 35 DAS (S_2); 45 DAS (S_3)]. Soxhlet-extracted guava leaves resulted in the highest seedling vigour, with 94% germination, 16.9 cm root length, 18.5 cm shoot length, 0.311 g/10 seedlings, and a vigour index of 3299 at 1% concentration. Under moisture stress (PEG 6000-0.2 MPa), quercetin @1% improved germination (62%), root length (13.7 cm), shoot length (15.3 cm), dry matter production (0.170 g/10 seedlings), and vigour index (1750). Enzyme activities, including peroxidase (0.165 U/mg protein/min) and superoxide dismutase (1.127 U/mg protein/min) were highest in this treatment. Significant increases in proline content were observed, ranging from 135–146 mg/g and 137–152 mg/g across both the seasons. Yield/plant increased to 16.7 g (4.4 kg/plot) in season 1 and 17.3 g (4.6 kg/plot) in season 2. Thus, quercetin @1%, infused in flax seed, effectively mitigates drought stress, offering a sustainable solution for organic cultivation.

Keywords: Abiotic stress, Decantation extraction, Guava leaves, HPTLC, Proline, Quercetin, Seed yield, Soxhlet extraction

Worldwide protein rich pulses are the second most important group of food crops, and are in the daily dietary menu of every human. According to the World Health Organization, each person should consume 80 g of pulses daily (ASSOCHAM 2012). Among different groups of pulses, greengram [*Vigna radiata* (L.) Wilczek] often known as mung, is the most significant grain legume that is commercially viable. Among the various seed production techniques, pre-sowing seed invigoration is one of the seed management techniques widely adopted with plant products. Admist, 84% of economic loss in agriculture was due to climate change-related disasters. Out of which, 86% of crop production loss was due to drought stress (FAO 2020), to overcome such condition, leaf powders of herbal plants are widely used for seed treatment (Anbarasan *et al.* 2016).

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The use of botanicals for seed pre-treatment is now receiving much attention in these days because of their proven advantages over the synthetic options. Seed treatment has the potential to deliver agents “in right amount, in right place and at the right time”. Hence, guava (*Psidium guajava* L.) is a widely cultivated fruit tree known for its nutritional properties. Guava leaves are known to contain bioactive compound, quercetin (Magar and Sohng 2020), which is a flavonoid with antioxidant properties. Quercetin has been reported to modulate the activities of various enzymes involved in seed germination processes, including amylases, proteases and lipases. These enzymes play essential role in breaking down storage reserves within seeds and mobilizing nutrients for growth (Abd *et al.* 2020). However, the effectiveness of guava leaf extracts largely depends on the extraction method employed, which influences phytochemical compositions. Therefore, standardization of extraction method is essential to ensure consistent quality and efficacy of guava leaf extracts for agricultural applications. Thus, the study was carried out to investigate the most suitable method of extraction of active ingredient of quercetin and the effect of guava leaf extract on germination and seedling survival of greengram seeds coated with organic polymer infused with quercetin @1% under abiotic stress condition in field level.

MATERIALS AND METHODS

A field experiment was conducted during the *zaid* seasons of 2022–23 and 2023–24 at Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. Fresh guava leaves were collected from Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. The collected leaves were subjected for shade drying (4–5 days) followed by sun drying for 3–5 days, then dried samples were powdered under hammer mill unit and subjected for two extraction method like decantation method, the leaf powder was immersed into distilled water in a ratio of 1 g/10 ml (Perry 2006). Various concentrations of 1, 2 and 3% of leaf powder extracts were prepared (Decantation extraction method) and the seeds were primed with the extracts for a period of 3 h with the seed to solution ratio of 1:0.5. Followed by Soxhlet extraction method, 5 g of finely powdered leaf sample were taken in a Whatman No.1 filter paper; finley powdered guava leaf sample was transferred to an extraction thimble along with the solvent petroleum ether and heated for 6 h until 6–8 siphoning were completed (Bascon and Castro 2020). Finally, leaf extract with active ingredient (quercetin) was diluted with 10 ml of hexane over completely randomized design (CRD) under laboratory condition (Fig. 1), using different treatments, viz. Control; Hydropriming; Guava leaf extract-Decantation extraction method (DEM) (1%, 2%, and 3%); and Guava leaf extract- Soxhlet extraction method (SEM) (1%, 2%, and 3%).

The seeds were subjected to priming for a period of 3 h at 24°C, followed by shade drying to restore original moisture level of 9%. Finally, the seeds were subjected for physiological trails (ISTA 2019) of germination test (%), root length (cm), shoot length (cm), dry matter production (g/seedlings) and vigour index-II. High Performance Thin Layer Chromatography (HPTLC) was done to quantify the quercetin content. Where, 0.5 g seeds of greengram were soaked for 3 h with 1% plant-based quercetin and commercial quercetin by macerating with 1.5 ml of HPTLC grade methanol. The solution was centrifuged at 10,000 rpm for 20 min (Sonia and Lakshmi 2017). The supernatant was filtered using 0.2 µm syringe filter. The HPTLC system was injected with 10 µl of sample solution under optimum chromatographic condition. The quantity of quercetin was determined by comparing the peak area in the chromatogram of each sample solution to that of standard quercetin.

In order to induce drought stress in the field level, moisture stress experiment was conducted in laboratory with polyethylene glycol 6000 (-0.2 MPa to -1.0 MPa). The seeds were treated with quercetin @1% and subjected to moisture stress condition. Based on 50% survival percentage of seedlings that emerged on final count, the concentration of PEG 6000 was selected to evaluate the seed quality parameters. Also, the biochemical parameters like peroxidase and superoxide dismutase activity (U/mg protein/min) were measured as per the method described by Malik and Singh (1980). After screening at laboratory level, quercetin @1% (active compound) was incorporated with organic polymer and evaluated under field condition by split-plot design (SPD). The treatments consisted of control (T_0), seeds coated with organic flax seed polymer (T_1), and seeds coated with organic flax seed polymer + quercetin @1% (T_2). Subplots had different irrigation schedules, including weekly irrigation (control) (S_0) and withholding irrigation for 15 days at different growth stages [25 DAS (S_1); 35 DAS (S_2); 45 DAS (S_3)]

The plants under stress were analysed for proline content through selective extraction method as described by Bates *et al.* (1973). Also, the yield attributes on number of pods/plants, pod yield/plant (g) and pod yield/plot (kg) were estimated under 2 seasons.

Statistical analysis: Duncan's Multiple Range Test was used to compare results and done an analysis of variance (DMRT) at *P*-values <0.05. The SPSS 16.0 program was used to conduct the statistical analysis (SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

In DEM, the seed quality parameters decreased gradually with increase in the concentration of leaf extract. In order to overcome the drawback of decantation method, SEM was tried. In SEM, significant difference was noticed due to high percentage of active ingredient when compared to DEM. In our study, greengram seeds primed with guava leaf extract (GLE) resulted in significant difference in physiological parameter. Maximum germination (94%) was recorded in SEM at 1% condition (Table 1). Similar, results were reported by Witayapan *et al.* (2010), who stated that increased germination was due to the presence of oxygen in the dried GLE. The current study aimed to explore the

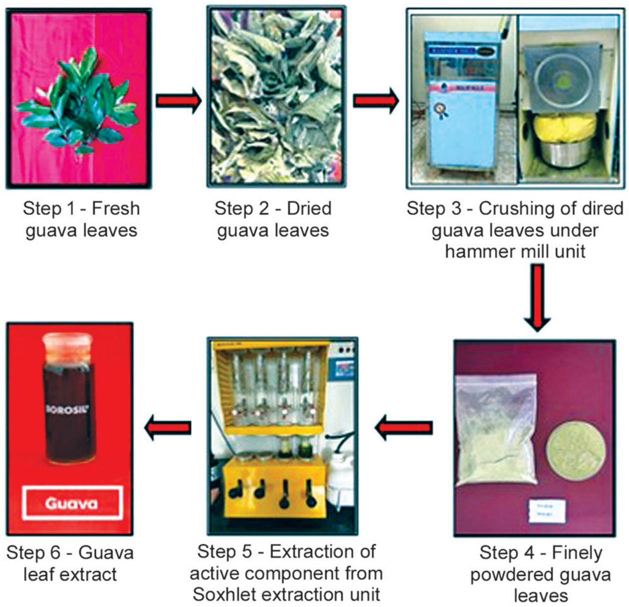

Fig. 1 Sequential steps for extraction of active ingredient from guava leaves through Soxhlet extraction method.

Table 1 Effect of guava leaf extract through different extraction method on physiological traits of greengram

Treatment		Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (g/10 seedlings)	Vigour index
Control		81 ± 0.24^{e}	15.5 ± 0.20^{efg}	17.8 ± 0.20^{cd}	0.203 ± 0.0013^{f}	2697 ± 22.27^{e}
Hydropriming		87 ± 0.58^{cd}	16.2 ± 0.22^{cd}	18.1 ± 0.32^{b}	0.220 ± 0.0025^{bc}	3019 ± 6.76^{c}
Guava leaf extract (DEM)	1%	66 ± 0.60^{fg}	15.5 ± 0.27^{efg}	15.5 ± 0.24^{k}	0.112 ± 0.0007^{ij}	2046 ± 3.73^{h}
	2%	67 ± 0.44^{fg}	15.3 ± 0.01^{efg}	15.9 ± 0.08^{ij}	0.111 ± 0.0018^{ij}	2090 ± 10.35^{f}
	3%	61 ± 1.26^{h}	14.4 ± 0.16^{h}	16.1 ± 0.16^{gh}	0.109 ± 0.0005^{k}	2105 ± 31.30^{g}
Guava leaf extract (SEM)	1%	94 ± 1.44^{a}	16.9 ± 0.15^{a}	18.5 ± 0.25^{a}	0.311 ± 0.0031^{a}	3299 ± 60.34^{a}
	2%	93 ± 0.42^{b}	16.4 ± 0.17^{b}	17.9 ± 0.21^{cd}	0.221 ± 0.0042^{bc}	3189 ± 22.70^{b}
	3%	86 ± 1.00^{cd}	16.2 ± 0.34^{cd}	17.1 ± 0.02^{e}	0.209 ± 0.0005^{de}	2864 ± 19.11^{d}

advantages of Soxhlet extract of GLE @1% concentration to increase the germination percent of greengram seeds, with increased root length (16.9 cm) and shoot length (18.5 cm) (Table 1), this study aligns with the findings of Seo *et al.* (2014). Vigour index was significantly influenced among two methods. Maximum vigour index was noticed in 1% SEM (3299) (Table 1). This is attributed to the presence of quercetin contents on *Psidium guajava* oil extracted with SEM (Coelho *et al.* 2021). Minimum germination percentage was noted in 3% DEM (61%) which, was lesser than control seeds with 81% germination (Table 1) due to limited efficiency in extracting of active component. DEM can be useful for simple separation; it may leave significant amount of desired compound in the original solution (Skoog *et al.* 2017). The lowest root length, shoot length was noticed in GLE @3% and 1% concentration on DEM (14.4 cm and 15.5 cm) (Table 1). Also, minimum dry matter was recorded in GLE @3% (0.109 g/10 seedlings). Since, the extracts from DEM on fresh leaves of guava contains inhibitory effect on germination of greengram (Jun *et al.* 2024). While, the minimum value on vigour index was recorded in GLE @1% concentration over DEM (2046) (Table 1), reduced vigour index in laboratory condition will have negative impact on crop production (Yuniastuti *et al.* 2018).

To confirm the presence of quercetin, GLS were analysed via HPTLC, with 1% Soxhlet extracted leaf sample. The presence of quercetin was detected with maximum peak area per cent of 0.00039 under the retention factor of 0.435, by comparing standard quercetin with 100% purity (Fig. 2). Thus, this result confirms the presence of quercetin in the Soxhlet extracted guava leaf sample, similar result was observed while quantifying quercetin in guava leaves using spectrophotometry and HPTLC analysis (Khan *et al.* 2011). According to Geetanjali *et al.* (2023) quercetin was identified in three onion varieties under the retention time of 64.5, 65.5 and 64.5 min, respectively. Also, the presence of active component cyanidin-3-glucoside and peonidin-3-glucoside in the black pigmented rice genotypes, viz. TTB Black Rice 7, Chakhao 1, Chakhao 2 and TTB Black Rice 11 were recorded under HPLC analysis (Surbhi *et al.* 2023).

Under drought stress condition, the findings confirmed that the seeds treated with quercetin @1% caused a noticeable difference in the proportion of greengram seedlings. Moisture stress considerably decreased the germination of control from 83% in normal condition to 62% under moisture stress condition (Table 2), reduced seed germination under stress condition is linked to metabolic diseases. The seedlings developed without moisture stress condition recorded maximum root length of 14.7 cm followed by moisture stress induced condition (13.7 cm) (Table 2). The same trend was noticed in shoot length on control seeds (16.3 cm) followed by PEG 6000 (-0.2 MPa) stress (15.3 cm). This decrease in genotype root and shoot elongation under low osmotic potential is consistent with the findings of Murillo *et al.* (2002) and can be attributed to PEG's inhibitory impact. Under moisture stress conditions, 1% quercetin treated greengram seeds (PEG 6000 -0.2 MPa) produced the least amount of dry matter (0.076 g/10 seedlings). In vigour index, control seeds recorded increased vigour index (2573), followed by moisture stress of -0.2 MPa (1740) (Table 2). Numerous research works, including Ji *et al.* (2012), Mostajeran and Rahimi (2009) have documented a comparable trend of decline in

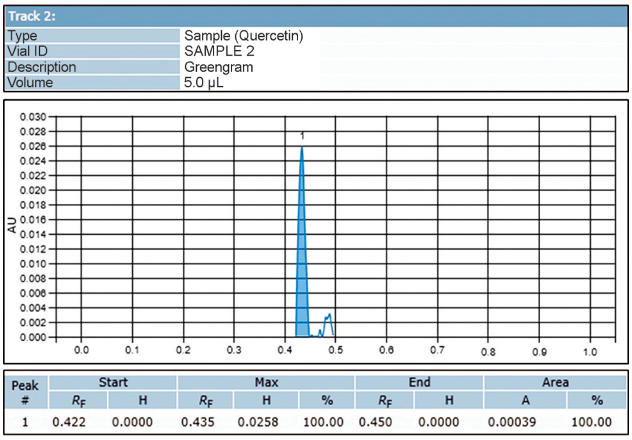

Fig. 2 Peak area per cent of quercetin content on guava leaf sample extract through HPTLC method.

Table 2 Changes in physiological activity under PEG 6000 induced moisture stress in 1% quercetin treated greengram seeds

Treatment	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (g/10 seedlings)	Vigour index
Control	83 ± 0.31[a]	14.7 ± 0.13[a]	16.3 ± 0.17[a]	0.199 ± 0.0004[a]	2573 ± 35.48[a]
PEG 6000 -0.2 MPa	62 ± 0.72[b]	13.7 ± 0.14[b]	15.3 ± 0.14[bc]	0.170 ± 0.0031[b]	1740 ± 14.08[b]
PEG 6000 -0.4 MPa	61 ± 0.46[cd]	13.3 ± 0.12[cd]	15.1 ± 0.18[d]	0.154 ± 0.0020[c]	1732 ±11.63[c]
PEG 6000 -0.6 MPa	60 ± 0.58[cd]	13.1± 0.06[cd]	15.2 ± 0.09[bc]	0.076 ± 0.0021[f]	1613 ± 2.41[e]
PEG 6000 -0.8 MPa	57 ± 0.43[e]	12.8 ± 0.17[e]	14.6 ± 0.10[f]	0.089 ± 0.0006[e]	1453 ± 3.52[f]
PEG 6000 -1.0 MPa	53 ± 0.73[f]	12.6 ± 0.18[f]	14.8 ± 0.15[e]	0.091 ± 0.0013[d]	1698 ± 16.81[d]

the dry and fresh weight of root and shoot during drought circumstances.

Under normal and moisture stress conditions, the peroxidase activity was influenced significantly. The increased peroxidase activity was seen in seeds treated with PEG 6000 -0.2 MPa (0.165 U/mg protein/min) and reduced amount of peroxidase activity was found in seeds treated with PEG 6000 -1.0 MPa (0.012 U/mg protein/min) (Fig. 3). Also, Seeds primed with PEG 6000 -0.2 MPa had the highest superoxide dismutase activity (0.127 U/mg protein/min (Fig. 3), while control seeds were having the lowest activity (0.046 U/mg protein/min) even under normal condition. 1% standard quercetin treated seeds showed maximal levels of superoxide dismutase and peroxidase activity. This explains their capacity to activate protective mechanism and to control the buildup of reactive oxygen species during the seed priming imbibition process *via* modifications to the seed's physiology and metabolism during the pre-germinative stage (Bailly 2004).

Various levels of moisture stress had an impact on enzyme activity. Notable differences were observed in proline content under moisture stress levels. Increased proline content was measured in T_0–T_2 under S_3 (ranging from 115–135 mg/g and 130–152 mg/g) in season 1 and season 2, respectively when, compared to S_0, which exhibited the reduced values (ranging from 115–135 mg/g and 117–137 mg/g). The maximum proline content (146 and 152 mg/g) was observed in T_2 treatment under S_3 conditions in both the seasons. In the present study, it was interpreted that under drought stressed field conditions, there was a significant increase in the accumulation of osmolytes, such as soluble carbohydrates and proline. Organic polymer with 1% quercetin (T_2) coated seeds has a potential to increase polyamines contributed to altering the osmotic potential by facilitating improved water uptake. As a result, the plants were able to grow and develop better even under drought stress conditions (Mumtaz *et al.* 2021). Drought tolerance mechanism can be described as the ability of crops to maintain productivity under extreme drought stress condition. The impact of stress on growth and yield parameters is manifested through it's influence on various physiological and developmental processes (Ali and Ahmadikhah 2009). For greengram, water stress during flowering, pollination and seed filling stages poses the greatest susceptibility to decline in yield (Lauer 2007). During critical periods, water deficit stress reduces carbon availability and dry matter partitioning into the pod, which directly influences the number of grains produced (Andrade *et al.* 2008). In the current study, significant differences were observed under drought stress condition, the number of pods/plant and pod yield/plant in S_0 ranges from T_0–T_2 32–34 pods/plant and 33–37 pods/plant (Table 3) and 14.3–16.7 and 14.9–17.3 pod yield/plant (Table 3) in both the seasons. When, compared to S_3, which exhibit reduced values ranging from 25–28 pods/plant and 24–31 pods/plant and 12.9–13.9 and 12.1–14.4 pod yield/plant (g) (Table 3) over extreme moisture condition over both the seasons.

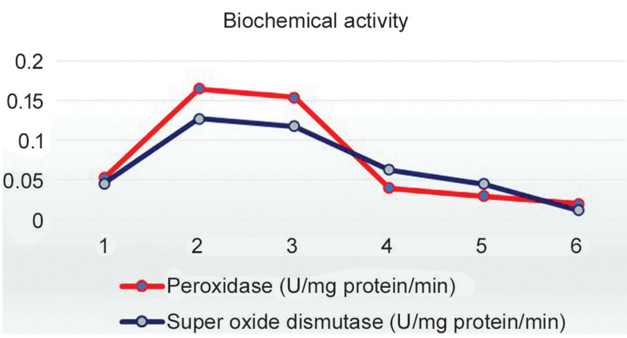

Fig. 3 Effect of seed priming with 1% quercetin on biochemical activity under PEG 6000 induced moisture stress in greengram.

Seed yield/plot of greengram (CO 8) under drought stress was observed with a significant difference at different level of moisture stress. Rise in moisture stress level from S_0–S_3 reduced the seed yield/plot (3.8–3.4 kg) (3.9–3.2 kg) in T_0. This reduction in seed yield/plot was increased (4.4–3.6 kg) (4.6–3.8 kg) by quercetin @1% along with organic polymer in T_2. Treatment T_2 recorded highest (4.4 and 4.6 kg) seed yield/plot under both the seasons. Since, quercetin plays a significant role in maintaining the balanced concentration of ROS and lipid peroxidation augmenting several physiological functions to confer extreme drought stress tolerance (Singh *et al.* 2021). Hence, the present study

Table 3 Effect of 1% quercetin seed treatment on number of pods/plants in greengram during *zaid* 2022–23 and 2023–24

Treatment	Number of pod/plants			
	2022–23			
	S_0	S_1	S_2	S_3
T_0	32 ± 0.57^{cd}	29 ± 0.16^{gh}	27 ± 0.30^{jk}	25 ± 0.35^{l}
T_1	33 ± 0.58^{b}	32 ± 0.47^{cd}	29 ± 0.01^{gh}	27 ± 0.18^{jk}
T_2	34 ± 0.09^{a}	31 ± 0.40^{e}	30 ± 0.31^{f}	28 ± 0.35^{i}
Treatment	*2023–24*			
T_0	33 ± 0.35^{ef}	28 ± 0.11^{i}	25 ± 0.11^{k}	24 ± 0.26^{l}
T_1	35 ± 0.18^{bc}	33 ± 0.40^{ef}	30 ± 0.04^{h}	27 ± 0.07^{j}
T_2	37 ± 0.40^{a}	35 ± 0.26^{bc}	34 ± 0.30^{d}	31 ± 0.16^{g}
Treatment	*Pod yield/plant (g)*			
	2022–23			
	S_0	S_1	S_2	S_3
T_0	14.3 ± 0.04^{fg}	14.1 ± 0.03^{h}	13.4 ± 0.08^{k}	12.9 ± 0.07^{l}
T_1	15.9 ± 0.05^{b}	15.1 ± 0.18^{d}	14.3 ± 0.10^{fg}	13.6 ± 0.03^{j}
T_2	16.7 ± 0.22^{a}	15.7 ± 0.05^{c}	14.9 ± 0.08^{e}	13.9 ± 0.24^{i}
Treatment	*2023–24*			
T_0	14.9 ± 0.05^{fg}	13.8 ± 0.02^{i}	12.7 ± 0.12^{k}	12.1 ± 0.10^{l}
T_1	16.1 ± 0.03^{c}	15.4 ± 0.13^{e}	14.9 ± 0.16^{fg}	13.5 ± 0.01^{j}
T_2	17.3 ± 0.20^{a}	16.8 ± 0.22^{b}	15.5 ± 0.23^{d}	14.4 ± 0.01^{h}

Treatment details are given under Materials and Methods.

concludes the importance of standardization in obtaining guava leaf extracts with optimal concentration. Soxhlet extraction proved to be the most efficient method for extracting bioactive compounds from guava leaves, resulting in extracts with superior antioxidant (quercetin) activity and promoting the germination and growth of greengram seeds and HPTLC analysis confirm the presence of quercetin. These findings highlight the potential of guava leaf extracts as natural bio-stimulants in agriculture, offering sustainable alternatives for enhancing crop productivity even under drought stress condition. Further research is warranted to elucidate the mechanisms underlying the effects of guava leaf extracts on plant growth and their potential applications in crop management practices.

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