Research article

Microenvironment created by *Plantago lagopus* L. may affect cover and diversity of coexisting species in urban vegetation

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A B S T R A C T

Some plant species may exhibit new microenvironments which lead to significant changes in the cover and diversity of the coexisting species. In this investigation, we evaluated the effects of *Plantago lagopus* L. on the cover and diversity of the associated plant species in the urban vegetation. A total of 70 plots were conducted in sites with- and without this species in urban gardens. Cover of the associated species and different diversity indices including species richness, Shannon-Wiener, evenness, and Simpson indices were measured. The allelopathic potential of *P. lagopus* was verified using its rhizosphere and non-rhizosphere soils on two target species existing within the same environment. Some soil criteria and seed sizes of the associated species were also determined. Most of the coexisting weeds were reduced in terms of their cover in plots with *Plantago*. The reduction of plant diversity depended on its cover. Besides, the aboveground biomass was reduced in sites comprising *Plantago*. The degree of inhibition was not related to the seed size of the species found. This species reduced the incident solar radiation and the local temperature over the soil surface. The locations exhibiting such species contained lower contents of available potassium and zinc. Rhizosphere soil of *P. lagopus* substantially inhibited germination and growth of *Amaranthus viridis*, but it didn't do so for *Medicago lupulina*. Reduction in cover, diversity, and biomass of the urban weeds associated with *P. lagopus* may be related to the reduction of received solar radiation, soil temperature, and nutrient availability. The allelopathic potential of *P. lagopus* may have a partial role in this reduction. These results suggest that *P. lagopus* may create a microenvironment of new conditions not favorable for most of the coexisting species.

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1. Introduction

The dominance of plant species in urban environments is affected by biotic interactions that occur among plants [1,2]. These interactions may be positive or negative. The positive ones occur when some plant species enhance the establishment, growth, and distribution of others through improving resource supply and microclimatic conditions [3,4]. Negative interactions fundamentally occur when a plant species inhibits the germination and growth of the neighboring species. These negative interactions comprise two main mechanisms: the first is competition for available resources like light, water, and some mineral nutrients [5] and the second is allelopathy. The latter is a chemically mediated interference that has received apparent interest in recent decades [6–8]. In addition, changes in microenvironments made by a certain species may experience effects on the neighbors [9]. Little attention has been paid to the ecological phenomena in urban vegetation. We, therefore, have to highlight this subject.

Microenvironment can be defined as the small-scale biotic and abiotic conditions the individual mediates, which may show significant variations from the average conditions prevailing within the same community. Variations brought onto microenvironments by a certain species can influence plant recruitment and distribution as well [10,11]. Besides, microenvironments created can affect plant communities for their composition, species diversity, and phenotypic variations by alterations in the abiotic factors such as soil water content, light availability, and available soil nutrients [12]. In most cases, ecological studies often overlook the effects of microenvironment on plant cover and diversity. Therefore, we will show how the studied species influence the microenvironment.

Allelopathy is an ecological interaction through which plant species influence other neighboring plants through the release of chemical compounds into the environment [13]. These allelochemicals can directly inhibit the germination, growth, or development of the neighboring species due to their effects on some physiological processes such as photosynthesis, respiration, cell division, and membrane permeability [13,14], or indirectly by their effects on soil properties and microbial community [15,16,17]. Therefore, these allelopathic plants may have wide-ranging impacts on the ecosystem. Besides, the allelopathic plants have a negative influence on the composition and diversity of the associated plants within the natural ecosystem [18]. It is also worth mentioning that the release of allelopathic compounds into the environment is essential for the dominance of the donor plants and for controlling the natural succession rate [15].

Plantago was recorded as an economic crop with good values in exportation, and with relatively high yield and mucilage contents which could be applicable in medicine industries [19]. In some Asian countries such as Iran, China, and India, *Plantago* seeds were used for potential treatment of dysentery, urinary problems, gonorrhea, fever, cold cough, and gastrointestinal malfunctions as an alternative method for chemical medicines such as antibiotics [20,21]. This genus in general, and *P. lagopus* in particular, had less attention based on ecology. We, therefore, give insights into this plant due to its ecological significance.

Plantago lagopus L. is an annual or short-lived perennial herb, native to the Mediterranean region and southwest Asia to Pakistan [22]. Leaves form rosettes along soil surface leading direct contact with rhizosphere soil. It has also been detected in urban plant communities [23]. In addition to being a refreshing source of food, it is widely used in traditional medicine in many different countries [24,25]. Previously, the aqueous extract of aerial parts of *P. lagopus* was reported to have bioactive compounds that show some biological activities including strong cytotoxic and radical scavenging activities that inhibit cancer cell proliferation [26]. Besides, the phytochemical analysis of these aerial parts showed the presence of the highest values of total phenolics, alkaloids, flavonoids, tannins, and saponins [26,27]. However, the allelopathic potential of this species has not been addressed under field conditions. In this respect, the previous study by Abd El-Gawad et al. [27] proved that the aqueous and methanolic extract of *P. lagopus* aerial parts exhibited significant inhibition on the germination and growth performance of the noxious weed *Bidens pilosa*. Nevertheless, a field study is still lacking, and the mentioned study couldn't be enough to conclude an allelopathic effect of *Plantago*. Therefore, it would be necessary to fill this scientific gap.

In our preliminary field observations in the urban gardens and parks of the study area, we noticed that *P. lagopus* species is widely

Table 1
Mean cover values (m^2) and the total aboveground biomass (g m^{-2}) (mean $\pm$ SE) of the plant species observed in the sites with and without *P. lagopus*. Values sharing the same letter within a column are statistically similar according to Tukey's test for normal data and Dunn's test for abnormal data.

Species	Family	Sites without *Plantago*	Sites with *Plantago*	Inhibition (%)	Seed mass (mg 100 seed^{-1})
Amaranthus viridis L. †	Amaranthaceae	1.69 ± 0.34	0.00 ** ± 0.00[a]	100 ± 0.00 e[a]	25.40 ± 1.72 a[a]
Bromus catharticus Vahl †	Poaceae	1.13 ± 0.22	0.17 * ± 0.09[a]	84.89 ± 0.56 d[a]	1393.60 ± 18.25 f[a]
Cynodon dactylon Pers. ††	Poaceae	1.44 ± 0.15	1.37 ± 0.12 [b]	4.53 ± 0.17 a[a]	30.60 ± 0.75 a[a]
Dichanthium annulatum (Forssk.) Stapf ††	Poaceae	2.04 ± 0.60	0.67 ± 0.47[a]	67.40 ± 0.19 c[a]	149.20 ± 6.78 e[a]
Digitaria sanguinalis (L.) Scop. †	Poaceae	0.97 ± 0.19	0.23 ** ± 0.07[a]	76.80 ± 0.63 d[a]	55.40 ± 1.80 b[a]
Eragrostis pilosa (L.) P.Beauv. †	Poaceae	2.61 ± 0.26	0.88** ± 0.25[a]	66.41 ± 0.08 c[a]	24.06 ± 1.00 a[a]
Euphorbia peplus L. †	Euphorbiaceae	1.10 ± 0.18	0.13 ** ± 0.10[a]	78.99 ± 0.46 d[a]	59.80 ± 1.40 b[a]
Euphorbia prostrata Aiton †	Euphorbiaceae	1.35 ± 0.40	0.29 * ± 0.08 [b]	78.97 ± 0.80 d [b]	111.40 ± 3.31 d[a]
Malva parviflora L. †	Malvaceae	0.57 ± 0.15	0.00 * ± 0.00 [b]	100 ± 0.00 e [b]	95.50 ± 1.85 cd[a]
Medicago lupulina L. †	Fabaceae	1.39 ± 0.30	1.10 ± 0.23 [b]	20.50 ± 0.22 b [b]	83.80 ± 2.76 c[a]
Oxalis corniculata L. ††	Oxalidaceae	2.84 ± 0.51	0.67 ** ± 0.28[a]	76.54 ± 0.45 d[a]	17.60 ± 1.63 a[a]
Aboveground biomass (g m^{-2})		176.66 ± 20.35	80.00 ± 4.25 **[a]		

*p < 0.05 and **p < 0.01. † Annual, †† Perennial.
[a] Data showing normality and homogeneity according to Kolmogorov–Smirnoff and Levene's tests, respectively.
[b] Data showing abnormality and heterogeneity according to Kolmogorov–Smirnoff and Levene's tests, respectively.

distributed, and its widespread adversely affects the cover of the associated species. In addition, when the *P. lagopus* was removed from the gardens and parks, other plant species could grow within the same season. Herein, we stated two main hypotheses. (1) *P. lagopus* individuals may modify the microenvironment in a way that experiences a reduction in cover and diversity of other species. Changes in the microenvironment may be related to the alteration of the incident sunlight onto the soil, soil temperature, soil water contents, and nutrient availability. These criteria will be measured to test this hypothesis. The cover and diversity of coexisting species were also measured and compared with those found in *Plantago*-free sites. The degree of inhibition may be related to the seed size of the species observed. The statement can be ensured by constructing a correlation between the seed mass of the different weeds detected and the inhibition (%) observed in their cover values. (2) The allelopathic effect of this weed may reduce the cover and floristic diversity of the urban vegetation. This hypothesis could be tested by showing the effect of rhizosphere soil of *Plantago* on germination and growth of some associated species and the results of such treatment can be compared with the effects of soil not affected with such plant. The principal objective of this study is to evaluate the impact of *P. lagopus* on the cover and floristic diversity of the associated plants in the urban ecosystem.

2. Results

In this study, eleven plant species belonging to six families were recorded. Five of them were listed as members of Poaceae, followed by two members of Euphorbiaceae. Each of the remaining species belongs to four different families of similar contributions. Eight of the species detected were annuals, while the remaining species were perennials (Table 1).

Generally, coverage of most of plant species monitored significantly declined in the sites influenced by *Plantago* (Table 1). Except for *Medicago lupulina*, all annuals were significantly reduced in sites with *Plantago*. Amongst them, *Amaranthus viridis* and *Malva parviflora* appeared to be absent from ranges with this weed. Amongst perennials, it was clear that the cover of *Oxalis corniculata* was significantly suppressed ($p < 0.01$) in the plots with this taxon. The aboveground biomass of the weeds associated with *Plantago* was reduced by about 55 % in comparison with those found in *Plantago*-free plots (Table 1). Concerning the seed size, six seed size classes were determined according to the LSD test. The smallest seed-sized species were *Amaranthus viridis, Cynodon dactylon, Eragrostis pilosa,* and *Oxalis corniculata*, whereas the largest seed size was noticed in *Bromus catharticus* (Table 1). The Spearman correlation indicated a non-significant correlation between the degree of inhibition in cover plant species and their seed sizes ($r = 0.19$).

For the different diversity indices checked, a tendency of reduction was noticed for them while the cover of *Plantago* increased (Fig. 1). The adverse effects of this weed on plant diversity contrasted according to the diversity index determined and cover values of *P. lagopus*. In particular, evenness was not affected at all. For the remaining indices, i.e. species richness, Shannon-Wiener, and Simpson indices, a significant reduction was observed where the cover of *Plantago* $\geq$ 70% (Fig. 1). Moreover, these three indices exhibited a negative correlation with the cover of *P. lagopus* (Table 2).

The physicochemical properties of the soil varied in the vegetation parts with- and without *Plantago* (Table 3). The soils under
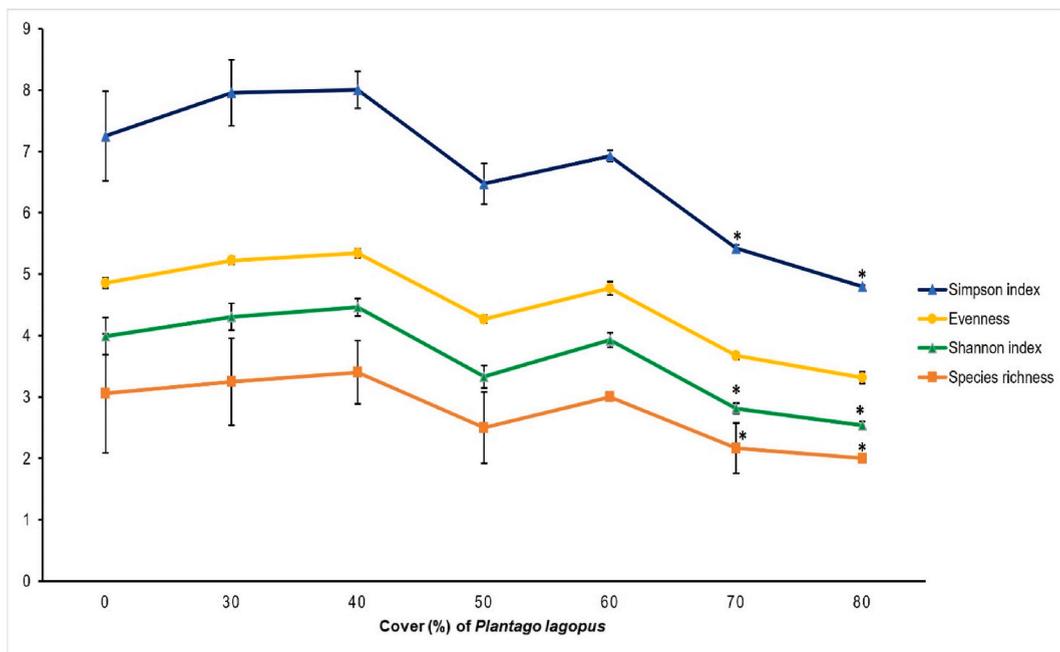

Fig. 1. The measured diversity indices (Mean ± SD) at the different cover values (%) of *P. lagopus*. Density at 0 value indicates the stands without *Plantago*. Bars represent standard deviations. All data exhibited normality and homogeneity.* Significant result from Plantago-free stands at p < 0.05.

Table 2

Correlation coefficients (r) between the values of diversity indices and the cover (%) of *P. lagopus*.

Diversity indices	Cover of *P. lagopus*
Species richness	−0.63[a]
Shannon-Wiener index	−0.70[a]
Evenness	−0.33
Simpson index	−0.76[a]

[a] p < 0.01.

Table 3

The measured physical and chemical properties of the soil (mean ± SE) in the sites with and without *Plantago*. **p < 0.01. All data exhibited normality and homogeneity.

Soil Properties	Sites without *Plantago*	Sites with *Plantago*
Soil temperature (°C)		
Summer	37.20 ± 2.58	31.5 ± 2.72 **
Autumn	31.33 ± 2.29	27.10 ± 2.52 **
Winter	22.38 ± 2.23	18.06 ± 2.30 **
Spring	30.33 ± 2.65	26.20 ± 1.92 **
Illumination on soil (Lux)	52114.30 ± 6861.12	15744.30 ± 2835.10**
Soil water content (%)	18.18 ± 4.27	13.72 ± 2.86
pH	7.61 ± 0.03	7.85 ± 0.10
EC (μS cm^{-1})	295.20 ± 8.35	318.40 ± 10.35
OC (%)	6.70 ± 0.11	6.90 ± 0.40
Available nutrients (mg kg^{-1} soil)		
N	35.42 ± 1.40	34.96 ± 2.20
P	26.00 ± 2.53	22.98 ± 1.36
K	478.18 ± 6.35	394.96 ± 7.15 **
Zn	7.34 ± 0.06	6.09 ± 0.06 **

P. lagopus were of lower temperatures during the different seasons. Similarly, the incident sunlight was also reduced by about 70 % in sites involving *Plantago*. However, the soil water content of these locations was not affected (Table 3). Regarding the available soil nutrients, both potassium and zinc were decreased in the sites with *Plantago* (Table 3).

2.1. Allelopathic effect of P. lagopus

The results revealed that the soil under *P. lagopus* had a non-significant effect on germination/emergence (%) and growth performance of *Medicago lupulina*. On the converse, the measured germination (%) and growth criteria were significantly reduced for *Amaranthus viridis* when planted in soil under *P. lagopus* (Fig. 2).

3. Discussion

This study revealed that the cover of most of the plant species coexisting with *Plantago lagopus* was significantly reduced. In addition, the diversity of these species mostly declined at the dense cover of such species, particularly at cover ≥70 %. A dense cover of *P. lagopus* may form a mat-like cover that may prevent or, at least, reduce, the penetration of solar radiation onto the soil, the result that we have stated in this study. A denser cover of *Plantago* may not permit fluctuation of sunlight and reduce, in turn, the level of temperature fluctuations over the soil. In this regard, it was proven that direct sunlight with relatively higher temperatures is a germination stimulus for many species [28]. The density of vegetation could be reduced by the effect of shading under field conditions [29]. The correlation constructed between the obtained inhibition under field conditions and the seed sizes of the different species was non-significant. Therefore, it is difficult to claim that the effect of *Plantago* depended upon the seed size. So, change in the micro-environment brought by this weed may have a role in such inhibition. On the other hand, the leaves of this species form rosettes over the soil surface, leading to a direct contact with the soil surface. This phenotype may cause a mechanical barrier to the seedling emergence of the associated species. This, indeed, can have negative effects as plant litter does, particularly at a denser cover. Plant litter may exhibit physical changes in the environment surrounding the growing plant individuals by modification of the sunlight, temperature, and the apparent wind on the soil surface [30,31]. It is also worth mentioning that plant litter reduces plant cover and diversity in urban gardens [32].

The results of the present study showed that the reduction in plant diversity depended mainly upon the cover/density of *P. lagopus*. The denser the cover of *Plantago* occurs, the less solar irradiance penetrates the soil surface. Therefore, light requirements could be delimited for other species. That is why the aboveground biomass could be reduced in the sites affected by *Plantago*. The morphology of the studied species may enable it to hold its leaves more horizontally, creating shallower penetration of light over the canopy, which reduces not only the canopy level for coexisting plants but also restricts the growth of those neighbors via shade [33]. In addition, more
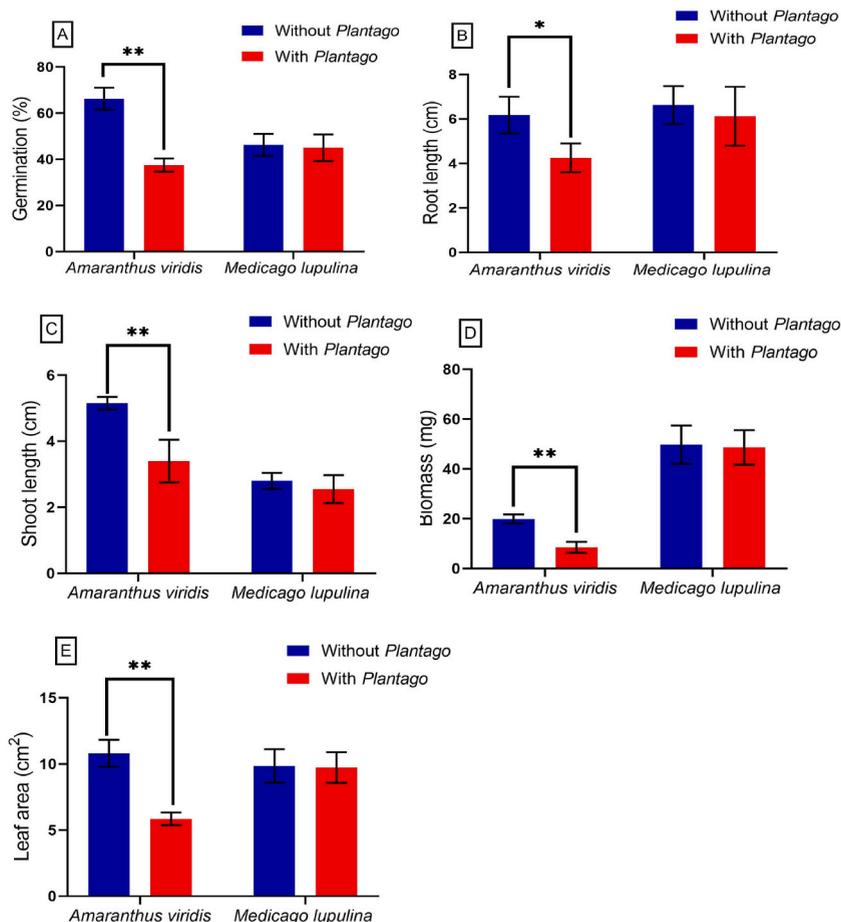
Fig. 2. [A] Germination (%) and the measured growth characteristics including [B] root length (cm), [C] shoot length (cm), [D] biomass (mg) and [E] leaf area (cm^2) for each individual of the selected target species when grown in soils with and without *Plantago*. (Means are represented by columns, and bars represent standard deviations). **p < 0.01. All data exhibited normality and homogeneity.

temperature may be reduced than required in sites comprising *Plantago*, particularly in relatively cold winters. On the other hand, higher densities of the donor species may enhance its phytotoxic effect; the concept of 'density-dependent phytotoxicity' [34,35]. Thus, the interference mechanism may be more effective at the dense cover of the donor species.

The greenhouse experiment indicated that seedling emergence and early development of *Amaranthus viridis* was significantly reduced when grown in the soil collected from the rhizosphere of *P. lagopus*, whereas *Medicago lupulina* was not. This result was substantially parallel to that observed under field conditions. This result also reflects the higher sensitivity of *A. viridis*, particularly when we noticed its complete absence from the sites involving *Plantago*. The obtained inhibition in the greenhouse trial could be attributed to phytotoxins released from the roots of *P. lagopus*. Reduction in nutrients may not inhibit germination. Therefore, there could be potential phytotoxins released from the roots of the donor species. Hassan [32] showed that *A. viridis* was highly sensitive to phytotoxins released into the environment. He also indicated that this species may completely disappear from sites involving allelopathic and mechanical effects of litter. On the other hand, continuous solar irradiance at a relatively higher temperature (at 35 °C) was recorded as optimum environmental stimuli for germination of *A. viridis* [36]. What is interesting in this case is that this optimum temperature may correspond to temperatures observed in July and August in the area of this study. Reduction in soil temperature, as an effect of *Plantago* cover, maybe a reason for suppression of *A. viridis* under field conditions. Therefore, the dual effect of *Plantago* cover, due to the changing microenvironment and phytotoxic potential, may have a role in the complete displacement of *A. viridis*.

Conversely, *Medicago lupulina* was not affected by the soil under *P. lagopus*. Maybe it could resist, or be insensitive to, the phytotoxic potential of *Plantago*. In comparison with the associated annuals, *M. lupulina* may remain alive for some time (6 years) in soil seed banks [37]. The dense cover of the rosette *Plantago* may provide a chilling requirement for *M. lupulina* which is crucial for better germination at relatively moderate temperatures (10–20 °C) occurring in winter of the study area, the fact proved by Van Assche et al. [38].

The result of such investigation showed that the soil chemical properties, in terms of the available nutrients, particularly for potassium and zinc, were significantly decreased in sites involving *Plantago*. Reduction of soil nutrients by plants is well-addressed [23]. This process may lead to a reduction in the criteria of the vegetation studied, particularly as a cause of the reduction of soil potassium as

a macronutrient. Therefore, the notion of competition for soil nutrients pronounced by *Plantago* may be relevant. Additionally, the reduction in soil nutrients in this study could be a modified microenvironment made by such species.

Some species of *Plantago* received some attention based on their chromosomal characteristics, variation in mucilage content, and grain yield traits [39]. However, various taxonomic studies depend on the plants' morphological and genetic variability with minimal consideration for the habitat conditions [40]. In addition, breeding strategies for increasing the productivity of *Plantago* species may be limited due to a lack of germplasm diversity characterization [41]. Therefore, future studies focusing on the genetic diversity of *Plantago lagopus* are recommended to organize the identity of ex situ collections for breeding and conservation efforts.

4. Conclusion

In general, the present study revealed that the occurrence of *Plantago lagopus* L. in the urban vegetation resulted in a noticeable decrease in cover values of most of the coexisting species. Amongst them, *Amaranthus viridis* and *Malva parviflora* likely disappeared from the plots affected by this weed. Reduction in the species richness, Shannon-Wiener, and Simpson indices depended upon the apparent density of *Plantago*. Besides, these indices manifested a negative correlation with the cover values of *Plantago*. Furthermore, the green areas involving this weed had lower values of the aboveground biomass. Interestingly, the occurrence of this species substantially reduced in the amounts of the incident solar irradiance on the soil surface, soil temperature, and the available soil potassium and zinc. The rhizosphere soil collected for the sites dominated by *P. lagopus* showed remarkable, but incomplete, suppression for emergence and early development of *A. viridis*, which may reflect another possible interaction from *P. lagopus* takes place under field conditions. The existence of *Plantago* had a non-significant effect on *Medicago lupulina* either in the field or in the greenhouse, indicating the lower sensitivity of the latter. The reduction observed in cover and diversity of most of the plants detected may be a cause of reduction in light availability, temperature over the soil, and some available nutrients mediated by *P. lagopus*. These results suggest that *P. lagopus* may create new biotic and abiotic conditions that may not favor the germination and growth performance of the associated plants in the urban vegetation.

5. Materials and methods

5.1. Field study

The field study was carried out in vegetation of the urban area described by Hassan [32] during the 2022 developing seasons. 70 plots were randomly selected in urban vegetation previously described by Hassan and Hassan [42]. Each of the designated plots was 1 × 5 m². This plot area was proportional to the sites covered by *Plantago lagopus* in this study area. A half of these plots (i.e. 35 plots) were conducted in the sites involving *P. lagopus* (the tested plots), whilst the remaining ones were sampled in *Plantago*-free sites (the control plots). From each plot, representative samples of *Plantago* were identified following Boulos [22] and Täckholm [43]. *P. lagopus* could be confused with *P. lanceolata*. Therefore, it was recommended to investigate plant samples well to ensure the entity of such species. *P. lagopus* was very common in Egyptian lands, and it was well characterized by oblanceolate leaves with wide sheathing bases and long hairy anterior sepals [22].

Both control and tested plots were close to each other (about 5 m apart) so that they shared the same environmental circumstances. The number of plots designed was comparable to the area covered by this weed. Besides, the size of the plots sampled was based on the average cover occupied by *Plantago* in each tested site. At each stop, the scientific names of the species found were enumerated. Boulos [44–46] were used to potentially identify the species monitored. The cover values of the observed species (per m²) were measured. Thereafter, the relative cover of each species (pi) (expressed as %) was calculated using the following equation:

Relative cover = [cover of a species i/cover of all species] × 100.

At each stop, the total aboveground biomass of the weeds other than *Plantago* was determined. The aboveground parts of the vegetation with- and free-from *Plantago* were cut using a rod cutter machete and dried in an oven at 70 °C until reaching a constant weight. The floristic diversity indices namely: species richness (S), Shannon-Wiener (H'), Evenness (E), and Simpson's indices (D), were also measured [47,48]. The species richness was determined by counting the number of plant species observed, while the remaining diversity indices were calculated as follows:

$$H' = - \sum_{i=1}^{s} (p_i \times \ln p_i)$$

$$E = \left[-\sum_{i=1}^{s} (p_i \times \ln p_i)\right]/\ln S$$

$$D = 1/C \text{ and } C = \sum_{i=1}^{s} p_i^2,$$

Where:

H' = Shannon-Weiner index, p_i = Relative cover of the species i, where pi = cover of a species i/total cover of all species, S = Number of species,

E = Evenness,

D = Simpson index for diversity, and

ln = the natural log.

To determine the seed size of each species detected in the field trial, seeds were collected at the late fruiting stage of every species. Five replicated samples of 100 seeds from different locations were weighed for each species. The seed mass was expressed as (mg per 100 seeds).

5.2. Soil analyses

Three soil samples were collected from each studied plot at 0–20 cm depth. These samples were pooled and combined as a single composite. After collection, measurement of soil water content was performed. The soil samples were virtually subjected to shade-drying and sieving by a 2 mm sieve. Thereafter, they have to be kept in plastic bags at room temperature for potential analyses. Different soil physicochemical characteristics including soil electrical conductivity (EC), pH, organic carbon (OC), and some available nutrients such as nitrogen (N), phosphorus (P), potassium (K), and zinc (Zn), were measured. As *P. lagopus* can form a rosette-shaped cover by its leaves, the soil temperature and illuminance on the soil, which may affect vegetation, were also measured.

Soil pH was measured in a soil-water extract (1:2.5 w/v) using a digital pH meter (3000, Szeged, Hungary), whereas soil electrical conductivity (EC) was determined in another soil extract (1:5 w/v) using a conductivity meter (Jenway 3305, UK). Walkley and Black's method was used for the possible determination of soil organic carbon [49]. Available soil nutrients were measured via Allen's methods [50]. Temperatures on the soil surface were determined using a mercury-glass thermometer, and the incident illuminance on the soil was detected using a digital lux meter (Victor 1010A, China).

5.3. Allelopathic potential of P. lagopus

5.3.1. Sampling and preparation of soil

Definite soil samples were carefully collected from the rhizosphere parts from *Plantago*-dominating locations. This type of soil was symbolized as the treatment soil. Untreated soil samples (symbolized as control soil) were gathered from the green areas where the cultivated Bermuda grass (*Cynodon dactylon*) dominates. This species has always been cultivated for urban greening. These samples were air-dried under shading conditions, sieved using a 2 mm sieve to remove plant residues, and shaken effectively in a plastic bag forming one composite. Both control and treated soils were retained in plastic pots, each of 10 cm diameter × 15 cm depth, for future planting of the tested target species.

5.3.2. The tested species

Amaranthus viridis (Linn.) and *Medicago lupulina* were chosen as the two tested target species as they are common species in this study area [42]. They belong to two different families. Therefore, variation in results may be predicted. Our preliminary field remarks indicated that *A. viridis* appeared to have very low cover in the sites with *P. lagopus*, whereas *M. lupulina* was one of the commonly associated species in the sites where *Plantago* coexists. The seeds of both weeds were picked up from different places representing the vegetation studied.

5.3.3. Test for allelopathy

Twenty seeds of the above-mentioned species were equally sown at 0.25 cm depth from soil surface in each pot. Pots were watered regularly by spaying method. Four replicated treated and control pots in a fully randomized design were retained for four weeks under the predominant environmental conditions. The emerging individuals were enumerated for the sake of calculating the percentage of germination for each species. Certain growth criteria including root depth (cm), shoot height (cm), total biomass (mg), and leaf area (cm^2) were determined for each plant [23].

5.4. Statistical analyses

Data normality and homogeneity were checked by application of Kolmogorov–Smirnoff and Levene's tests, respectively. The results involving comparisons between the plots with- and without *Plantago* and showing requirements of normality and homogeneity were analyzed via independent samples *t*-test, whereas for those exhibiting abnormality and heterogeneity, the Mann–Whitney U test was carried out as a non-parametric one. On the other hand, the degree of inhibition (%) in the cover of the species detected was classified by applying one-way ANOVA, followed by the least significant difference (LSD) test for normal data, and the Kruskal-Wallis H test for abnormal data. Dunn's test was performed for a pairwise comparison of the abnormal data. Therefore, these tests were conducted to manifest the gradual inhibitory effect of *Plantago* on the associated species. Both tests substantially perform pairwise comparisons between each independent group and tell which groups are statistically significantly different from another. Similarly, the same tests were applied to classify the species found according to their seed sizes. To evaluate a relationship between the cover of *P. lagopus* and the measured diversity indices, a Spearman correlation was achieved. Such correlation was also performed to construct the relationship between the inhibition in plant cover and the seed size of each species. The statistical analyses were conducted by the SPSS program, version 23.0 (IBM Corporation, N.Y., USA).

Data availability statement

All the data used for this research have been described in the Manuscript.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Mahmoud O. Hassan: Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization. **Ibtisam Mohammed Alsudays:** Writing – review & editing, Methodology, Conceptualization. **Howida Y. Mohamed:** Writing – original draft, Software. **Asmaa A. Abdelhameed:** Software. **Suliman Mohammed Suliman Alghanam:** Writing – review & editing, Supervision. **Sami Asir Al-Robai:** Writing – review & editing, Conceptualization. **Esraa M. Genidy:** Methodology. **Siada H. Nasr:** Methodology. **Mohamed A. El-Tayeb:** Project administration, Funding acquisition. **Mohammad K. Okla:** Project administration, Funding acquisition. **Bushra Hafeez Kiani:** Funding acquisition. **Amany H. A. Abeed:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

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

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