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DEVELOPMENT AND EVALUATION OF MULTI-BOTANICAL FORMULATIONS FOR SEED TREATMENT IN SUNFLOWER

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ABSTRACT

The present study aimed to develop and evaluate multi-botanical seed coating formulations for improving seed quality in sunflower. Seven formulations comprising different combinations of neem, tulsi, garlic, and cinnamon extracts were prepared using guar gum, kaolin and glycerol and compared with untreated and fungicide-treated controls. The formulations were characterized for their physicochemical properties and evaluated for coating performance, germination, seedling vigour, seed health and field emergence. Significant differences were observed among the treatments. The formulation containing garlic (2%) + tulsi (2%) + cinnamon (2%) exhibited superior physicochemical characteristics, including higher viscosity and coating adherence, along with the highest germination, seedling length, seedling dry weight, Seed Vigour Index-I, Seed Vigour Index-II and field emergence. The results indicate that the multi-botanical formulation is an effective and eco-friendly seed coating for enhancing seed quality and crop establishment in sunflower and has potential as a sustainable alternative to conventional chemical seed treatments.

Key words : Botanical seed treatment, Fungicide, Sunflower seed quality, Bio-coating formulation, Sustainable seed protection, Eco-friendly pest management.

Introduction

Sunflower (*Helianthus annuus* L.) is one of the world's leading oilseed crops, cultivated for its high-quality edible oil, balanced fatty acid composition, and diverse industrial applications (FAOSTAT, 2024). Sunflower oil is rich in unsaturated fatty acids, tocopherols, phytosterols, and phenolic compounds, making it nutritionally valuable and beneficial for human health (Guo *et al.*, 2017). Seed quality is a major determinant of crop establishment, influencing germination, seedling vigour, field emergence, and ultimately seed yield (Copeland and McDonald, 2012; Taylor *et al.*, 1998). However, sunflower seeds are susceptible to physiological deterioration during storage and infection by seed-borne fungi such as *Alternaria*, *Aspergillus*, *Fusarium*, and *Penicillium*, which reduce germination and seedling establishment (Neergaard, 1979; Ruksana Afzal *et al.*, 2010).

Seed treatment is an economical and effective

strategy for protecting seeds against seed- and soil-borne pathogens and enhancing early crop establishment (Taylor *et al.*, 1998; Munkvold, 2009). Although synthetic fungicides have been widely used for seed protection, their repeated application has raised concerns regarding environmental contamination, fungicide resistance, and pesticide residues, creating a demand for safer and sustainable alternatives (Lamichhane *et al.*, 2016). Plant-derived botanicals have attracted considerable attention because they are biodegradable, environmentally benign, and rich in bioactive metabolites including phenolics, terpenoids, flavonoids, alkaloids, and sulfur-containing compounds that exhibit antimicrobial and antioxidant activities (Sohail *et al.*, 2022; Hossain and Schlosser, 1993).

Among the various botanicals, neem has received considerable attention because of its potent antimicrobial and pesticidal properties attributed to bioactive constituents such as azadirachtin, nimbin, salannin, and

gedunin (Biswas *et al.*, 2002; Schmutterer, 1990). Neem extracts have been reported to inhibit the growth of several seed-borne fungi while improving seed germination and seedling vigour in different crop species (Koul *et al.*, 2008; Gahukar, 2014). Similarly, tulsi is rich in eugenol, ursolic acid, rosmarinic acid, and other phenolic compounds possessing strong antioxidant and antimicrobial activities (Pandey *et al.*, 2014). Tulsi extracts have demonstrated inhibitory effects against a broad range of phytopathogenic fungi and bacteria and have also been reported to enhance seed germination and seedling growth (Sinha and Kumar, 2019). Garlic contains allicin and other organosulfur compounds that exhibit broad-spectrum antifungal and antibacterial activity by disrupting microbial cell membranes and metabolic pathways (Borlinghaus *et al.*, 2014). Likewise, cinnamon is an important medicinal spice containing cinnamaldehyde, eugenol and polyphenolic compounds with remarkable antifungal, antioxidant and preservative properties (Yoon *et al.*, 2013; Rao and Gan, 2014). These bioactive constituents collectively contribute to the suppression of pathogenic microorganisms while protecting seeds from oxidative deterioration during storage (Gahukar, 2014).

Although numerous studies have investigated individual botanical extracts as seed treatments, comparatively limited attention has been given to the development of standardized multi-botanical formulations that integrate complementary plant extracts into a single seed treatment product (Koul *et al.*, 2008; Gahukar, 2014; Isman, 2020). The combination of botanicals possessing diverse bioactive constituents may provide broader antimicrobial activity, complementary modes of action, enhanced antioxidant potential and improved seed physiological performance compared with individual plant extracts (Isman, 2020). Experimental studies have shown that binary combinations of botanical extracts, such as neem–tulsi and neem–garlic, can improve seed germination, seedling vigour, and suppress seed-borne pathogens more effectively than untreated seeds, indicating the potential benefits of combining botanicals for seed treatment applications. Likewise, several studies have demonstrated the efficacy of neem, garlic, tulsi and other botanicals in reducing seed-borne fungal infection and improving seed quality in different crops, although these investigations have largely focused on individual extracts or simple combinations rather than standardized multi-component formulations. Therefore, systematic studies on formulations integrating neem, tulsi, garlic, and cinnamon for sunflower seed treatment remain scarce, highlighting the need for developing scientifically standardized multi-botanical seed treatment formulations.

Materials and Methods

Sunflower hybrid Tilhan Tech SUNH-1 seed was procured from the ICAR-Indian Institute of Oilseeds Research (IIOR), Rajendranagar, Hyderabad, India.

Preparation of botanical extracts and formulation development

Fresh leaves of neem (*Azadirachta indica*) and tulsi (*Ocimum sanctum*), garlic (*Allium sativum*) cloves, and cinnamon (*Cinnamomum verum*) bark were collected from local sources and used for the preparation of aqueous extracts. Botanical extracts were prepared by the hot maceration method described by Abubakar and Haque (2020). Shade-dried powdered plant material was extracted and 10g of each powdered sample was extracted with 100 mL distilled water (1:10 w/v ratio) at 55°C for 24 h. The extracts were filtered through Whatman No. 1 filter paper, stabilised by adding 10 mL methanol, heated for an additional 30 min, cooled to room temperature, and used as stock extracts as shown in Fig. 1.

Multi-botanical liquid seed coating formulations were prepared by incorporating the botanical extracts with guar gum (0.5 g 100 ml⁻¹) as an adhesive, kaolin clay (1.5 g 100 ml⁻¹) as a filler and glycerol (2 ml 100 ml⁻¹) as a plasticiser. The ingredients were mixed thoroughly to obtain homogeneous formulations as shown in Fig. 1. Sunflower seeds were coated with the botanical formulation treatments at 6 ml kg⁻¹, shade-dried for 24 h and used for subsequent laboratory and field evaluations.

Treatment details : T₁- Neem 5% + Tulsi 3%; T₂- Cinnamon 2% + Tulsi 3%; T₃- Neem 5% + Cinnamon 3%; T₄- Garlic 2% + Cinnamon 3%; T₅- Neem 3% + Garlic 1.5% + Tulsi 1.5%; T₆- Garlic 2% + Tulsi 2% + Cinnamon 2%; T₇- Neem 2% + Garlic 1.5% + Tulsi 1.5% + Cinnamon 1%; T₈-Control; T₉- Treated control (Fungicide (Carbendazim + Mancozeb)).

Characterization of botanical formulations

The botanical formulations were characterized for

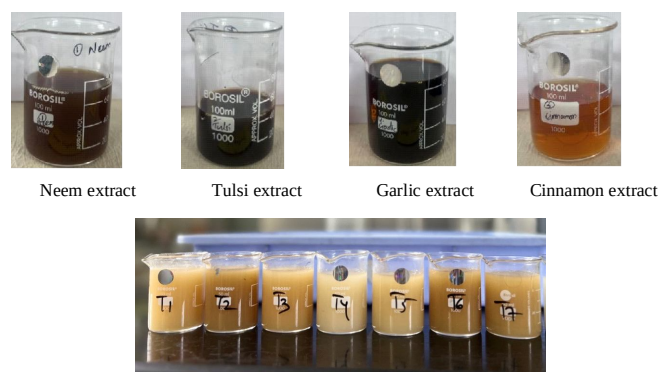


Fig. 1 : Botanical extracts and their formulations.

extraction yield, colour, viscosity, sedimentation stability, pH and drying time. Botanical extraction yield was calculated as the percentage of extract recovered after filtration (Abubakar and Haque, 2020). Colour parameters (L^* , a^* , b^* , chroma and hue angle) were determined using the CIELAB colour system (Popa *et al.*, 2008). Viscosity was measured using a Brookfield DV3T LV rheometer at $25.5 \pm 1^\circ\text{C}$ and 1.5 rpm following Prasanna Kumara *et al.* (2018). Formulation stability was evaluated by determining sedimentation volume after 7 and 14 days of storage according to Kolo *et al.* (2018). The pH of freshly prepared formulations and those stored for seven days was measured using a calibrated digital pH meter (Thawong and Kangsopa, 2025). Drying time was recorded as the time required for complete drying of the coating film under ambient laboratory conditions (Arif *et al.*, 2023).

Seed coating characteristics : Seed coating performance was evaluated based on adherence (%), the weight of the seed before and after coating, and uniformity score. Adherence (%) and uniformity score were assessed according to Roth (2014), while the weight of the seed before and after coating was calculated as the percentage increase in seed weight after coating, following Amirkhani *et al.* (2016).

Seed quality parameters : Seed germination was determined by the rolled paper towel method (ISTA, 2022) and recorded on the tenth day as a percentage. Seedling length and dry weight were measured from ten randomly selected normal seedlings per replication, with dry weight recorded after oven-drying at $100 \pm 1^\circ\text{C}$ for 24 h. Seed vigour indices were calculated as per Abdul-Baki and Anderson (1973) by multiplying germination percentage with seedling length (SV-I) and dry weight (SV-II). Field emergence was evaluated by sowing hundred seeds per

treatment in three replications under field conditions, with counts recorded on the tenth day and expressed as a percentage.

Statistical analysis : The experiment was conducted in a completely randomized design (CRD) with nine treatments and three replications. Data were analysed using GRAPES software, and treatment means were compared using the critical difference (CD) test at the 5% significance level.

Results and Discussion

Extraction yield and physicochemical characterization of Botanical Formulations

Extraction yield varied significantly among botanicals, ranging from 45.40% (tulsi) to 54.50% (neem), followed by cinnamon (52.70%) and garlic (50.90%). This variation was likely attributed to differences in phytochemical composition, solvent affinity and extractable polar constituents, consistent with earlier reports (Hashim *et al.*, 2021; Ahmad *et al.*, 2020; Ferioli *et al.*, 2020; Cha *et al.*, 2019; Chaudhary *et al.*, 2020).

Colour analysis (Table 1) revealed significant differences among treatments. T_6 (Garlic 2% + Tulsi 2% + Cinnamon 2%) showed the lowest lightness ($L^* = 43.87$) and highest redness ($a^* = 5.24$) and yellowness ($b^* = 24.30$), followed by T_7 and T_2 , indicating darker formulations attributable to phenolics, flavonoids, carotenoids and cinnamaldehyde, whereas the untreated control (T_8) was the lightest ($L^* = 73.90$). These trends aligned with the findings of Ciriello *et al.* (2023) and Lipan *et al.* (2025) on cinnamon-rich formulations. Viscosity ranged from 2060 cP (T_1) to 13,080 cP (T_6), with T_4 (11,500 cP) and T_3 (10,140 cP) also showing high values, likely due to soluble polysaccharides and phenolics that enhanced intermolecular interactions (Karazhiyan *et al.*,

Table 1 : Evaluation of botanical formulations for physical characteristics.

Treatments	Visual characteristics of formulation				Stability of formulation		pH	
	Colour (CIELAB colour space L* a* b* values)			Viscosity (cP)	Sedimentation volume (%) 7 th day	Sedimentation volume (%) 14 th day	Initial 0 th day	Final 7 th day
	L*	a*	b*					
T ₁	56.65 ^c	1.50 ^e	14.16 ^d	2060 ^h	6 ^{bc}	6.8 ^e	5.28	5.12
T ₂	49.82 ^d	4.99 ^a	21.25 ^b	5540 ^f	6.4 ^b	7.6 ^c	5.47	5.30
T ₃	48.88 ^d	3.47 ^c	21.14 ^b	10140 ^c	7.2 ^a	8.4 ^b	6.18	5.98
T ₄	63.72 ^b	0.71 ^f	8.84 ^e	11500 ^b	3.8 ^e	5.2 ^f	5.48	5.29
T ₅	59.70 ^{bc}	2.84 ^d	19.00 ^c	2820 ^g	5.4 ^d	6.8 ^e	5.79	5.60
T ₆	43.87 ^e	5.24 ^a	24.30 ^a	13080 ^a	5.8 ^{cd}	7.2 ^d	6.26	6.05
T ₇	48.56 ^d	4.42 ^b	22.29 ^b	7820 ^e	7.6 ^a	9.2 ^a	6.15	5.94
T ₈	73.90 ^a	-1.13 ^g	7.23 ^e	8060 ^d	2.4 ^f	4 ^g	5.04	4.96

Table 2 : Effect of botanical seed coating formulations on drying time, adherence, coating uniformity and weight of the seed before and after coating.

Treatments	Drying time (minutes)	Adherence (%)	Uniformity score	Weight of the seed before and after coating (g)
T ₁	10	35.49 ^e	3	0.51 ^g
T ₂	10	36.53 ^d	4	0.54 ^f
T ₃	7	37.30 ^c	5	0.57 ^e
T ₄	12	38.36 ^b	6	0.61 ^d
T ₅	10	37.09 ^c	5	0.66 ^c
T ₆	13	41.63 ^a	7	0.78 ^a
T ₇	10	35.70 ^e	3	0.69 ^b
T ₈ (Control)	0	0 ^g	0	0 ⁱ
T ₉ (Fungicide control)	0	6.83 ^f	1	0.14 ^h

Means within a column followed by different superscript letters differ significantly at $P \leq 0.05$ according to Duncan's Multiple Range test.

Table 3 : Effect of different seed treatments on germination and seedling vigour of sunflower seeds.

Treatments	Seed germination (%)	Seedling length (cm)	Seedling dry weight (mg)	SV-I	SV-II
T ₁	87 ^{cd}	31.57 ^a	45.32 ^e	2756 ^b	3958 ^e
T ₂	83 ^e	29.01 ^{bc}	44.70 ^f	2418 ^c	3725 ^f
T ₃	86 ^d	25.95 ^d	43.59 ^g	2232 ^d	3749 ^f
T ₄	89 ^{bc}	32.37 ^a	49.06 ^b	2870 ^{ab}	4350 ^b
T ₅	83 ^e	29.88 ^b	41.82 ^h	2470 ^c	3457 ^g
T ₆	91 ^a	32.44 ^a	50.58 ^a	2962 ^a	4619 ^a
T ₇	87 ^{cd}	27.95 ^c	46.61 ^d	2441 ^c	4071 ^d
T ₈ (Control)	88 ^c	25.02 ^d	47.41 ^c	2202 ^d	4172 ^c
T ₉ (Fungicide treated control)	90 ^{ab}	31.83 ^a	49.13 ^b	2865 ^{ab}	4422 ^b

Values are means of three replications. Means were compared using one-way ANOVA followed by Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

2009; Hanumantharaju *et al.*, 2020; Iqbal *et al.*, 2023).

Sedimentation volume after 7 and 14 days ranged from 2.4–7.6% and 4.0–9.2%, respectively, with T₇ and T₃ showing the highest sedimentation and T₈ (control) the lowest. The acceptable stability observed across treatments was attributed to guar gum, kaolin and glycerol, which aided particle suspension and limited aggregation, in agreement with Faers (1999) and Langlet *et al.* (2024).

Initial pH ranged from 5.04 (T₈) to 6.26 (T₆) and declined marginally (0.08–0.21 units) after 7 days (4.96–6.05), with T₆ and T₃ consistently recording the highest

values. This slight decline reflected gradual oxidation and degradation of phytochemicals and indicated good physicochemical stability, consistent with previous reports (Baugh and Ignelzi, 2000; Meena *et al.*, 2022; Prasad *et al.*, 2020; Liang *et al.*, 2026).

Coating characteristics of Sunflower seeds

Drying time ranged from 7 min (T₃) to 13 min (T₆), the longer duration in T₆ reflecting its higher viscosity and coating thickness (Tang *et al.*, 2024; Teichert *et al.*, 2026) (Table 2). Adherence was highest in T₆ (41.63%), followed by T₄ (38.36%) and T₃ (37.30%), while the fungicide-treated control (T₉) showed minimal adherence (6.83%) and T₈ showed none, consistent with binder concentration effects on adhesion (Baroni *et al.*, 2020; Accinelli *et al.*, 2021). The uniformity score ranged from 0 to 7, with T₆ recording the highest score (7), followed by T₄ (6), while the lowest score was 0 in T₈ (Roth, 2014; Rocha *et al.*, 2024). Seed weight gain after coating was greatest in T₆ (0.78 g) and least in T₉ (0.14 g), with T₈ showing no change, reflecting effective deposition of coating materials (Xavier *et al.*, 2015; De Sousa *et al.*, 2017).

Seed Quality parameters

Germination ranged from 83–91%, with T₆ recording the highest value (91%), on par with T₉ (90%) and T₄ (89%), exceeding the untreated control T₈ (88%), likely due to the antifungal activity of the botanical blend (Sinha and Kumar, 2019; Suganthi *et al.*, 2022; Rao *et al.*, 2026).

T₆ also recorded the greatest seedling length (32.44 cm), dry weight (50.58 mg) and vigour indices (SV-I: 2962; SV-II: 4619), compared with lower values in T₈, indicating enhanced reserve mobilization and reduced pathogen interference (Rao *et al.*, 2026; Suganthi *et al.*, 2022; Devi *et al.*, 2021; Sinha and Kumar, 2019; Ahmed, 2023). Field emergence was highest in T₆ (83%), followed by T₄ (82%) and T₂ (81%), exceeding both T₉ (77%) and T₈ (75%), reflecting improved seed health and vigour (Bhati *et al.*, 2025; Agyetoah *et al.*, 2025; Nwachukwu and Umechuruba, 2001).

Conclusion

This study demonstrated the successful development of multi-botanical seed coating formulations using neem, tulsi, garlic and cinnamon extracts for sunflower seed treatment. The formulations exhibited desirable physicochemical properties, confirming their suitability for seed coating applications. Among all treatments, T₆ (Garlic 2% + Tulsi 2% + Cinnamon 2%) consistently showed superior coating performance, germination, seedling vigour and field emergence, performing on par with or better than the fungicide-treated control. These improvements were likely attributable to the synergistic action of bioactive phytochemicals in the combined botanical extracts. Further evaluation under varied storage conditions and field environments is warranted before recommending its large-scale agricultural application.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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