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Living mulches as a strategy for weed control and improved *Nigella sativa* L. production in salt-affected soils☆

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Abstract – Expanding the cultivation of underutilized oilseed crops on marginal lands necessitates innovative agronomic strategies to maintain lipid quality while ensuring stable yields. This study explored the use of living mulch systems for the sustainable production of black cumin (*Nigella sativa* L.), a promising yet weed-sensitive oilseed crop, in salt-affected soils in Iran. Through a two-year field experiment, the effectiveness of berseem clover (*Trifolium alexandrinum* L.) and barley (*Hordeum vulgare* L.) mulches, paired with strategic mowing timings, was evaluated. The results showed that berseem clover mulch significantly reduced the biomass of dominant weeds including *Amaranthus retroflexus*, *Chenopodium album*, and *Portulaca oleracea*, by 47–74.3% compared to weedy control plots. The highest reduction (74.3%) observed in plots with *T. alexandrinum* living mulch mowed before the black cumin flowering stage. The oil yield of black cumin cultivated under berseem clover living mulch (with post-establishment mowing) increased by 12.4% relative to weed-free control plots. Concurrently, the fatty acid profile improved, with a 3.47% increase in unsaturated fatty acids and a 13.35% higher unsaturated-to-saturated fatty acid ratio compared to the control. These results demonstrate a dual improvement in both oil yield and quality under the living mulch system. The optimal management strategy involved removing berseem clover after crop establishment, which led to a 12.4% and 45.7% increase in oil yield compared to the weed-free and weedy plots, respectively. These findings provide the first evidence-based protocol for managing weeds in black cumin under saline conditions, offering oilseed producers an ecological alternative to herbicides that maintains both yield quantity and lipid quality in marginal environments.

Keywords: 1000-grain weight / black oil / competition / fatty acid profile / weed management / yield

Résumé – Les couverts végétaux vivants comme stratégie de lutte contre les adventices et d'amélioration de la production de *Nigella sativa L.* dans des sols salins. L'expansion de la culture de plantes oléagineuses sous-utilisées sur des terres marginales nécessite des stratégies agronomiques innovantes afin de maintenir la qualité des lipides tout en assurant des rendements stables. Cette étude a examiné l'utilisation de systèmes de couverts végétaux vivants pour la production durable de nigelle (*Nigella sativa L.*), une plante oléagineuse prometteuse mais sensible aux adventices, dans des sols salins en Iran.
Au cours d'une expérimentation de deux ans sur le terrain, l'efficacité des couverts de trèfle d'Alexandrie (*Trifolium alexandrinum L.*) et d'orge (*Hordeum vulgare L.*), associés à différents calendriers de fauche, a été évaluée. Les résultats ont montré que le couvert de trèfle d'Alexandrie réduisait significativement la biomasse des adventices dominantes, notamment *Amaranthus retroflexus*, *Chenopodium album* et *Portulaca oleracea*, de 47 à 74,3% par rapport aux parcelles témoins non désherbées. La plus forte réduction (74,3%) a été observée dans les parcelles où le trèfle d'Alexandrie avait été fauché avant la floraison de la nigelle.
Le rendement en huile de la nigelle cultivée avec un couvert vivant de trèfle d'Alexandrie (fauché après l'implantation de la culture) a augmenté de 12,4% par rapport aux parcelles témoins désherbées. Parallèlement, le profil en acides gras s'est amélioré, avec une augmentation de 3,47% des acides gras insaturés et un ratio acides gras insaturés/saturés supérieur de 13,35% par rapport au témoin. Ces résultats démontrent une double amélioration de la teneur et de la qualité de l'huile grâce au système de couvert vivant.

☆ Contribution to the Topical Issue: "Innovative Cropping Systems / Systèmes innovants de culture oléoprotéagineux".
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La stratégie de gestion optimale a consisté à supprimer le trèfle d'Alexandrie après l'établissement de la culture, ce qui a permis une augmentation du rendement en huile de 12,4% et 45,7% comparativement aux parcelles désherbées et au témoin non désherbé, respectivement.
Ces résultats fournissent le premier protocole fondé sur des données probantes pour la gestion des adventices dans la culture de nigelle en conditions salines, offrant ainsi aux producteurs d'oléagineux une alternative écologique aux herbicides, qui préserve à la fois le rendement et la qualité de l'huile dans les environnements marginaux.

Mots-clés : poids de 1000 grains / huile de nigelle / compétition / profil en acides gras / gestion des adventices / rendement

Highlights

Trifolium alexandrinum living mulch reduces weeds by 47-74% in saline-grown *Nigella sativa* while increasing oil yield by 45.7% and maintaining lipid quality (31.1-34.3%). This first IWM protocol for black cumin in salt-affected soils offers herbicide-free production on marginal lands.

1 Introduction

Grain oils have various applications in food, cosmetic, and pharmaceutical industries. They are important for human health and nutrition, as they provide fatty acids and oil-soluble vitamins. They also have some functions and biological activities that help humans prevent some diseases (Hamed *et al.*, 2017). They reduce the risk of heart disease, diabetes, auto-immunity and many other chronic diseases. Grains of *Nigella sativa* L., an annual herbaceous plant from Ranunculaceae, contain 30–48% valuable plant oil rich in thymoquinone and unsaturated fatty acids (Randhawa and Alghamdi, 2011). Grains of this plant have been traditionally used to treat fever, headache, anxiety, diarrhea, gastrointestinal disorders, asthma, hypertension, diabetes and stroke for years (Randhawa and Alghamdi, 2011). The protective and therapeutic effects of *N. sativa* oil have been well documented (Al-Okbi *et al.*, 2015). Besides bioactive compounds such as phenolics with high antioxidative activity (Mariod *et al.*, 2009; Meziti *et al.*, 2012; Dalli *et al.*, 2021), *N. sativa* grains also contain sterols and tocols (Ketenoglu *et al.*, 2020).

N. sativa originates from Southwest Asia, North Africa and Southern Europe (Sultana *et al.*, 2015; Kooti *et al.*, 2017). It is now cultivated in South Asia, including Pakistan and India, the Middle East such as Saudi Arabia and Syria, the Mediterranean region like Turkey and Southern Europe, and extends to other areas (Sultana *et al.*, 2015). *N. sativa* can tolerate salinity stress (Papastylianou *et al.*, 2018). This makes it a valuable plant for agricultural development and land use in arid and semi-arid areas where soil and water salinity are problems. To grow functional industrial plants in salt-affected lands, we need to understand the specific needs of the crops in these areas and manage various factors, such as water resources, mineral nutrients, pests and diseases, and weeds.

Weeds are a major threat to crop production, with yield reductions ranging from 30 to 90%, depending on weed species, crop type, and management practices (Chauhan, 2022). Weeds can affect the growth and production of secondary metabolites in medicinal plants due to competition, allelopathic effects, and as a biological stress factor. The impact of weeds on the secondary metabolites of medicinal plants can be very significant and varied. These impacts can be either positive or negative, depending on the type of weed and the medicinal plant involved (Gaba *et al.*, 2014; Macías *et al.*, 2019; Shan *et al.*, 2023).

Herbicides are commonly used to control weeds in arable crops and medicinal plants such as *N. sativa* (Zia Ul Hag *et al.*, 2024). However, they can affect the quality of medicinal plants and harm the environment. Therefore, there is a need to reduce the use of herbicides in growing medicinal plants. Although hand weeding can be extremely effective in managing weeds, especially for targeted elimination, it is often constrained by high costs associated with labor and time (Kudsk and Mathiassen, 2020; Peerzada *et al.*, 2022). A better approach to reduce weed competition is to fill the ecological niches that weeds would occupy. "Living mulches" are cover crops that grow at the same time and place as the main crops (Bhaskar *et al.*, 2021). Living mulches have many benefits over dead mulches, *i.e.* straw and hay. Living mulches enhance the competitive ability of crops by suppressing weeds from 34% to 96% (Ghosheh *et al.*, 2005; Mohammadi, 2010). Furthermore, living mulches positively influence the soil ecosystem by impacting both surface and subterranean layers, thereby enhancing soil biological activity (Nakamoto and Tsukamoto, 2006). Living mulches can either reduce (Carof *et al.*, 2007; Jędrszczyk and Poniedziałek, 2007) or increase (Adamczewska-Sowińska *et al.*, 2009) the productivity of the main crop. These different outcomes depend partly on the species, sowing date, and management method of living mulches. We conducted this study to assess the feasibility of growing *N. sativa* in salt-affected soils by evaluating its oil yield and composition. Furthermore, since this plant has low competitive power against weeds, we investigated the effect of growing barley (*Hordeum vulgare* L.) and berseem clover (*Trifolium alexandrinum* L.) as living mulches on weed control, yield and oil quality of *N. sativa* in the field.

2 Materials and methods

2.1 Experimental site

We carried out the experiments at the research farm of the Abouraihan College of Agricultural Technologies (35° 28 N, 51° 36 E and 1020 masl), University of Tehran, Iran. This site is in an arid region according to the de Martonne climate

Table 1. Mean temperature and precipitation in the experimental site during the growing season

		March	April	May	June	July
2017	Temperature (°C)	12.7	20	27	32	34
	Precipitation (mm)	52	22	22	1	0
2018	Temperature (°C)	17	17	22	30	35
	Precipitation (mm)	3	30	9	5	0

Table 2. Physiochemical properties of the soil of the experimental site in depth of 0–30 cm.

N (%)	K (mg kg^{-1})	P (mg kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})	Mn (mg kg^{-1})	B (mg kg^{-1})
0.14	732	79	4.23	2.3	0.83	8.94	1.34
pH	Clay (%)	Silt (%)	Sand (%)	Texture	EC (ds m^{-1})	OC (%)	TNV (%)
8.21	28	50	22	Sandy silt	7.57	1.4	20.25

EC: Electrical conductivity; OC: Organic carbon; TNV: Total neutralizing value

classification. It has hot and dry summers and mild winters with an annual rainfall and temperature of 141 mm and 15.6 °C, respectively (Tab. 1). The soil type and properties in this experimental field are shown in Table 2.

2.2 Experimental treatments and agronomic practices

We conducted the experiment in a completely randomized block design with six treatments and four replications. We repeated the experiments in two consecutive years 2017 and 2018. The treatments included two living mulch species: barley and berseem clover, and two living mulch mowing times: (1) mowing after *N. sativa* establishment at the 6-leaf stage (Ct1) and (2) mowing before the *N. sativa* flowering stage (Ct2). We also had two control treatments: weed-free (WF): full weeding during the growth stages of *N. sativa*, and weed-infested (WI): no weeding during the growth of *N. sativa*. The barley cultivar used (*Hordeum vulgare* L. cv. 'Baharan'/'Bahman') was a high-yielding six-row winter barley developed by the Iranian Seed and Plant Improvement Institute for semi-cold climates with clay-loam soils typical of the region. For berseem clover (*Trifolium alexandrinum* L.), locally adapted ecotypes from Isfahan province were used as representative genotypes of central Iran's agricultural ecosystems.

The moldboard plowing (25–30 cm) in the spring was followed by twice disking before planting. Each plot was 4 m in length and 2 m in wide with 4 rows at a distance of 50 cm. A 50 cm space was considered between each experimental unit and each replication. The seedbed preparation operations were the same in both years. The seeds of *N. sativa* (local landraces which were obtained from Isfahan region of Iran) were sown by hand on 14 April 2017 and 16 April 2018. Seeds were planted using a 50 cm row spacing and 5 cm in-row spacing (400,000 seeds ha^{-1}). Due to a 60% germination percentage, the final plant density achieved was 240,000 plants ha^{-1}. Seeds of the living mulch plants were planted simultaneously with *N. sativa*. They were planted manually and randomly between *N. sativa* rows and covered with sand. The seed rates used for barley and berseem clover were 150 kg ha^{-1} and 30 kg ha^{-1}, respectively. The first irrigation was performed immediately after planting. Until the establishment of *N. sativa*, irrigation was done weekly, and then, the irrigation interval was considered 10 days. No chemical fertilizers or pesticides were used in the field. A picture of the *N. sativa* plant is provided in Supplementary Figure S1, showing key morphological features.

2.3 Weed assessment

At the end of *Nigella sativa*'s flowering stage, weed sampling was conducted using three randomly placed 50 cm × 50 cm quadrats per plot. To avoid margin effects, quadrats were placed no closer than 1 m from plot edges. Within replicates, sampling locations were systematically varied to ensure that every plot was adequately sampled and representative coverage is achieved. We examined weed species composition, total weed density and weed biomass. To evaluate the biomass per unit area, the weeds were cut from the ground surface and dried at 75 °C for 73 h.

2.4 Yield related traits and fatty acids profile

Seeds were harvested at physiological maturity which took place 97 ± 3 days after sowing. These seeds were then shade-dried at a temperature of 25 ± 2°C until moisture content reached 8%. For oil extraction, 10 g of the seeds from each treatment from both years were mixed, grounded and put in extraction paper bags. The samples were placed in a distillation flask in a Soxhlet extraction unit and 300 ml of petroleum benzene was added. The boiling temperature range of the Soxhlet apparatus was kept at 40–60 °C for 4 h (Akbari *et al.*, 2020; Movahhedy-Dehnavy *et al.*, 2009). The extracted oil was filtered and dehydrated and the oil content of the seeds was calculated as the percentage of seed dry weight (Ashraf *et al.*, 2006; Movahhedy-Dehnavy *et al.*, 2009; Hosseini *et al.*, 2019).

Table 3. Weed species and their relative frequency in the experimental field.

Relative frequency(%)	Scientific Name
36	*Amaranthus retroflexus* L.*
29	*Chenopodium album* L.*
21	*Portulaca oleracea* L.*
3	*Cynodon dactylon* L. (Pers)
2	*Echinochola crus–galli* (L.) P. Beauv.
2	*Malva sylvestris* L.
2	*Sorghum halepense* L. (Pers).
1	*Rumex crispus* L.
1	*Cyperus longus* L.
1	*Convolvulus arvensis* L.
1	*Sonchus arvensis* L.
1	*Xanthium strumarium* L.

* The field dominant weeds

To determine the fatty acid composition of the oil, the samples were prepared according to the method described by Metcalfe *et al.* (1966). The samples were analyzed by a gas chromatograph (Unicam 4600, Cambridge, England) equipped with an FID detector after derivatization to fatty methyl esters (FAME). The capillary column was BPX70 (30 m × 0.22 mmi. d.) with a 0.25 μm film thickness (from SGE). The column and the injector temperature were 180 and 240 °C, respectively. The detector temperature was 200 °C (Movahhedy-Dehnavy *et al.*, 2009). FAMEs were identified and quantified based on the comparison of the retention times of the samples with those of standards from Aldrich or Sigma (USA). The percentage of each identified fatty acid was calculated based on the total value of the fatty acids. The value of each sample was determined based on the average of two injections (Movahhedy-Dehnavy *et al.*, 2009).

To compare the *N. sativa* oil characteristics obtained from different treatments, total values of unsaturated, mono unsaturated, poly unsaturated and saturated fatty acids were calculated. Double bond index (DBI) as an indicator of the unsaturation fatty acid fraction and Iodine value (IV) was also determined for each treatment. DBI and IV were calculated based on Eq. 1 and 2, respectively (Akbari *et al.*, 2020).

$$DBI = 0 \times ([14:0] + [15:0] + [16:0] + [18:0] + [20:0]) + 1 \times ([16:1] + [18:1] + [20:1]) + 2 \times ([18:2] + [20:2]) + 3 \times ([18:3]) + 4 \times ([20:4]), \quad (1)$$

$$IV = (\%\,oleicacid \times 0.8601) + (\%\,linoleicacid \times 1.7321) + (\%\,eicosenoicacid \times 0.7854), \quad (2)$$

2.5 Statistical analyses

Analysis of variance (ANOVA) on density and dry mass of weeds; and one thousand seed weight and oil yield of *N. sativa* data was performed using the general linear model (GLM) procedure in the RStudio software. The least significant differences test was applied to compare means at a 5% probability level. Bartlett's test was used to test for homogeneity of variances of the experimental errors of the results of the two years, and considering that the difference between the error variances was not significant, the combined variance analysis was performed for two years of experiment. The year-specific data presented in Supplementary Table S1, also show consistent trends between the two growing seasons. The Shapiro–Wilk test was used to assess data normality and the Breusch–Pagan test was used for homoscedasticity.

3 Results

3.1 Weed composition

Table 3 shows the common weed species and their relative frequency in the experimental field in both years. *Amaranthus retroflexus*, *Chenopodium album* and *Portulaca oleracea* accounted for 86% of the weeds in the *N. sativa* plots.

3.2 Weed density

The ANOVA showed that weed density was significantly affected by the weed management systems (Tab. 4). Living mulches reduced weed densities significantly (Fig. 1A). The lowest weed density was observed in TCt2 and TCt1 (berseem clover living mulch: mowing before the *N. sativa* flowering stage and mowing after *N. sativa* establishment, respectively) which reduced weed density by 74.3% and 70.8% compared with the weed-infested treatment (WI), respectively. In HCt1 (barley living mulch, mowing after black cumin establishment) and HCt2 (barley living mulch, mowing before the *N. sativa* flowering stage) plots, weed density decreased by 52.0% and 46.8% compared with the WI treatment, respectively.

3.3 Weed growth

Weed management systems had a significant effect on weed dry mass (Tab. 4). Weed dry mass decreased by 74.3% and 70.3% in TCt2 and TCt1 treatments, respectively, compared to the WI treatment (Fig. 1B). HCt1 and HCt2 reduced weed dry mass by 50% compared with the WI treatment (Fig. 1B).

3.4 Grain weight of *N. sativa*

The weed management systems affected 1000-grain weight of *N. sativa* significantly (Table 4). The TCt1 (berseem clover mowing after *N. sativa* establishment) had the highest 1000-grain weight of *N. sativa* (1.71 g) while WI (weed-infested) had the lowest 1000-grain weight (1.41 g) (Fig. 1A). Likewise, the highest seed yield was recorded for the TCt1 treatment as (366.15 kg ha^{-1}) and the lowest yield was recorded for WI treatment (268.85 kg ha^{-1}) (Supplementary Figure S2). TCt1 increased 1000-grain weight by 7.7% compared with WF (weed-free). There was no significant difference between HCt2 (barley mowing before the black cumin flowering stage) and WF (Fig. 1A).

Table 4. Combined analysis of variance (mean squares) for the effect of treatments on density and biomass of weeds, and yield indices of black cumin.

S.O.V	*df*	Weed density	Weed dry mass	1000-grain weight	Seed oil content percentage	Oil yield
Year	1	184.0 ns	3650.2 ns	0.0003 ns	0.0030 ns	0.24ns
Block (Year)	6	552.5 *	1733.3 ns	0.0057 ns	0.0008 **	466.24**
Treatments	5	6365.6**	147269.5**	0.0817**	14.720**	1311.34**
Year×Treat.	5	37.2 ns	431.6 ns	0.0005 ns	0.0004*	24.10ns
Error	30	217.9	2292.5	0.0028	0.0001	117.46

ns, * and **: non –significant, and significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

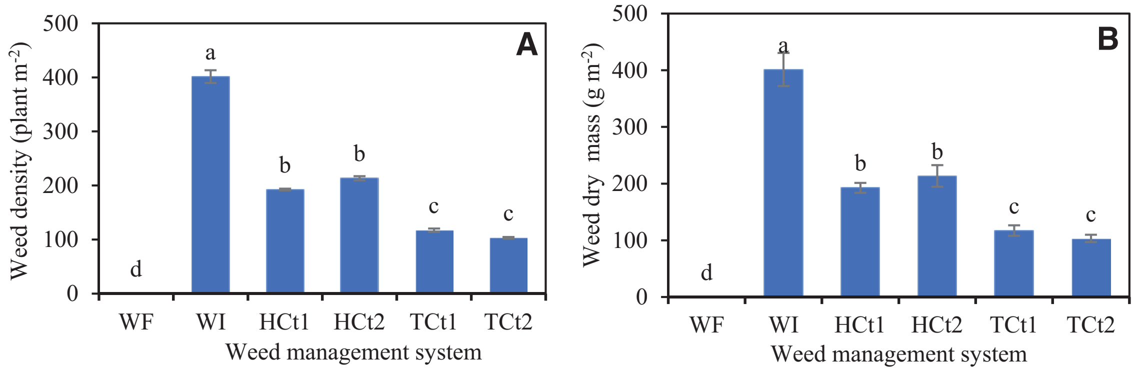

Fig. 1. The effects of weed management systems on density (A) and dry mass (B) of weeds in the black cumin field in two cropping years. The values represent the means of 2017 and 2018. WF: weed free plot; WI: weed infest plot; HCt1: *H. vulgare* living mulch, mowing after black cumin establishment; HCt2: *H. vulgare* living mulch, mowing before the black cumin flowering stage. TCt1; *T. alexandrinum* living mulch, mowing after black cumin establishment; TCt2: *T. alexandrinum* living mulch, mowing before the black cumin flowering stage.

3.5 *N. sativa* oil yield

The ANOVA indicated that, the weed management systems significantly affect the oil content of *N. sativa* seeds (Tab. 4). The lowest oil content was observed in the seeds of plants in the weed infested (WI) plots. The weed management systems increased the percentage of oil content compared with WI and weed free (WF) treatments. The highest oil content was observed in the HCt2 treatment, which was 2.9% more than the WF treatment (Fig. 2C).

The effect of weed management systems on the oil yield of *N. sativa* plots was significant (Tab. 4). The lowest oil yield was detected in the WI plot. The highest oil yield was obtained from TCt1 and HCt1 treatments (Fig. 2B). The oil yield in TCt1 plots was increased by 12.4% and 45.7%, compared to those in WF and the WI plots, respectively.

3.6 Fatty acid composition

Table 5 represents the fatty acid profile of *N. sativa* oil in response to different weed management systems at two years of the experiment. Six unsaturated fatty acids including linoleic, oleic, palmitoleic, α-linolenic, arachidonic, and eicosenoic fatty acids, and five saturated fatty acids including myristic, palmitic, stearic, arachidic, and pentadecanoic were identified and quantified in *N. sativa* oil (Tab. 5). Linoleic acid (52.8%), oleic acid (23.1%) and palmitic acid (12.9%) were the main components of the *N. sativa* oil.

The weed management systems significantly affect the profile of fatty acids in *N. sativa* oil (Tab. 5). Living mulch treatments increased the amount of linoleic and oleic acids. The highest content of linoleic acid was obtained in the HCt2 treatment which showed a 1.85% increase compared to the WF treatment. Oleic acid in the HCt1 and HCt2 plots was 10.34% and 4.84% higher than the WF treatment, respectively.

Unsaturated (ΣUFA) and saturated (ΣSFA) fatty acids accounted for 79.5% and 20.5% of the total fatty acids of *N. sativa* oil, respectively (Tab. 6). The living mulch treatments, increased ΣUFA in *N. sativa* oil up to 3.25% compared with that in the WF treatment. The highest amount of ΣUFA with 80.9% belonged to the TCt1 plot (Tab. 6). A noteworthy point is that the amount of ΣUFA in the WI treatment was also higher than the WF treatment. In contrast, the presence of living mulches decreased the ΣSFA. Among the investigated treatments, TCt1 with 12.8% less saturated fatty acids compared with WF, had the least ΣSFA content. In HCt1 and HCt2 plots, 6.85% less ΣSFA were obtained, compared with the WF plot.

4 Discussion

The results of our two-year experiment showed that the living mulches played a significant role in controlling salt-tolerant weeds in the *N. sativa* field. With the application of living mulches, a 61% reduction in weed population was observed. Verret *et al.* (2017) meta-analyses revealed that

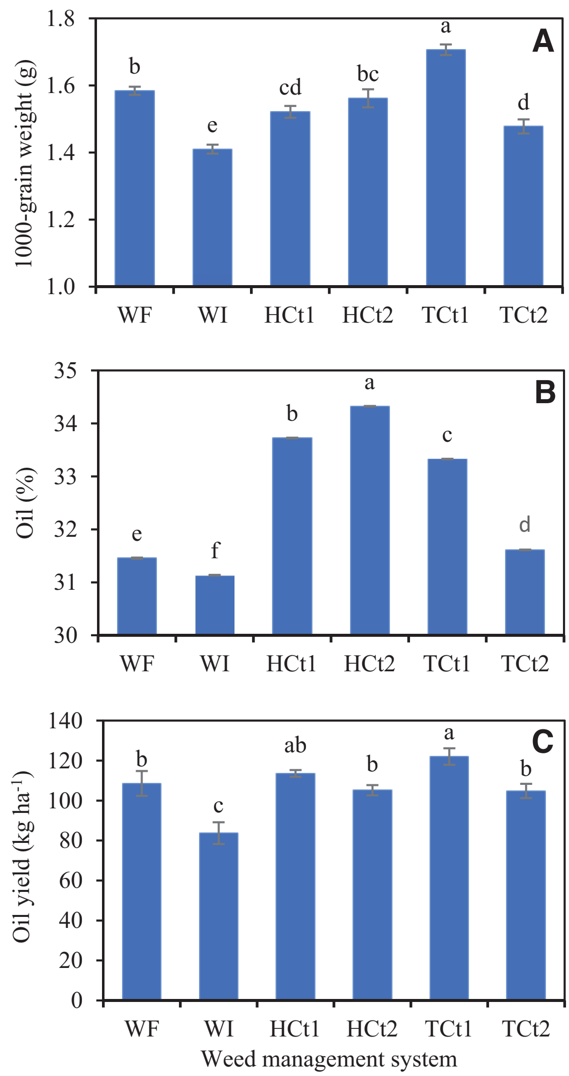

Fig. 2. The effects of weed management systems on thousand seed weight (A), oil percentage (B), and oil yield (C) of black cumin in two cropping years. The values represent the means of 2017 and 2018. WF: weed free plot; WI: weed infest plot; HCt1: *H. vulgare* living mulch, mowing after black cumin establishment; HCt2: *H. vulgare* living mulch, mowing before the black cumin flowering stage. TCt1; *T. alexandrinum* living mulch, mowing after black cumin establishment; TCt2: *T. alexandrinum* living mulch, mowing before the black cumin flowering stage.

intercropping living mulches, relay cropping, or co-planting of cover crops can reduce weed population by 82% compared to the WI plots. It has been reported that living mulch could decrease weed densities in cereal crops by up to 55% (Bhaskar *et al.*, 2014; Den Hollander *et al.*, 2007; Gerhards, 2018). Mulching, by intercepting the light, suppresses the germination and growth of weeds. By absorbing red light, living mulch reduces the ratio of red to far-red light on the soil surface, which prevents the reception of environmental signals for the germination of weed seeds (Mechergui *et al.*, 2021).

The oil content of *N. sativa* (31.1–34.3%) was in the range reported in previous studies (Ashraf *et al.*, 2006; Seyyedi *et al.*, 2016). These results indicated that *N. sativa* produces a comparable yield in saline soils and therefore, it can be utilized in the rehabilitation and exploitation of salt affected lands. However, weed infestation reduced the 1000-grain weight and the *N. sativa* oil content of the seeds that eventually led to a decrease in the oil yield per unit area. Meena *et al.* (2019) and Mehni *et al.* (2020) also reported the effect of weeds on reducing the *N. sativa* yield. The reduction of *N. sativa* oil content in the presence of weeds was also reported by other researchers (Kirici *et al.*, 2021; Seyyedi *et al.*, 2016; Hossein *et al.*, 2019). Weeds affect growth and productivity of the crop by competition with the crop for limited common resources (Meena *et al.*, 2014). These results emphasized the low competition capacity of N. sativa with weeds. Due to its slow growth, short height, and open canopy structure, the plant exhibits little competitive power compared to most weeds, so that contamination by weeds can destroy 60–80% of the *N. sativa* crop (Kifelew *et al.*, 2017). Tweaking essential elements of a nutrition program, like the ratios of nutrients and their timing, may control weed competition and help to increase oil yield by enhancing crop growth (Seyyedi *et al.*, 2016). However, an effective weed management program is required to obtain maximum yield in *N. sativa* farms.

The main weed species in this study included *A. retroflexus* (Redroot pigweed), *C. album* (lamb's quarters), and *P. oleracea* (purslane). Although the weed composition is affected by various factors such as production sites, year and management practices (Tursun *et al.*, 2007), these species were reported by Seyyedi *et al.* (2016) in a two-year cultivation of *N. sativa* in the northeastern part of Iran. This similarity in the results was due to the climatic similarity of the experimental sites and the spread and adaptability of these weeds in arid and semi-arid areas.

A. retroflexus is one of the most important weed species in arid and semi-arid regions (Amini *et al.*, 2014). A C4 photosynthesis system is one of the factors that give this plant the ability to survive and grow in arid environments and salt-affected soils. This plant causes a significant decrease in the yield of agricultural plants and creates problems in harvesting operations (Aguyoh and Masiunas, 2003; Amini *et al.*, 2014). *C. album* is an annual species that has an almost global distribution and is one of the most important weeds in the world. It is a difficult weed in many agricultural systems because of high seed production and an extended germination period (DeGreeff *et al.* 2018). *A. retroflexus* and *C. album* are fast-growing and allelopathic plants that can drastically reduce the yield of crops in infested fields (Bajwa *et al.*, 2019). *P. oleracea* (Portulacaceae) is considered one of the 10 most aggressive weeds in the world (CABI, 2022). This plant is an annual weed that exhibits both C4 and CAM photosynthesis systems and therefore shows great compatibility with dry and saline soils (Ozturk *et al.*, 2021). This plant grows in different types of soils and soil pH from acidic to alkaline and tolerates dry and saline soils (Yao *et al.*, 2010). *C. album* is an intermediate form of C3-C4 (Yorimitsu *et al.*, 2019), possibly an evolutionary step that facilitates range expansion under climate change. *P. oleracea* severely reduces the yield of monocot and dicot crops (Silva *et al.*, 2007). Various allelochemicals have been identified in this plant (Zhu *et al.*, 2012) that can suppress the germination and growth of crops (Silva *et al.*, 2007). In addition, its leaf and root extracts impair the antioxidant defense system and damage

Table 5. The effects of weed management systems on fatty acid composition of black cumin oil. The values represent the means of 2017 and 2018.

Weed management	Palmitic acid (C16:0)	Palmitoleic acid (C16:1)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)	ɑ–linolenic acid (C18:3)
WF	13.23 a	0.13 c	2.63 e	22.53 e	52.40 e	0.07 d
WI	12.99 b	0.14 b	2.80 d	22.61 d	53.25 c	0.14 b
HCt1	12.66 cd	0.14 b	2.96 b	22.62 cd	53.33 b	0.21 a
HCt2	12.65 d	0.14 b	2.95 b	22.63 c	53.37 a	0.20 a
TCt1	12.68 c	0.14 b	2.87 c	24.86 a	52.53 d	0.14 b
TCt2	12.98 b	0.15 a	3.28 a	23.62 b	52.14 f	0.09 c
Weed management	Eicosenoic acid(C20:1)	Myristicb acid(C14:0)	Pentadecanoic acid(C15:0)	Arachidic acid(C20:0)	Arachidonic acid(C20:4)	
WF	0.270 a	2.35 a	3.67 a	0.12 d	3.00 b	
WI	0.260 b	1.60 d	3.08 b	0.15 c	2.96 c	
HCt1	0.250 c	1.62 c	2.99 d	0.18 a	3.00 b	
HCt2	0.190 d	1.58 e	3.02 d	0.17 b	2.80 d	
TCt1	0.260 b	0.68 f	2.67 c	0.12 d	3.05 a	
TCt2	0.250 c	1.69 b	2.66 c	0.00 e	3.06 a	

Means within each column of each section followed by the same letter are not significantly different ($P < 0.05$). Values represent the relative percentage of each fatty acid normalized to the total identified fatty acids (unidentified peaks excluded). WF: weed free plot; WI: weed infest plot; *H*Ct1: *H. vulgare* living mulch, mowing after black cumin establishment; *H*Ct2: *H. vulgare* living mulch, mowing before the black cumin flowering stage. *T*Ct1; *T. alexandrinum* living mulch, mowing after black cumin establishment; *T*Ct2: *T. alexandrinum* living mulch, mowing before the black cumin flowering stage.

Table 6. Comparison of saturated fatty acids (ΣSFA), unsaturated fatty acids (ΣUFA), double bond index (DBI) and iodine value (IV) of *Nigella sativa* L. seed oil in different weed management systems at two years of experiment.

Weed management	ΣSFA	ΣUFA	ΣUFA/ΣSFA	DBI	IV
WF	21.9 a	78.1 f	2.53 e	139.0 f	110.3 f
WI	20.6 c	79.4 d	2.73 c	141.9 d	111.9 d
HCt1	20.4 d	79.6 c	2.78 b	142.5 c	112.0 c
HCt2	20.4 d	79.6 b	2.78 b	142.6 b	112.1 b
TCt1	19.1 e	80.9 a	2.92 a	142.8 a	112.6 a
TCt2	20.8 b	79.2 e	2.65 d	140.4 e	110.8 e

WF: weed free plot; WI: weed infest plot; HCt1: *H. vulgare* living mulch, mowing after black cumin establishment; HCt2: *H. vulgare* living mulch, mowing before the black cumin flowering stage. TCt1; *T. alexandrinum* living mulch, mowing after black cumin establishment; TCt2: *T. alexandrinum* living mulch, mowing before the black cumin flowering stage.

photosynthetic pigments in other plants (El-Shora and Abd El-Gawad, 2015). The adaptation of these three species to arid and saline soils caused them to prevail over other weeds and black cumin. Due to their ability to tolerate salinity, such plants can show a higher competitive ability than agricultural plants in soils that have the potential for salinity stress and cause a decrease in crop growth and yield.

The use of berseem clover as a cover crop (until the establishment of *N. sativa*) effectively controlled the weeds. Proper density of berseem clover reduced weed emergence and growth by minimizing empty niches that are conducive to weed growth. The greater effectiveness of clover in limiting weeds compared to barley was probably due to the greater shade area of clover. The higher biomass of berseem clover compared to barley showed the higher competition capability of this plant with other species. Our results are in line with Wang *et al*. (2016) and Bhaskar *et al*. (2021) who stated that the application of legumes as living mulch is an effective ecological approach to weed suppression and soil fertility improvement. Clover is an ideal living mulch plant because of its nitrogen-fixing ability, long-term perennial growth, and weed suppression potential (Fracchiolla *et al*. 2022). However, the rapid establishment, high competition ability, and allelopathic properties make barley a desirable plant for weed suppression (Kremer and Ben-Hammouda, 2009). Its rapid establishment and competition capacity largely depend on the absorption of soil moisture during the early stages of growth. Shading and release of allelopathic chemicals could be the reasons for weed growth inhibition in Barley plots (Asghari and Tewari, 2007).

N. sativa yield in presence of the living mulches was higher than the weed-infested and weed free plots. It is well established that mixed cultivation compared to monoculture, improves performance and maximizes the use of resources and more appropriate use of them in the agricultural ecosystem, due to the increase in diversity (Huss *et al.*, 2022). As a result, using living mulches increases yield by reducing the consumption of expensive inputs. Usually, such improvements are obtained in crops where the length of the growing period of plants is different (Ahmed *et al.*, 2020). Cultivation of plants with different morphological and physiological characteristics together facilitates the optimal use of environmental factors (Dong *et al.*, 2018). Our results showed that in the *N. sativa* mixed cultivation, the use of berseem clover had more beneficial effects than barley. These results can be due to the ability of leguminous plants to fix nitrogen and increase the availability of this element in the soil, which causes the improvement of soil fertility and ultimately the plant productivity (Campiglia *et al.*, 2010). A weed-free system, while eliminating competition, lacks these positive ecological interactions. Therefore, the observed superiority of berseem clover as a cover crop (until the establishment of *N. sativa*) over the weed-free control in grain yield may be attributed to the mentioned cases. It was also reported that the yield of cereals in the cultivation system with living mulch was 14–22% higher than the system without living mulch (Bhaskar *et al.*, 2014). The use of living mulch also increased the percentage of seed oil. Seed oil content of fennel (*Foeniculum vulgare* L.) increased by intercropping with legume plants (Rezaei-Chiyaneh *et al*. 2020). Due to the increase in seed yield and the percentage of oil in seed, oil yield per unit area also increased in living mulch plots.

The yield of *N. sativa* oil in the plots where the clover was cut in the early growth stages was higher than the plots where the clover was kept until *N. sativa* flowering stage. These results showed that if berseem clover is permitted to grow until the flowering period of *N. sativa*, it will contest with *N. sativa* for resources like nutrients, water, or light for a prolonged duration, which subsequently decreases the yield of black seed oil. In contrast, cutting clover early minimizes this competition. This interpretation aligns with our results, where early cutting of berseem clover improved oil yield by 7.42% compared to plots where clover was retained longer. While the open canopy structure of barley makes it less competitive with the main crop. In this study, the fatty acid composition of the *N. sativa* oil was similar to the values reported by Tulukcu (2011) and Soleimanifar *et al*. (2019). Linoleic acid (52.1–53.4%) was the predominant fatty acid in the oil of *N. sativa* seeds. This is an essential polyunsaturated omega-6 fatty acid which constitutes more than 50% of the total fatty acids in the oil of *N. sativa* (Ashraf *et al.*, 2006). Yimer *et al*. (2019) reported that linoleic acid content in the oil of *N. sativa* was 64.6%. Matthäus and ÖzCaN (2011) also reported the linoleic acid content of *N. sativa* between 56.7% and 58.9%. Oleic acid was the second predominant fatty acid in the *N. sativa* oil (22.5–24.9%). The oleic acid content of *N. sativa* has been reported 18.7 and 23.7% in previous studies (Matthäus and ÖzCaN, 2011). The fatty acid composition of the oil seed of *N. sativa* varied by the climatic conditions and genotypes (Amin *et al.*, 2010). However, the present study showed that the *N. sativa* cultivation in saline soil does not significantly change the fatty acid profile of this plant.

Our results indicated that the weed management systems did not affect the relative content of the dominant fatty acids of the *N. sativa* oil. However, the percentage of unsaturated fatty acids in black cumin oil in the presence of living mulches and weeds was higher than the plots without living mulch and the weed-free plots. The results revealed that the presence of another plant species (weed or living mulch) increases the ΣUFA in *N. sativa* oil. Crops and weeds may coexist without economic loss of yield, and some relationships between crops and weeds may have beneficial effects (Koehler *et al.*, 2020). The yield of *N. sativa* oil and the content of each fatty acids in its oil are under the influence of environmental conditions (Bayati *et al.*, 2020). Different plants in the vicinity of each other do not compete for the absorption of a specific element. In other words, the effect of interspecific competition is equal or less than intraspecific competition (Adler *et al.*, 2018). When interspecific competition is weaker than intraspecific competition, each species in a community limits growth of its own population more than its competitors. The result is negative frequency dependence: the rarer a species becomes in a community, the more its population growth rate increases, protecting it from competitive exclusion. A variety of symbiotic mechanisms, such as differential responses to spatial and temporal environmental changes, resource partitioning, and species-specific natural enemies, lead to differences and negative frequency dependence (Adler *et al*. 2007). One of the ways that two plants are complementary is the time difference in the growth period of the plants. If the length of growth of plants is different from each other, they will provide their required materials (aerial and terrestrial) at different times (Dong *et al.*, 2018).

5 Conclusion

N. sativa exhibited a low competitive ability with weeds since the presence of weeds reduced its seed and oil production capacity. Although weed infestation did not affect the relative amounts of fatty acids in the *N. sativa* oil, it decreased oil yield per unit area by reducing the grain weight and the percentage of oil in the seed. *Amaranthus retroflexus*, *Chenopodium album* and *Portulaca oleracea* were the major weeds in the experimental site. These are highly salt and drought tolerant plants that may cause serious problems in agricultural lands in the future. Berseem clover and barley as living mulch showed acceptable performance in controlling the dominant weeds. These plants performed well in the hot and dry condition and the salt-affected soil of the site. Berseem clover was more effective in controlling weeds than barley, thereby of its dense cover over the soil and nitrogen fixation activity. However, the results indicated that berseem clover may compete with the main crop in the long term. Thus, berseem clover cultivation as a living mulch and mowing it after *N. sativa* establishment is recommended to manage weeds of *N. sativa* and improve its productivity.

Conflicts of interest

The authors have no conflicts of interest to declare.

Supplementary material

Figure S1. Image of a complete *N. sativa* plant, its flowers and seeds. (https://commons.wikimedia.org/wiki/File:Nigella_sativa).

Figure S2. The effects of weed management systems on seed yield of black cumin in two cropping years. The values represent the means of 2017 and 2018. WF: weed free plot; WI: weed infest plot; HCt1: *H. vulgare* living mulch, mowing after black cumin establishment; HCt2: *H. vulgare* living mulch, mowing before the black cumin flowering stage. TCt1; *T. alexandrinum* living mulch, mowing after black cumin establishment; TCt2: *T. alexandrinum* living mulch, mowing before the black cumin flowering stage.

Table S1. Year-wise treatment effects on weed parameters and yield indices of black cumin. Values represent Mean ± Standard Deviation and different lowercase letters indicate significant differences (P±0.05) among treatments within each year and parameter.

The Supplementary Material is available at https://www.ocl-journal.org/10.1051/ocl/2025024/olm.

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