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Original article

Potential Use of Essential Oil Nanoemulsions as Bee Attractants in Seed Sunflower (*Helianthus annuus* L.) Production

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Abstract

This study was conducted under field conditions in 2024 to determine the effects of certain essential oils on enhancing the pollination efficiency of honeybees in hybrid sunflower seed production. In the study, nanoemulsions (NEs) of lemongrass (*Cymbopogon citratus*), lavender (*Lavandula angustifolia*), rosemary (*Rosmarinus officinalis*), and sage (Salvia officinalis) essential oils were prepared at a 5% concentration and applied via drone at doses of 1000 and 2000 ppm to the male and female sunflower parent lines at the beginning of the flowering period. At 50% flowering, the highest bee activity was observed in the plots treated with 2000 ppm C. citratus essential oil NE, while bee visits in plots treated with 1000 ppm R. officinalis and S. officinalis essential oil NEs and Tween-80 - chitosan NE were similar to the control. Applications of essential oil NEs resulted in increases of up to 28% in the filled seed ratio, 34% in seed number, and 38% in seed yield. The study concluded that 2000 ppm C. citratus NE enhanced bee activity, thereby improving seed set in sunflowers, and can be practically used in hybrid sunflower seed production.

Keywords: Sunflower, Bee activity, Attractant, Essential oil, Nanoemulsion

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INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an agriculturally strategic crop worldwide, valued for both its oilseed and seed production. In seed production systems, obtaining high-quality and genetically pure seeds largely depends on an efficient pollination process. Sunflower is generally an entomophilous (insect-pollinated) species, and both yield and seed quality are highly dependent on bee activity. However, in recent years, the decline in bee populations in agricultural landscapes, environmental stress factors, and extensive monoculture practices have resulted in insufficient natural pollination, leading to significant yield and quality losses in seed production. Wind pollination (anemophily) in sunflower is limited due to morphological and physiological differences between male and female organs, self-incompatibility mechanisms involved in the breeding process, and the weak adaptation of pollen to wind dispersal. Therefore, honeybees (*Apis mellifera* L.) represent the most important group of pollinators in sunflower, as they visit flowers more frequently than other insects due to their need to sustain their colonies (Morgado et al., 2002).

The yields of many agricultural crops increase in the presence of pollinators (Silva et al., 2018; Mazzei et al., 2021; Santone et al., 2022; Mota et al., 2024). The growing dependence of numerous cultivated species on pollinators, along with the alarming decline in both managed and wild bee populations (IPBES, 2016; Dicks et al., 2021), highlights the urgent need to investigate the effects of pollination on crop yield and seed quality in modern agricultural systems (Bartual et al., 2018; Silva et al., 2018). Sunflower, one of the world's most important oilseed crops, relies heavily on insect pollination for successful seed production (Silva et al., 2018; Mota et al., 2024). Exclusion (pollinator-blocking) experiments with hybrid sunflower cultivars have demonstrated that bee pollination can increase seed set and yield by more than 90% (Silva et al., 2018).

In Türkiye, in addition to oilseed production, a substantial amount of sunflower seed production is also carried out. In hybrid seed production, cytoplasmic male sterile (CMS) lines are used, and pollination is mainly achieved by honeybees. To ensure adequate and efficient pollination, an average of 9 honeybee colonies per hectare is typically placed in seed production fields. Numerous studies have reported that biotic pollination by honeybees significantly increases seed set and yield in sunflower seed production (Torretta and Poggio, 2013; Chamer et al., 2015; Susic and Farina, 2015). Nevertheless, in many parental lines used in hybrid seed production, pollination and seed set rates often remain as low as 30–40%. Factors that may contribute to this include the low attractiveness of the maternal line's flower color to bees, insufficient pollen production by paternal lines, the potentially bitter taste of pollen, the presence of alternative pollen sources nearby, and the tendency of bees to remain in hives under high temperatures (>30-32 °C) (Bartual et al., 2018; Amarilla et al., 2025). In this context, developing new and environmentally friendly strategies to enhance the attractiveness of flowers to bees is of critical importance for sustainable sunflower seed production.

Essential oils are bioactive compounds naturally synthesized by plants, predominantly composed of terpenoids. These substances act as natural signaling molecules in plant–plant and plant–insect communication, playing key roles in attracting pollinators such as bees and butterflies. Recent ecological and chemical ecology studies have demonstrated that certain volatile constituents of essential oils can have attractive or orienting effects on bee behavior. The use of essential oils as natural bee attractants in sunflower seed production offers a promising and eco-friendly alternative to synthetic attractants, contributing to both enhanced pollination efficiency and environmental sustainability. Most plant-derived bioactive compounds, however, are highly volatile and unstable, showing strong sensitivity to oxygen, light, and heat. In addition, their hydrophobicity, reactivity, and volatility impose certain limitations on their direct use. NE systems, characterized by high optical clarity and long-term stability, have shown great potential for improving the water solubility and bioavailability of lipophilic active compounds, protecting them from environmental degradation, and enabling controlled release (Prasad et al., 2019). Moreover, owing to their small droplet size, NEs can exhibit biological activity even at lower concentrations (Ghazy et al., 2021).

The aim of this study was to determine the potential use of selected essential oil NEs as bee attractants in sunflower seed production, and to evaluate their effects on bee activity, seed yield, and seed quality.

MATERIAL and METHOD

Experimental Site

Field experiments were conducted during 2024 growing seasons in sunflower seed production fields located in Seyitgazi District, Eskişehir Province, Türkiye (39º 56′ 58″ N, 30º 74′ 98″ E, 925 m above sea level). The total annual precipitation (292 mm) was lower than the long-term average (334 mm). The annual mean temperatures (15.7 °C) were slightly higher than the long-term average (15.4 °C), while relative humidity levels (51.3%) were similar to the long-term mean value (51.5%).

Essential Oil Extraction and GC-MS Analysis

Lemongrass (*Cymbopogon citratus*) essential oil used in the study was purchased from Sebat Ltd. Co., while lavender (*Lavandula angustifolia*), rosemary (*Rosmarinus officinalis*), and sage (*Salvia officinalis*) essential oils were obtained by hydrodistillation of the flowers and herbs using a Clevenger-type apparatus at 100 °C for 3 hours (European Pharmacopoeia, 1997). The chemical compositions of the essential oils were determined using a GC–MS system (QP Shimadzu 2010 Plus). For analysis, 10 µL of essential oil was dissolved in 1 mL of *n*-hexane and injected into a CP-Wax 52 CB column (50 m × 0.32 mm i.d., 0.25 µm film thickness). The oven temperature was programmed from 60 °C to 220 °C at a rate of 10 °C/min and held at 220 °C for 10 min. The injector and detector temperatures were maintained at 240 °C and 250 °C, respectively. The ionization energy was 70 eV, with helium as the carrier gas at a flow rate of 20 mL/min. Compound identification was performed by comparing the obtained mass spectra and retention indices with those in the NIST and Wiley libraries (Rostad and Pereira, 1986; Adams, 2007).

According to GC-MS results, the major constituents of the essential oils were as follows: *S. officinalis*: 44.6% α-thujone and 18.4% camphor; *C. citratus*: 42.3% geranial and 35.1% neral; *R. officinalis*: 66.3% 1,8-cineole and 5.9% camphor; *L. angustifolia*: 40.2% linalool and 11.7% linalyl acetate.

Preparation of NEs

NE formulations (5% w/v) were prepared using lemongrass, lavender, rosemary, and sage essential oils (EOs). Each EO was first mixed with Tween-80 at a 1:3 (v/v) ratio (Campolo et al., 2022) under continuous stirring at 1000 rpm for 10 min. To achieve slow release of the oils, the pre-emulsions were added dropwise into a 1% chitosan solution, forming macroemulsions. These macroemulsions were then homogenized at 25,000 rpm for 10 min using a high-speed homogenizer to obtain NE. NEs containing only Tween-80 and chitosan were used as control formulations.

Experimental Design

The experiment was arranged in randomized complete block design with three replications. Sowing was performed in April using a pneumatic seeder at a spacing of 70 × 25 cm, with four female (A-line) rows alternating with two male (R-line) rows over 50-decare fields. Prior to sowing, 20 kg/da of diammonium phosphate (DAP; 18% N and 46% P_2O_5) was applied, followed by 20 kg/da of ammonium nitrate (30% N) at the stem elongation stage. Plants were irrigated via drip irrigation four times: when they reached 25–30 cm height, at the onset of head formation, at the beginning of flowering, and at the start of grain filling. Weed control was achieved with a pre-emergence herbicide application (RACER® 25 EC, 250 g/L flurochloridone, 300 g/da). As the parental line's seeds were pre-treated against soil pests and fungi, no additional pest control measures were required, and no pest or disease incidences were observed during the experiments.

Eight NE treatments (4 EOs × 2 concentrations: 1000 and 2000 ppm) and two controls (negative control: NE containing only Tween-80 and chitosan; positive control: untreated) were evaluated, for a total of ten treatments. NEs were applied at 2 L/da during the 10% flowering stage using a crop spraying drone (40 L), targeting 12 rows of female and 4 rows of male plants (approximately 1200 m²). To prevent cross-contamination of odors among treatments, a buffer zone consisting of 12 female and 4 male rows (100 m in length) was left between each treatment plot. Control plots were established in each block by leaving a gap of three plots (approximately 3600 m²) from treated plots. Prior to flowering, one honeybee colony per decare was placed in the field.

Observations and Measurements

The effects of essential oil nanoemulsions (NEOs) used as bee attractants on seed set and yield in sunflower were evaluated according to the method described by Osman and Siham (2015). Observations were made on head samples collected from the middle four female rows in each plot (3 female and 2 male parents). Starting one day after application, bee activity was recorded daily for five consecutive days at the same time each morning (10:00 a.m.) by counting the number of bees visiting ten randomly selected flower heads per plot, and mean values were calculated. At harvest, the total number of seeds per head was determined for ten randomly selected heads per plot, and the mean number of seeds per head was calculated. Ten heads per treatment were threshed, and seed weight per plant was measured with an electronic balance to determine average seed yield per plant. The 1000-seed weight was obtained by weighing four replicates of 100 dried seeds per treatment. In addition, filled and unfilled seeds from ten heads per plot were separated, and the percentage of filled seeds was calculated using the formula:

Filled seed ratio (%) = (Number of filled seeds / Total number of seeds per head) × 100

Seed yield (kg/da) was calculated by threshing and weighing all heads harvested from a 10 m-long section in each plot.

Statistical Analysis

All data were analyzed according to the randomized complete block design using the General Linear Model (GLM) procedure in SAS software (SAS Institute, 2009). Analysis of variance (ANOVA) was performed, and treatment means were compared using the Least Significant Difference (LSD) test.

RESULTS and DISCUSSION

Effect of Essential Oil NEs Applications on Bee Attractiveness

The effects of NEOs applications on honeybee activity on sunflower heads are presented in Table 1. Prior to treatment, the number of bees per head was similar across all plots (ranging from 8.8 to 9.6 bees per head). However, after application, a marked increase in bee visitation was observed, particularly in plots treated with C. citratus NEs. On the first day after application, the highest bee density was recorded in the C. citratus NE 2000 ppm treatment (18.2 ± 0.57 bees per head), followed by C. citratus NE 1000 ppm (14.3 ± 0.33) and L. angustifolia NE 2000 ppm (14.7 ± 0.84). In contrast, the Tween-80–chitosan NE control and the untreated control plots exhibited a declining trend in bee numbers, which became more pronounced by day 7. The same trend persisted on days 3, 5, and 7 after application, with C. citratus NE 2000 ppm consistently maintaining the highest bee counts (17.3 ± 0.25, 17.7 ± 0.63, and 18.2 ± 0.94 bees per head, respectively). The low-concentration (1000 ppm) applications of R. officinalis and S. officinalis NEs were ineffective in enhancing bee visitation, whereas their 2000 ppm treatments showed a slight improvement compared to the control. In both the Tween-80–chitosan carrier control and the untreated control plots, bee numbers gradually declined over time, reaching 7.2–7.4 bees per head by day 7 (Table 1).

Table 1. Effects of NEOs applications on honeybee activity in sunflower fields

Treatments	Prior Treatment	1. Day	3. Day	5. Day	7. Day
C. citratus NE 1000 ppm	9.2 ± 0.14	14.3 ± 0.33	16.2 ± 0.19	16.5 ± 0.78	15.9 ± 0.72
C. citratus NE 2000 ppm	9.6 ± 0.22	18.2 ± 0.57	17.3 ± 0.25	17.7 ± 0.63	18.2 ± 0.94
L. angustifolia NE 1000 ppm	9.1 ± 0.17	12.5 ± 0.78	13.7 ± 0.11	13.9 ± 0.93	14.1 ± 0.52
L. angustifolia NE 2000 ppm	8.8 ± 0.34	14.7 ± 0.84	14.2 ± 0.37	14.5 ± 0.57	15.0 ± 0.62
R. officinalis NE 1000 ppm	8.9 ± 0.28	8.9 ± 0.43	9.2 ± 0.55	8.6 ± 0.24	9.3 ± 0.69
R. officinalis NE 2000 ppm	9.2 ± 0.42	11.5 ± 0.69	12.2 ± 0.73	12.7 ± 0.77	12.4 ± 0.83
S. officinalis NE 1000 ppm	9.6 ± 0.77	10.2 ± 0.72	9.4 ± 0.18	9.3 ± 0.41	10.1 ± 0.47
S. officinalis NE 2000 ppm	9.4 ± 0.26	12.1 ± 0.45	12.5 ± 0.21	12.4 ± 0.68	11.9 ± 0.71
Tween-80 – Chitosan NE	9.3 ± 0.65	8.7 ± 0.22	8.8 ± 0.42	7.1 ± 0.15	7.4 ± 0.18
Control	9.5 ± 0.39	9.1 ± 0.69	8.3 ± 0.29	7.4 ± 0.19	7.2 ± 0.32

The strong attractiveness of C. citratus EO can be attributed to its high citral (neral + geranial) and limonene content, which strongly stimulates the olfactory receptors of bees, triggering flower-searching behavior. These compounds are among the key odor components that elicit orientation and foraging responses in honeybees (Mas et al., 2018). Moreover, due to the similarity of citral to natural floral scent profiles, it has been reported to mimic the positive behavioral responses that bees exhibit toward flower odors (Conesa et al., 2012). Rahman et al. (2020) also demonstrated that C. citratus EO produced a pronounced orientation response in honeybees, which gave a positive electroantennographic reaction to this volatile compound. Similarly, the increase in bee numbers observed in L. angustifolia NE treatments is consistent with the known attractant properties of linalool and linalyl acetate-major constituents of lavender oil-on bees. Mishra et al. (2020) reported that linalool enhances learning and olfactory memory mechanisms in honeybees, thereby promoting persistence in floral preference. Hernández et al. (2019) further noted that plant volatiles released at low concentrations can activate bee olfactory receptors, resulting in increased flower visitation frequency. On the other hand, the relatively lower attractiveness of R. officinalis and S. officinalis EOs may be attributed to their high contents of 1,8-cineole, camphor, and borneol, which are known to be neutral or even repellent to bees at higher concentrations (Núñez et al., 2018). Cook et al. (2007) similarly reported that the high volatility of 1,8-cineole causes rapid dissipation of the scent in the environment, thereby reducing its long-term attractiveness to bees. Veeranjaneyulu et al. (2024) also found that applications of lemongrass, cumin, and orange essential oils at 0.5% and 1.0% concentrations significantly enhanced bee activity in sunflower fields, although the attractive effect declined notably by the fifth and seventh days after application. The absence of a decline in bee numbers over the seven-day observation period following NE application indicates that NEs reduced the evaporation rate of the essential oils, thereby enhancing the environmental stability of their active components. This suggests that volatile compounds persisted longer in the field environment, allowing bees to detect and respond to treated plants over an extended period. Several researchers have also emphasized that NE systems slow down the oxidative degradation of essential oils and prolong their biological activity (Majeed et al., 2021; Kumar et al., 2025). The gradual decrease in bee numbers observed in the carrier control (Tween-80–chitosan) and negative control plots confirms that the observed increases in bee activity were solely due to the essential oil constituents. This finding demonstrates that the physical properties of the NE alone had no significant effect on bee behavior.

Seed Number per Capitulum

The highest number of seeds per capitulum was observed in the *C. citratus* NE 2000 ppm treatment (1027 seeds/capitulum). This treatment was followed, within the same statistical group, by *C. citratus* NE 1000 ppm (988 seeds/capitulum) and L. angustifolia NE 2000 ppm (957 seeds/capitulum) applications. All three treatments significantly increased the seed number compared to the control. The effects of *R. officinalis* and *S. officinalis* NEs at 1000 ppm on seed number were insignificant, with mean seed numbers comparable to Tween-80–chitosan NE and control treatments (782–815 seeds/capitulum) (Table 2). Seed number per capitulum is an important indicator of pollination efficiency and seed set capacity. The results indicate that essential oil NE applications enhance bee activity, leading to increased pollen transfer per flower and, consequently, higher seed formation. Similarly, Free (1993) and Bosch & Blas (1994) reported that higher pollinator density increases seed number in sunflower. In our study, *C. citratus* NE applications particularly enhanced bee attractiveness, which positively reflected on the seed number per capitulum. Overall, our findings demonstrate that seed number per capitulum is influenced by bee visitation intensity and essential oil NE applications. This suggests that essential oil NEs may serve as a potential strategy to optimize sunflower yield by increasing not only the proportion of filled seeds but also total seed production.

Capitulum Yield

Significant differences ($P<0.01$) were observed among NEO applications in terms of capitulum yield. The highest yields were recorded for *C. citratus* NE 2000 ppm and 1000 ppm treatments (43.7 g/capitulum and 41.8 g/capitulum, respectively), which belonged to the same statistical group. *L. angustifolia* NE applications at both doses, as well as *R. officinalis* and *S. officinalis* NE at 2000 ppm, also significantly increased seed yield per capitulum (36.8–39.8 g), although not to the same extent as *C. citratus* NE. The 1000 ppm applications of *R. officinalis* and *S. officinalis* NE did not significantly affect capitulum yield, with values similar to Tween-80–chitosan NE and control treatments (Table 2). The observed yield increases can be attributed to higher bee activity, increased seed numbers, and higher filled seed ratios in these treatments. Specifically, *C. citratus* NEs attracted more bees during the flowering period, enhancing pollination efficiency and increasing both seed number and seed fill per capitulum, directly translating to higher seed yield. In contrast, *L. angustifolia* NE treatments exhibited a less persistent effect, likely due to the rapid volatilization of lavender essential oils and shorter duration of activity (Robu et al., 2011; Hassiotis and Vlachonasios, 2025).

Filled Seed Ratio

Significant differences (P<0.01) were observed among treatments in terms of filled seed ratio. The highest filled seed ratios were achieved with *C. citratus* NE at 1000 and 2000 ppm (67.8% and 70.2%, respectively) and *L. angustifolia* NE at 2000 ppm (66.8%). These treatments increased the filled seed ratio by more than 24% compared to the control. *R. officinalis* and *S. officinalis* NEs at 2000 ppm slightly increased the filled seed ratio relative to the control (37.6% and 36.8%, respectively), whereas their lower-dose applications showed no significant difference. Tween-80–chitosan and control treatments exhibited filled seed ratios of 54.5% and 53.6%, respectively (Table 2). These results suggest that NEOs enhance bee attraction, leading to higher pollen transfer per flower and more effective pollination. Previous studies have reported that increased pollinator density improves both the filled seed ratio and seed set (Free, 1993; Bosch & Blas, 1994; Amarilla et al., 2025). In this context, the high filled seed ratio observed in *C. citratus* NE applications may be associated with increased bee visitation to the capitulum. *L. angustifolia* NE showed moderate filled seed ratios (64–67%), while *R. officinalis* and *S. officinalis* NE exhibited lower ratios (56–60%). This indicates that the chemical composition and application dose of essential oils are critical factors influencing bee activity, pollination efficiency, and seed filling (Abrol, 2012).

Thousand-Seed Weight

NEO treatments significantly affected 1000-seed weight (P<0.05), ranging from 41.6 g to 43.4 g depending on the application. Both doses of *C. citratus* NE reduced 1000-seed weight relative to the control, while other treatments exhibited similar values to the control (Table 2). The decrease in 1000-seed weight in *C. citratus* NE treatments can be explained by the higher seed number per capitulum, as assimilates produced via photosynthesis are distributed among a larger number of seeds. Similarly, Kalyar et al. (2021) reported that under low pollination conditions, a decrease in seed number may increase 1000-seed weight.

Seed Yield

The effects of different essential oil NEs on sunflower seed yield are presented in Table 2. Significant differences (P<0.01) were observed among treatments. The highest seed yield (172.5 kg/da) was obtained with *C. citratus* NE 2000 ppm, followed by *C. citratus* NE 1000 ppm (162.7 kg/da) and *L. angustifolia* NE 2000 ppm (161.4 kg/da). These three treatments provided approximately 38%, 31%, and 30% higher seed yields than the control, respectively. In contrast, low-dose *R. officinalis* and *S. officinalis* NE, as well as Tween-80–chitosan NE and control treatments, formed the lowest-yield group (123.0–128.3 kg/da) (Table 2). Increased seed yield following essential oil application has been reported by Ghazoul (2006) and Chittka & Raine (2006), who noted that yield components (seed number, capitulum yield, and oil content) rise significantly under conditions of enhanced pollinator activity. Similarly, Sharma et al. (2021) and Sabir et al. (2022) reported significant increases in seed set and yield in sunflower following NEO applications. In our study, *C. citratus* and *L. angustifolia* NEs, which exhibited high bee activity, also resulted in high seed yield. Conversely, low bee-attractive *R. officinalis* and *S. officinalis* NEs were associated with lower seed yields. These findings indicate that high bee-attractive treatments enhance pollination efficiency, which in turn increases seed yield.

Table 2. Mean values of seed number per capitulum, capitulum yield, filled seed ratio, 1000-seed weight, and seed yield in sunflower plants treated with NEOs

Treatments	Seed Number per Capitulum	Capitulum Yield (g)	Filled Seed Ratio (%)	1000-Seed Weight (g)	Seed Yield (kg/da)
C. citratus NE 1000 ppm	988 a	41.8 ab	67.8 ab	41.7 b	162.7 ab
C. citratus NE 2000 ppm	1027 a	43.7 a	70.2 a	41.6 b	172.5 a
L. angustifolia NE 1000 ppm	885 b	38.0 cd	64.0 bc	42.1 ab	152.6 bc
L. angustifolia NE 2000 ppm	957 ab	39.8 bc	66.8 ab	41.9 ab	161.4 b
R. officinalis NE 1000 ppm	815 d	34.9 ef	57.2 de	42.6 ab	127.3 f
R. officinalis NE 2000 ppm	879 bc	37.6 cde	60.4 cd	42.0 ab	139.5 de
S. officinalis NE 1000 ppm	782 d	33.3 f	56.5 de	42.4 ab	123.0 f
S. officinalis NE 2000 ppm	850 cd	36.8 cde	60.3 c	42.5 ab	146.7 cd
Tween-80 – Chitosan NE	793 cd	32.2 f	54.5 e	43.2 ab	128.3 f
Control	767 d	32.6 f	53.6 e	43.4 a	124.5 f
CV (%)	6.5	4.9	4.8	3.2	7.2
Lsd	88.2**	3.0**	4.0**	1.6*	10.4**

Conclusion

This study investigated the effects of essential oil-based NE applications on bee-attracting activity and seed yield parameters in sunflower. The findings indicate that NEO applications, particularly C. citratus at higher concentrations, significantly enhance bee visitation, positively influencing seed number per capitulum, filled seed ratio, capitulum yield, and seed yield per hectare. The C. citratus NE 2000 ppm treatment produced the highest values across all parameters, demonstrating greater seed production and yield potential compared to other treatments. L. angustifolia NE applications exhibited moderate effects, whereas R. officinalis and S. officinalis NEs at 2000 ppm showed lower efficacy; their lower-dose applications had no significant impact on seed set or yield in sunflower. Overall, the results suggest that C. citratus and L. angustifolia essential oil NE possess high bee-attractive potential and can enhance pollinator activity in entomophilous crops such as sunflower, thereby improving seed formation and overall yield. These NEOs may represent a promising strategy to increase bee activity and optimize seed production in hybrid seed sunflower cultivation.

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