ALLELOPATHIC EFFECT OF AQUEOUS EXTRACTS OF WEEDS ON MEDICINAL PLANTS

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ABSTRACT
A pot experiment was carried out in 2020 to assess the allelopathic influence of various weed extracts on different medicinal plants. The experiment was laid out in a two factorial CR design replicated three times. Factor A comprised of medicinal plants viz. *Linum usitatissimum*, *Nigella sativa*, *Foeniculum vulgare*, *Plantago ovata*, and *Matricaria chamomilla*; while factor B included aqueous extracts of *Silybum marianum, Parthenium hysterophorus*, and *Broussonetia papyrifera*, along with a herbicide (atrazine) and a control. Data was recorded on germination percentage, root and shoot lengths (cm), fresh and dry biomasses (g), antioxidant activity, total phenolic content, total flavonoid content, chlorophyll content, and antibacterial activity. The results showed that extracts of weeds had stimulatory effects on the growth of medicinal plant species, a phenomenon called hormesis. The aqueous extracts of the tested weeds enhanced the seed germination and significantly affected all the parameters tested in the experiment. The highest shoot length was of *L. usitatissimum* treated with *S. marianum* extract and the lowest was recorded also in *L. usitatissimum* with atrazine application. Similarly, the root length of *L. usitatissimum* was highest under *P. hysterophorus* extract application and the lowest was again in *L. usitatissimum* under *B. papyrifera* extract. The highest fresh biomass was of *M. chamomilla* and lowest in *P. ovata* under *P. hysterophorus*. Similarly, dry biomass was found highest in *M. chamomilla* in control, while lowest was of *L. usitatissimum* under *B. papyrifera* treatment. The antioxidant content was maximum in *F. vulgare* under *B. papyrifera* and minimum in *P. ovata* under *Parthenium*. The total flavonoid content was on top in *F. vulgare* under *B. papyrifera* and the least flavonoid content was in *L. usitatissimum* under *Parthenium*. The total phenolic content was greatest in *M. chamomilla* under Parthenium and the lowest in *F. vulgare* treated with atrazine. Moreover, the greatest *Escherichia coli* content was found in *F. vulgare* under *B. papyrifera* the smallest in *M. chamomilla* under *Silybum* extract. The highest content of *Staphylo coccus* bacteria was in *N. sativa* under atrazine and lowest in *F. vulgare* under *S. marianum*. The Agro-bacterium activity was highest in *N. sativa* under control and lowest in *M. chamomilla* under atrazine. The *Citro bacter* was highest in *F. vulgare* treated with *B. papyrifera* while lowest in *P. ovata* under *P. hysterophorus*. The chlorophyll content was highest in *L. usitatissimum* and lowest in *P. ovata* under *S. marianum*.

Keywords: allelopathy, aqueous extract, growth, medicinal plants, weeds
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INTRODUCTION

Pakistan has got diverse ecological zones from the highest peaks to the sea level, with huge biodiversity of flora and fauna. About 12% of the plants in Pakistan are used for the cure of a number of diseases. To make herbal medicines, 350 to 400 plant

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species are utilized by different manufacturers (Khan *et al*., 2021; Mlilo and Sibanda, 2022). Several medicinal plants are exported from Pakistan, and many others are commonly found in the local markets (Islam *et al.,* 2021). The flora of Pakistan is loomed unintentionally by the native collectors, herbal manufacturers and merchants (Mlilo and Sibanda, 2022; Cahyaningsih *et al.*, 2021).

The following is the brief description of the different medicinal plants tested in the research. *Foeniculum vulgare,* a member of Apiaceae, commonly known as fennel, has a wide range of use against respiratory, digestive, and gastrointestinal disorders. Its medicinal properties include antibacterial, antifungal and antioxidant capabilities (Rather *et al*., 2012; Ahmed and Akhtar, 2016). *Matricaria chamomilla* (also chamomile), belonging to Asteraceae is widely used as cosmetic, nutritional and multitherapeutic values (Aziz *et al*., 2017). *Nigella sativa* is used to treat different animal and human illnesses. The main component found in its seeds is thymoquinone, which helps cure heart disorders, sugar, mental illness, infertility, cancer, and viral, bacterial, parasitic and fungal diseases, and especially used in treatment of HIV\AIDS (Chawuke *et al*., 2021). *Linum usitatissimum* (flax seed) belongs to Linaceae and is very beneficial in cancer, cardiac disorders, and diabetes.

There are certain weeds competing with these medicinal plants, and these weeds are famous for their allelopathic effect in various crops. *Parthenium hysterophorus* (Parthenium) is a member of Asteraceae, and an invasive herbaceous broadleaf weed (Shi and Adkins, 2020). *Silybum marianum* (milk thistle) also belongs to Asteraceae whose allelopathic effect has been observed on various crops like canola and ryegrass (Tsiaousi *et al.*, 2019). *Broussonetia papyrifera* is used for making of ropes, paper and for cattle feeding (Maan *et al.*, 2021).

MATERIALS AND METHODS

Plant specimens of the three weeds, *Silybum marianum*, *Broussonetia papyrifera* and *Parthenium hysterophorus* and five medicinal plants viz. *Foeniculum vulgare* (fennel), *Matricaria chamomile* (chamomile)*, Nigella sativa* (black cumin), *Plantago ovata* (psyllium) and *Linum usitatissimum* (Flax seed) were collected from the Non-Timber Forest Department, district of Peshawar, Khyber Pakhtunkhwa, Pakistan and were identified in the herbarium of the department of Weed Science, The University of Agriculture Peshawar. Leaf samples were washed for removal of dust and other impurities. The aqueous extracts of the weed species were tested against the five medicinal plants for their allelopathy.

The plant leaves were dried in an oven at 70°C for 48 h. Then the dried material was ground with a grinder and passed through a 40-mesh screen. The allelopathic water extract were prepared by adding 100 g powder of each plant to 1 liter of distilled water and kept at 25°C. After 24 hours, the solutions were filtrated and centrifuged at 12000 rpm, after which clean and pure extracts were collected. Seeds germination test of the five medicinal plants was performed in petri dishes in the lab of Weed Science department. Ten viable seeds of each of the five medicinal plants were put in the petri dishes and three mL of each of the prepared extracts of the weeds be applied, herbicide was applied as separate treatment and distilled water was used as control treatment. The extracts of 10 ml each were sprayed three times a day. The petri dishes were repeated four times.

There were total of five treatments, out of which the first three were weed extracts of *Silybum marianum, Parthenium hysterophorus* and *Broussonetia papyrifera*, the fourth was Atrazine herbicide and the fifth was a weedy control. The volume of each treatment used was 5 ml and was sprayed three times a day. During the experiment, data was recorded on the following parameters.

Data collection procedures

Germination (%):

The germinated seeds in each pot were counted when sprayed three times a day and the percentage was computed for each treatment by the formula,

$$\text{Germination percentage} = \frac{\text{Germinated seed x 100}}{\text{Total seeds}}$$

Shoot length (cm):

Shoot length of the crop seedlings was measured with a ruler in centimeters (cm) for all the germinated plants in each treatment and then means were computed.

Root length (cm):

Root length was measured with a ruler in centimeters (cm) for all the germinated plants in each treatment and then means were recorded.

***Fresh and dry biomasses plant^{-1} (g)*:**

The fresh and dry biomasses data of all plants were taken with the help of an electric balance in grams (g) and subsequently means plant^{-1} will be calculated for each treatment. The fresh biomass was then dried in oven at 65^{o}C for 48 hours. The dry biomass was taken similar to the fresh biomass.

***Antioxidant activity determination by DPPH scavenging assay*:**

Free radical scavenging activities of crude fungal extract was determined by using a DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical. 0.1 ml of the extract solution was mixed with 3.9 ml of methanol and 1.0 ml of DPPH solution. After 30 min, Absorption was measured at 517nm with the help of spectrophotometer. The same procedure was followed for the standard of ascorbic acid. The scavenging effect was determined by the following Equation.

$$\text{DPPH scavenging \%} = 100\text{x}\frac{\text{Ao} - \text{A1}}{\text{Ao}}$$

Where A0 = abs of control, A1 = abs of samples

***Total phenolic content*:**

Total phenolic constituents of plant extracts were performed employing the literature methods involving Folin-Ciocalteure reagent and gallic acid as standard. Extract solution (0.1 ml) containing 1000 µg extract was taken in a volumetric flask, 46 ml distilled water and 1 ml Folin-Ciocalteure agent were added, and flask was shaken thoroughly. After 3 min, 3 ml of solution 2% Na2CO3 was added, and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm. The same procedure was repeated to all standard gallic acid solutions (0–1000 mg, 0.1 ml-1) and standard curve was obtained. TPC values were calculated on the base of gallic acid standard curve and results were expressed as µg gallic acid equlence (GAE) per mal of extract.

***Total flavonoid content*:**

The total flavonoid content (TFC) of each extract was investigated using the aluminum chloride colorimetry method with slight modifications. In brief, the extract sample was diluted with methanol until 100 mg mL^{-1}. The calibration curve was prepared by diluting quercetin in methanol (0e100 mg mL^{-1}). The diluted extract or quercetin (2.0 mL) was mixed with 0.1 mL of 10% (w/v) aluminum chloride solution and 0.1 mL of 0.1 mM potassium acetate solution. The mixture was kept at room temperature for 30 minutes. Then the maximum absorbance of the mixture was measured at 415 nm using a UVeVIS spectrophotometer. TFC was expressed as milligram quercet in equivalent per ml of extract (mg QCE/ml).

Anti-bacterial activity:

Antibacterial activity of different fractions of the selected plant parts was evaluated through well diffusion method. Nutrient agar was used as a growth media for growth of

bacterial strands. Selected pathogenic bacteria were inoculated on nutrient agar with four 8mm bores in solid media. Aliquots extract free of solvents were solubilized in DMSO (v/v). 60 ul of each solvent fraction was introduced into every well. Appearance of clear zone around the well indicated the inhibition of growth of pathogenic bacteria. Streptomycin and DMSO was used as positive and negative control, respectively.

Chlorophyll content (µmol):

The chlorophyll content was measured with the help of chlorophyll meter. The model used was SPAD-502 Plus, manufactured in country Japan. Its function is to measure the chlorophyll content of plant leaves. Its unit is called SPAD value, which is internationally known.

1 SPAD value = 1mg per micro-mole

STATISTICAL ANALYSIS

The experiment was laid out in a two factorial completely randomized design with four repetitions. The factor A comprised of five medicinal plants and factor B of the aqueous extracts of three weed species mentioned above along with a herbicide and a control treatment. The block size was, 5 plants species (medicinal plants) x 5 treatments (3 extracts, a herbicide and a control) = 25. Total number of experimental units were 25 x 4 (replications) = 100.

The recorded data for each trait were subjected individually to the ANOVA technique by using Statistix 8.1 computer software for the data analyses. Also, Excel 2010 was used for confirmation of the data analyses.

RESULTS AND DISCUSSION

Germination percentage

The germination was found to be more effective in the pots which were exposed to extracts treatments of *S. marianum*, *P. hysterophorus* and *B. papyrifera*. Control and herbicide treatments had the minimum growth of the medicinal plant species (Table-1). After two weeks of treatments application, the highest germination was in *F. vulgare* (21%) under treatment of *B. papyrifera*; while the lowest germination was in *M. chamomilla* in the control. On completion of 4 weeks after application, the germination was this time highest in *F. vulgare* (25.25%) in *B. papyrifera* treatments and the lowest in *M. chamomilla* under control. In week 6, the germination of *F. vulgare* was highest (24.75%), under *P. hysterophorus*. On the other hand, the germination was lowest in *M. chamomilla* treated with the herbicide atrazine. The interaction effect was significant in all the assessed weeks where *P. hysterophorus* played stimulatory role and control behaved as inhibitory (Tab- 1 A-D). In the last week of assessment, the *L. usitatissimum* showed highest germination (20.25%) treated with extract of *B. papyrifera* and the germination was lowest *M. chamomilla* in control treatment. The interaction was significant but here *B. papyrifera* played stimulatory role and control as inhibitory. Shi and Adkins (2020) studied similarly on the stimulation and inhibition bioactivity of Parthenin (a phytochemical derived from *P. hysterophorus*).

Table 1(A). Effect of treatments (*S. marianum*, *P. hysterophorous*, *B. papyrifera*, Atrazine (herbicide) and control) on % germination of medicinal plants. Interaction effect of medicinal plants (Linseed (*L. usitatissimum*), Black Cumin (*N. sativa*), Fennel (*F. vulgare*), Psyllium (*P. ovata*) and Chamomila (*M. chamomilla*) and treatments on % germination of the seedlings in week 2^{nd},4^{th},6^{th} and 8^{th} week respectively.

Medicinal Plants	Treatments					Mean
	silybum marianum	*Parthenium hysterophorous*	*Betula apyrifera*	Atrazine (Herbicide)	Control	
L. usitatissimum	10 defg	16.25 abcd	17.75 abc	7.25 efgh	2.75 gh	11.05 a
N. sativa	3.50 fgh	12.25 bcde	5.25 efgh	4.25 fgh	4.75 fgh	6.00 b
F. vulgare	18 ab	16.25 abcd	21 a	6 efgh	10 defg	14.2 a
P. ovata	10.5 defg	15.75 abcd	14.75 abcd	9.50 defg	6.50 efgh	11.4 a
M. chamomila	0 h	0 h	0 h	0 h	0 h	0.00 c
Mean	8.40 b	12.3 a	11.7 a	5.40 bc	4.80 c	

Means in same column followed by same letters are statistically similar at 5% level of significance,
LSD (0.05) for MP= 3.32.
LSD (0.05) for TREATMENTS = 3.32.
Interaction (MP x TREATMENTS) = 7.43.

Table 1(B). Effect of treatments (*S. marianum*, *P. hysterophorous*, *B. papyrifera*, Atrazine (herbicide) and control) on % germination of medicinal plants. Interaction effect of medicinal plants (Linseed (*L. usitatissimum*), Black Cumin (*N. sativa*), Fennel (*F. vulgare*), Psyllium (*P. ovata*) and Chamomila (*M. chamomilla*) and treatments on % germination of the seedlings in week 2^{nd},4^{th},6^{th} and 8^{th} week respectively.

Medicinal Plants	Treatments					Mean
	silybum marianum	*Parthenium hysterophorous*	*Betula apyrifera*	Atrazine (Herbicide)	Control	
L. usitatissimum	11.5 def	19 b	16 bcd	9.25 efg	6.25 fgh	12.4 b
N. sativa	3.25 hi	9.75 efg	7.00 efgh	5.75 gh	6.25 fgh	6.40 c
F. vulgare	17.25 b	17.0 bc	25.25 a	8.00 efgh	8.75 efg	15.2 a
P. ovata	11.5 def	15.25 bcd	11.75 cde	9.00 efg	1.75 gh	11.05 b
M. chamomila	0.00 i	0.00 i	0.00 i	0.00 i	0.00 i	0.00 d
Mean	8.70 b	12.20 a	12.00 a	6.40 bc	5.80 c	

Means in same column followed by same letters are statistically similar at 5% level of significance,
LSD (0.05) for MP= 2.40.
LSD (0.05) for TREATMENTS = 2.40.
Interaction (MP x TREATMENTS) = 5.38.

Table 1(C). Effect of treatments (*S. marianum*, *P. hysterophorous*, *B. papyrifera*, Atrazine (herbicide) and control) on % germination of medicinal plants. Interaction effect of medicinal plants (Linseed (*L. usitatissimum*), Black Cumin (*N. sativa*), Fennel (*F. vulgare*), Psyllium (*P. ovata*) and Chamomila (*M. chamomilla*) and treatments on % germination of the seedlings in week 2^{nd},4^{th},6^{th} and 8^{th} week respectively.

Medicinal Plants	Treatments					
	silybum marianum	*Parthenium hysterophorous*	*Betula apyrifera*	Atrazine (Herbicide)	Control	Mean
L. usitatissimum	12.75 c	20.5 b	8.75 de	2.50 ghi	0.25 hi	8.95 b
N. sativa	6.00 f	7.25 ef	6.00 f	2.50 ghi	0.75 hi	4.50 d
F. vulgare	24.2 a	24.7 a	26.5 a	2.50 hi	0.75 hi	15.15 a
P. ovata	11.25 cd	4.75 fg	13.75 c	1.00 hi	0.75 hi	6.30 c
M. chamomila	2.00 hi	2.75 gh	2.75 gh	1.00 hi	0.00 i	1.50 e
Mean	11.25 a	12.00 a	10.95 a	1.70 b	0.50 b	

Means in same column followed by same letters are statistically similar at 5% level of significance,
LSD (0.05) for MP= 1.16.
LSD (0.05) for TREATMENTS = 1.16.
Interaction (MP x Treatments) = 2.59.

Table 1(D). Effect of treatments (*S. marianum*, *P. hysterophorous*, *B. papyrifera*, Atrazine (herbicide) and control) on % germination of medicinal plants. Interaction effect of medicinal plants (Linseed (*L. usitatissimum*), Black Cumin (*N. sativa*), Fennel (*F. vulgare*), Psyllium (*P. ovata*) and Chamomila (*M. chamomilla*) and treatments on % germination of the seedlings in week 2^{nd},4^{th},6^{th} and 8^{th} week respectively.

Medicinal Plants	Treatments					
	silybum marianum	*Parthenium hysterophorous*	*Betula apyrifera*	Atrazine (Herbicide)	Control	Mean
L. usitatissimum	9.5 d	3.75 h	20.2 a	14.0 b	6.50 f	10.80 a
N. sativa	13.2 b	8.25 e	8.25 e	6.00 fg	1.25 ij	7.40 b
F. vulgare	11.0 c	7.00 f	5.00 g	5.00 g	1.00 ijk	5.80 c
P. ovata	1.25 ij	3.75 h	3.00 h	0.25 jk	0.25 jk	0.95 d
M. chamomila	0.00 k	0.00 k	1.5 i	0.00 k	0.00 k	0.30 e
Mean	7.60 a	3.80 d	7.60 a	5.05 c	1.80 e	

Means in same column followed by same letters are statistically similar at 5% level of significance,
LSD (0.05) for MP = 0.501
LSD (0.05) for TREATMENTS = 0.501.
Interaction (MP x Treatments) = 1.122.

Shoot length (cm):

The different medicinal plants species, weeds aqueous extracts and their interaction had a highly significant effect

(P<0.05) on shoot length of the seedlings of medicinal plants. Regarding the individual effect of the medicinal plant's species, the highest shoot length (8.79 cm) was found in *P. ovata* seedlings and the lowest (5.04 cm) in *F. vulgare* seedlings (Table-2). Thus, the shoot of *P. ovata* grew longer than the rest of the tested species. Among the weed control treatments, there was the highest shoot length (8.21 cm) in seedlings treated with aqueous extract of *P. hysterophorus* and it was minimum (5.55 cm) in seedlings sprayed with aqueous extract of *B. papyrifera*. This indicated that *P. hysterophorus* had a stimulatory and *B. papyrifera* an inhibitory effect on the shoot length of the growing seedlings in pots.

For the interaction effect, the shoot length of *L. usitatissimum* (11.56 cm) under treatment of aqueous extract of *S. marianum* was highest, followed by *L. usitatissimum* (10.0 cm) under *P. hysterophorus* and *P. ovata* (9.57 cm) under *S. marianum* and the shoot length was lowest for *L. usitatissimum* (2.37 cm) under herbicide treatment (Table-2). The overall interaction effect revealed that *S. marianum* mostly played stimulatory role in all the medicinal plants for their shoot lengths as compared to the control pots. Parthenium played inhibitory role in the interaction effect on the shoot length of the tested medicinal plants species. Belz and Cedergreen (2010) tested *Lactuca sativa* with parthenin applied at low dosage that gave good growth conditions for shoot elongation.

Table 2. Effect of treatments (*S. marianum*, *P. hysterophorous*, *B. papyrifera*, Atrazine (herbicide) and control) on shoot length (cm) of medicinal plants. Interaction effect of medicinal plants (Linseed (*L. usitatissimum*), Black Cumin (*N. sativa*), Fennel (*F. vulgare*), Psyllium (*P. ovata*) and Chamomila (*M. chamomilla*) and treatments on shoot length of the seedlings.

Medicinal Plants	Treatments					
	silybum marianum	*Parthenium hysterophorous*	*Betula apyrifera*	Atrazine (Herbicide)	Control	Mean
L. usitatissimum	11.5 a	`10 b	9.53 b	2.37 i	7.60 d	6.96 c
N. sativa	8.58 c	5.42 fg	8.62 c	5.50 fg	6.48 e	6.29 d
F. vulgare	7.64 d	7.58 d	7.43 d	6.33 e	6.50 e	5.04 e
P. ovata	9.57 b	6.47 e	9.37 b	5.0 gh	6.50 e	8.79 a
M. chamomila	6.60 e	5.33 g	5.43 fg	6 ef	4.37 h	8.08 b
Mean	8.21 a	7.38 b	5.55 d	7.10 bc	6.92 c	

Means in same column followed by same letters are statistically similar at 5% level of significance
LSD (0.05) for MP= 0.29
LSD (0.05) for TREATMENTS = 0.29.
Interaction (MP x TREATMENTS) = 0.65.

Root length (cm)

The different medicinal plants species, weeds aqueous extracts and their interaction had a highly significant effect (P<0.01) on root length of the medicinal plants' seedlings. For the individual effect of medicinal plants species, the highest root length (5.24 cm) was found in *L. usitatissimum* seedlings and the lowest (1.9127 cm) in *P. ovata* seedlings (Table-3). Consequently, the root of *L. usitatissimum* grew longer than the rest of the tested species. For the weed control treatments, the root length was highest (4.98 cm) in pots sprayed with aqueous extract of *P. hysterophorus* whereas it was minimum (2.71 cm) in pots treated with aqueous extract of *B. papyrifera*. This indicated that *P. hysterophorus* had a stimulatory effect and *B. papyrifera* had an inhibitory effect on the shoot length. For the interaction effect,

the root length for *N. sativa* treated with aqueous extract of *P. hysterophorus* was highest (8.50 cm); while the lowest root length was recorded in *F. vulgare* in control (1.45 cm). The interaction effect revealed that *P. hysterophorus* played stimulatory role among the medicinal plants for root length development when compared with control (Table-3). This phenomenon is termed hormesis which is the response to increase in any biological substance or condition (Belz and Cedergreen, 2010). Hormesis is the stimulatory effect of a low rate of a toxic substance in plants exposed to herbicides and other phytotoxins. The mechanisms of stimulatory responses are discussed with positive and negative implications of hormesis for crops. Belz and Cedergreen (2010) performed an experiment on *Lactuca sativa* by applying *Parthenin* at a low dosage which gave good growth conditions for root elongation. Here, *B. papyrifera* application inhibited the root length of the tested medicinal plants species. All the medicinal plants are uniformly affected by the weed control treatments in terms of the root length of the plant seedlings of the target species.

Table 3. Effect of treatments (*S. marianum*, *P. hysterophorous*, *B. papyrifera*, Atrazine (herbicide) and control) on shoot length (cm) of medicinal plants. Interaction effect of medicinal plants (Linseed (*L. usitatissimum*), Black Cumin (*N. sativa*), Fennel (*F. vulgare*), Psyllium (*P. ovata*) and Chamomila (*M. chamomilla*) and treatments on root length of the seedlings.

Medicinal Plants	Treatments					
	silybum marianum	*Parthenium hysterophorous*	*Betula apyrifera*	Atrazine (Herbicide)	Control	Mean
L. usitatissimum	3.46 de	8.43 a	1.6 f	7.36 a	5.33 bc	5.24 a
N. sativa	4.46 cd	3.5 de	2.36 ef	3.53 de	6.12 b	4.10 a
F. vulgare	4.53 cd	3.46 cd	5.43 bc	1.83 f	3.5 de	3.75 b
P. ovata	2.33 ef	2.0 f	1.66 f	2.11 f	1.45 f	1.91 c
M. chamomila	3.36 de	2.50 ef	2.46 de	1.47 f	2.32 ef	2.42 c
Mean	3.63 b	4.10 a	2.70 c	3.27 b	3.74 b	

Means in same column followed by same letters are statistically similar at 5% level of significance
LSD (0.05) for MP= 0.54.
LSD (0.05) for TREATMENTS = 0.54.
Interaction (MP x TREATMENTS) = 1.22.

Fresh biomass (g)

Statistical analysis of the data declared that the extracts of *S. marianum*, *P. hysterophorus* and *B. papyrifera* had a significant effect on fresh biomass of medicinal plants species (Table-4). The herbicide treatment had a negative effect on the fresh weed biomass as it led to chlorosis of the plant. The weed biomass was highest (2.0 g) in *N. sativa* in control treatment, followed by *L. usitatissimum* (1.36 g) in control, and the lowest value was of *N. sativa* (0.17) under treatment of *P. hysterophorus*. The interaction effect was also significant (Table-4). The highest fresh biomass was found in *M. chamomilla* (0.63 g) followed by *P. ovata* (0.56 g). The lowest biomass was recorded for *N. sativa* (0.38 g). The treatments interaction showed that control had the highest biomass (0.99 g) followed by *S. marianum* (0.61 g), and the lowest was of *B. papyrifera* (0.29 g) (Table4).

Table 4. Effect of treatments (*S. marianum*, *P. hysterophorous*, *B. papyrifera*, Atrazine (herbicide) and control) on fresh biomass in grams of medicinal plants. Interaction effect of medicinal plants (Linseed (*L. usitatissimum*), Black Cumin (*N. sativa*), Fennel (*F. vulgare*), Psyllium (*P. ovata*) and Chamomila (*M. chamomilla*) and treatments on fresh biomass in grams of medicinal plants.

Medicinal Plants	Treatments					
	silybum marianum	*Parthenium hysterophorous*	*Betula apyrifera*	Atrazine (Herbicide)	Control	Mean
L. usitatissimum	1.02 bc	0.27 d	0.23 d	0.33 cd	0.34 cd	0.44 a
N. sativa	0.71 bcd	0.16 d	0.26 d	0.34 cd	0.42 cd	0.39 a
F. vulgare	0.47 cd	0.43 cd	0.36 cd	0.34 cd	0.71 bcd	0.49 a
P. ovata	0.61 cd	0.33 cd	0.33 cd	0.18 d	1.36 ab	0.55 a
M. chamomila	0.23 d	0.36 cd	0.27 d	0.26 d	2 a	0.62 a
Mean	0.61 b	0.31 bc	0.29 c	0.30 c	0.99 a	

Means in same column followed by same letters are statistically similar at 5% level of significance
LSD (0.05) for MP= 0.31
LSD (0.05) for TREATMENTS = 0.31.
Interaction (MP x TREATMENTS) = 0.70.

Dry biomass (g)

The extracts of *S. marianum*, *P. hysterophorus* and *B. papyrifera* had a significant influence on dry biomass of the medicinal plant's species (Table-5). The herbicide treatment had a negative effect on the fresh weed biomass as it led to chlorosis of the plant. The highest value of 1.17 g of *M. chamomilla* was recorded in the control pots, followed by *L. usitatissimum* (0.96 g) under *S. marianum* while the lowest dry biomass (0.13 g) was found in *N. sativa* under *B. papyrifera*. The interaction was non-significant (Table-5). The highest dry biomass (0.42) was in *L. usitatissimum* followed by *M. chamomilla* (0.42 g), and the lowest dry biomass was of *N. sativa* (0.29 g). For the interaction effect, the highest value was of control (0.61 g) and lowest of *B. papyrifera* (0.20 g) (Table-5).

Table 5. Effect of treatments (*S. marianum*, *P. hysterophorous*, *B. papyrifera*, Atrazine (herbicide) and control) on dry biomass in grams of medicinal plants. Interaction effect of medicinal plants (Linseed (*L. usitatissimum*), Black Cumin (*N. sativa*), Fennel (*F. vulgare*), Psyllium (*P. ovata*) and Chamomila (*M. chamomilla*) and treatments on dry biomass in grams of medicinal plants.

Medicinal Plants	Treatments					
	silybum marianum	*Parthenium hysterophorous*	*Betula apyrifera*	Atrazine (Herbicide)	Control	Mean
L. usitatissimum	0.96 ab	0.17 c	0.13 c	0.26 c	0.58 abc	0.42 a
N. sativa	0.63 abc	0.13 c	0.13 c	0.23 c	0.35 bc	0.29 a
F. vulgare	0.44 bc	0.36 bc	0.26 c	0.31 bc	0.32 bc	0.34 a
P. ovata	0.53 abc	0.26 c	0.23 c	0.14 c	0.59 abc	0.35 a
M. chamomila	0.13 c	0.33 bc	0.24 c	0.26 c	1.16 a	0.42 a
Mean	0.54 ab	0.25 bc	0.20 c	0.23 c	0.60 a	

Means in same column followed by same letters are statistically similar at 5% level of significance
LSD (0.05) for MP= 0.29.
LSD (0.05) for TREATMENTS = 0.29.
Interaction (MP x TREATMENTS) = 0.66.

Antioxidant activity determination by DPPH scavenging assay

The different medicinal plants species, aqueous extracts of weeds and their interaction had a highly significant effect (P<0.01) on the antioxidant activity of the plants. The highest antioxidant activity was recorded in *F. vulgare* (91.03) under *B. papyrifera*, then by *M. chamomilla* (83.00) treated with extract of *B. papyrifera* (Table-6). The overall interaction effect revealed that *B. papyrifera* played stimulatory role in antioxidant activity as compared to control (Table-6). While in the interaction effect, *P. hysterophorus* played inhibitory role. All the medicinal plants were uniformly affected by the weed control treatments in terms of the antioxidant activity of the plant seedlings of the target species. Isildak *et al.* (2022) reported similar findings in determination of DPPH radical scavenging activity of plant extracts.

Table 6. Effect of treatments (*S. marianum*, *P. hysterophorous*, *B. papyrifera*, Atrazine (herbicide) and control) on antioxidant activity of medicinal plants. Interaction effect of medicinal plants (Linseed (*L. usitatissimum*), Black Cumin (*N. sativa*), Fennel (*F. vulgare*), Psyllium (*P. ovata*) and Chamomila (*M. chamomilla*) and treatments on antioxidant activity of medicinal plants.

Medicinal Plants	Treatments					
	silybum marianum	*Parthenium hysterophorous*	*Betula apyrifera*	Atrazine (Herbicide)	Control	Mean
L. usitatissimum	76.2 g	82.0 d	78.0 e	73.2 j	75.2 h	76.9 a
N. sativa	74.0 i	64.0 o	72.1 k	66.1 m	72.0 k	69.7 d
F. vulgare	73.1 j	67.23 l	91.0 a	74.0 i	67.2 l	74.5 c
P. ovata	67.3 l	72.1 k	76.1 g	73.2 j	65.1 n	70.0 e
M. chamomila	83 c	77.1 f	85.1 b	63.2 p	66.0 m	74.9 b
Mean	74.5 b	72.5 c	80.5 a	67.0 e	69.1 d	

Means in same column followed by same letters are statistically similar at 5% level of significance
LSD (0.05) for MP= 0.25.
LSD (0.05) for TREATMENTS = 0.25.
Interaction (MP x TREATMENTS) = 0.56.

Total phenolic content

The different medicinal plants species, weeds aqueous extracts and their interaction had a highly significant effect (P<0.01) on the total phenolic content of the medicinal plants (Table-7). Regarding the individual effect of the medicinal plant's species, the highest phenolic content was found in *M. chamomilla* treated with *P. hysterophorus* aqueous extracts, followed by *L. usitatissimum* (202.80) treated with *P. hysterophorus*, and the lowest content was in *F. vulgare* (114.33) treated with atrazine application. For the interaction effect, *P. hysterophorus* had a stimulatory effect for the total phenolic content as compared to control (Table-7). While *B. papyrifera* had inhibitory effect on the antioxidant activity of the tested medicinal plants species. However, the phenolic content was statistically at par in all the medicinal plants. Khawory *et al.* (2020) reported similar results of anti-phenolic content using

bioassay to determine the total phenols of extracts of plants. This experiment proved that plants studied might be potential source of phenols.

Table 7. Effect of treatments (*S. marianum*, *P. hysterophorous*, *B. papyrifera*, Atrazine (herbicide) and control) on phenolic content of medicinal plants. Interaction effect of medicinal plants (Linseed (*L. usitatissimum*), Black Cumin (*N. sativa*), Fennel (*F. vulgare*), Psyllium (*P. ovata*) and Chamomila (*M. chamomilla*) and treatments on phenolic content of medicinal plants.

Medicinal Plants	Treatments					
	silybum marianum	*Parthenium hysterophorous*	*Betula apyrifera*	Atrazine (Herbicide)	Control	Mean
L. usitatissimum	173 abcdef	202 a	161 abcdef	185 abcde	177 abcdef	180.1 a
N. sativa	181 abcdef	178 abcdef	141 fg	143 efg	174 abcdef	163.9 ab
F. vulgare	158 bcdef	157 cdef	199 abc	114 g	165 abcdef	159.1 b
P. ovata	188 abcd	175 abcdef	200 ab	141 fg	172 abcdef	175.77 ab
M. chamomila	175 abcdef	203 a	177 abcdef	155 defg	168 abcdef	176.1 ab
Mean	175.5 a	183.5 a	175.9 a	171.6 a	148.4 b	

Means in same column followed by same letters are statistically similar at 5% level of significance
LSD (0.05) for MP = 19.01.
LSD (0.05) for TREATMENTS = 19.01.
Interaction (MP x TREATMENTS) = 42.52.

Total flavonoid content

The different medicinal plants species, weeds aqueous extracts and their interaction had a highly significant effect ($P<0.01$) on the total flavonoid content of the medicinal plants (Table-8). For the individual effect, the highest flavonoid content was in *F. vulgare* (76.47) applied with extract of *B. papyrifera*, followed by *P. ovata* (66.07) treated with *S. marianum extract,* and the lowest value was for *P. ovata* under treatment of *B. papyrifera*. The interaction effect showed that *S. marianum* played a stimulatory effect on the medicinal plants for the antioxidant activity as compared to the control (Table-8). However, the effect was statistically at par for the flavonoid content of all the medicinal plants. Khawory *et al*. (2020) indicated similar findings for anti-flavonoid properties using bioassay for the total flavonoid content in the plants. This experiment proved that plants studied might be potential source of flavonoids.

Table 8. Effect of treatments (*S. marianum*, *P. hysterophorous*, *B. papyrifera*, Atrazine (herbicide) and control) on flavonoid content of medicinal plants. Interaction effect of medicinal plants (Linseed (*L. usitatissimum*), Black Cumin (*N. sativa*), Fennel (*F. vulgare*), Psyllium (*P. ovata*) and Chamomila (*M. chamomilla*) and treatments on flavonoid content of medicinal plants.

Medicinal Plants	Treatments					
	silybum marianum	*Parthenium hysterophorous*	*Betula apyrifera*	Atrazine (Herbicide)	Control	Mean
L. usitatissimum	61.1 d	42.56 n	65.8 b	45.4 n	56.6 ef	53.7 b
N. sativa	55.1 fgh	53.2 i	54.7 gh	43.0 n	48.3 i	50.8 c
F. vulgare	63.3 c	46.4 m	76.4 a	55.4 fg	63.3 c	60.9 a
P. ovata	66.0 b	52.0 j	0.00 o	42.7 n	50.1 k	42.2 d
M. chamomila	50.1 k	54.1 hi	56.5 ef	63.0 c	46.0 m	53.9 b
Mean	59.14 a	49.68 d	50.70 c	59.54 d	52.76 b	

Means in same column followed by same letters are statistically similar at 5% level of significance
LSD (0.05) for MP= 0.46.
LSD (0.05) for TREATMENTS = 0.46.
Interaction (MP x TREATMENTS) = 1.03.

Anti-bacterial activity

There was a highly significant effect (P<0.01) of the different medicinal plant's species, weeds aqueous extracts and their interaction on the anti-bacterial activity of the medicinal plants (Table-9 (A-D)). The highest anti-bacterial activity was recorded for *Citro bacter* on *F. vulgare* (15.73) treated with *B. papyrifera,* which was followed by *Citro bacter* activity in *L. usitatissimum* (14.00) under the treatment of *P. hysterophorus*, and then *Escherichia coli* activity in *F. vulgare* (13.56) under treatment of *B. papyrifera*. The lowest value of *Escherichia coli* activity was recorded in *M. chamomilla* under atrazine herbicide. The interaction effect showed that *S. marianum* had a stimulatory effect for anti-bacterial activity in all the medicinal plants as compared to the control.

Table 9 (A). Effect of treatments (*S. marianum*, *P. hysterophorous*, *B. papyrifera*, Atrazine (herbicide) and control) on anti-bacterial activity of medicinal plants. Interaction effect of medicinal plants (Linseed (*L. usitatissimum*), Black Cumin (*N. sativa*), Fennel (*F. vulgare*), Psyllium (*P. ovata*) and Chamomila (*M. chamomilla*) and treatments on anti-bacterial activity of medicinal plants.

(A: *Escherichia coli*)

Medicinal Plants	Treatments					
	silybum marianum	*Parthenium hysterophorous*	*Betula apyrifera*	Atrazine (Herbicide)	Control	Mean
L. usitatissimum	11.43 d	11.4 d	12.26 bc	10.26 ef	8.1 i	10.6 ab
N. sativa	9.5 gh	11.33 d	11.7 cd	10.2 efg	9.26 h	10.40 bc
F. vulgare	9.9 efgh	10.23 ef	13.56 a	9.93 efgh	11.43 d	11.01 a
P. ovata	12.3 bc	10.4 e	9.63 fgh	11.2 d	8.53 i	10.41 bc
M. chamomila	12.8 b	10.2 efg	10.2 efg	8.3 i	10 efg	10.30 c
Mean	11.18 a	10.71 b	11.47 a	9.98 c	9.46 d	

Means in same column followed by same letters are statistically similar at 5% level of significance
LSD (0.05) for MP = 0.32.
LSD (0.05) for TREATMENTS = 0.32.
Interaction (MP x TREATMENTS) = 0.71.

Table 9 (B). Effect of treatments (*S. marianum*, *P. hysterophorous*, *B. papyrifera*, Atrazine (herbicide) and control) on anti-bacterial activity of medicinal plants. Interaction effect of medicinal plants (Linseed (*L. usitatissimum*), Black Cumin (*N. sativa*), Fennel (*F. vulgare*), Psyllium (*P. ovata*) and Chamomila (*M. chamomilla*) and treatments on anti-bacterial activity of medicinal plants.

(B: *Staphylo coccus*)

Medicinal Plants	Treatments					Mean
	silybum marianum	*Parthenium hysterophorous*	*Betula apyrifera*	Atrazine (Herbicide)	Control	
L. usitatissimum	9.80 gh	10.5 cd	6.63 l	5.80 m	9.86 fgh	8.52 cd
N. sativa	6.8 l	9.7 h	10.3 cde	12.3 a	12.3 a	10.28 a
F. vulgare	5.8 m	6.73 l	11.1 b	10.2 def	8.3 j	8.42 d
P. ovata	7.00 kl	9.86 fgh	10.1 efg	7.3 k	10.6 c	8.97 b
M. chamomila	8.00 j	10.33 cde	9.16 i	9.00 i	6.8 l	8.66 c
Mean	7.48 c	9.42 a	9.46 a	8.92 b	9.57 a	

Means in same column followed by same letters are statistically similar at 5% level of significance
LSD (0.05) for MP = 0.17.
LSD (0.05) for TREATMENTS = 0.17.
Interaction (MP x TREATMENTS) = 0.38.

Table 9 (C). Effect of treatments (*S. marianum*, *P. hysterophorous*, *B. papyrifera*, Atrazine (herbicide) and control) on anti-bacterial activity of medicinal plants. Interaction effect of medicinal plants (Linseed (*L. usitatissimum*), Black Cumin (*N. sativa*), Fennel (*F. vulgare*), Psyllium (*P. ovata*) and Chamomila (*M. chamomilla*) and treatments on anti-bacterial activity of medicinal plants.

(C: *Agro bacterium*)

Medicinal Plants	Treatments					Mean
	silybum marianum	*Parthenium hysterophorous*	*Betula apyrifera*	Atrazine (Herbicide)	Control	
L. usitatissimum	7.2 hi	10.96 ab	9.76 cd	6.7 ijk	7.8 gh	8.48 b
N. sativa	9.2 de	10.16 bc	10.4 abc	5.82 jkl	11.23 a	9.36 a
F. vulgare	8.5 efg	9.76 cd	7.8 gh	10.86 ab	7.53 hi	8.89 b
P. ovata	10.56 abc	7.76 gh	6.76 ij	5.8 kl	5.4 l	7.26 c
M. chamomila	8.86 def	8.06 fgh	7.13 hi	5.2 l	6.63 ijk	7.18 c
Mean	8.86 b	9.34 a	8.37 c	6.87 e	7.72 d	

Means in same column followed by same letters are statistically similar at 5% level of significance
LSD (0.05) for MP = 0.43.
LSD (0.05) for TREATMENTS = 0.43.
Interaction (MP x TREATMENTS) = 0.96.

Table 9 (D). Effect of treatments (*S. marianum*, *P. hysterophorous*, *B. papyrifera*, Atrazine (herbicide) and control) on anti-bacterial activity of medicinal plants. Interaction effect of medicinal plants (Linseed (*L. usitatissimum*), Black Cumin (*N. sativa*), Fennel (*F. vulgare*), Psyllium (*P. ovata*) and Chamomila (*M. chamomilla*) and treatments on anti-bacterial activity of medicinal plants.

(D: *Citro bacter*)

Medicinal Plants	Treatments					
	silybum marianum	*Parthenium hysterophorous*	*Betula apyrifera*	Atrazine (Herbicide)	Control	Mean
L. usitatissimum	13 d	14 b	13.3 c	10.2 o	12.3 fg	12.56 a
N. sativa	11.1 l	13.2 c	12.13 gh	12.5 e	11.3 jk	12.06 c
F. vulgare	11.5 i	11.5 i	15.73 a	12.1 h	11.5 i	12.42 b
P. ovata	12.4 ef	10.0 o	11.3 jk	10.1 o	11.3 jk	11.06 d
M. chamomila	10.8 m	11.46 ij	11.2 kl	10.6 n	11.5 i	11.11 d
Mean	11.76 c	12.05 b	12.73 a	11.13 e	11.54 d	

Means in same column followed by same letters are statistically similar at 5% level of significance
LSD (0.05) for MP = 0.08.
LSD (0.05) for TREATMENTS = 0.08.
Interaction (MP x TREATMENTS) = 0.18.

Chlorophyll Content (µmol)

The highest value of chlorophyll content was found in *F. vulgare* (19.75) under the treatment of *B. papyrifera,* followed by *L. usitatissimum* (18.750) under the treatment of *S. marianum*, while the lowest chlorophyll content was of *P. ovata* (5.12) under treatment of *S. marianum* (Table-10). The interaction effect was significant where *P. hysterophorus* played a stimulatory role and the herbicide played inhibitory role (Table-10).

Table 10. Effect of treatments (*S. marianum*, *P. hysterophorous*, *B. papyrifera*, Atrazine (herbicide) and control) on chlorophyll content of medicinal plants. Interaction effect of medicinal plants (Linseed (*L. usitatissimum*), Black Cumin (*N. sativa*), Fennel (*F. vulgare*), Psyllium (*P. ovata*) and Chamomila (*M. chamomilla*) and treatments on chlorophyll content of medicinal plants.

Medicinal Plants	Treatments					
	silybum marianum	*Parthenium hysterophorous*	*Betula apyrifera*	Atrazine (Herbicide)	Control	Mean
L. usitatissimum	18.75 ab	15.1 abcde	11.02 bcdefg	15.7 abcde	10.3 cdefg	14.1 ab
N. sativa	13.67 abcdefg	18.7 ab	17.4 abcde	8.95 efg	13.05 abcdefg	14.3 ab
F. vulgare	18.07 abc	18.8 ab	19.75 a	14.02 abcd	13.47 abcdef	16.8 a
P. ovata	5.12 g	6.35 fg	6.65 fg	11.77 abcdefg	7.65 efg	7.50 c
M. chamomila	17.5 abc	14.17 abcdef	12.17 abcdefg	10.32 cdefg	9.15 defg	12.6 b
Mean	14.62 a	14.6 ab	13.4 ab	10.7 b	10.7 b	

Means in same column followed by same letters are statistically similar at 5% level of significance

LSD (0.05) for MP = 3.73.
LSD (0.05) for TREATMENTS = 3.73.
Interaction (MP x TREATMENTS) = 8.34

CONCLUSION

The weed extracts used on medicinal plants had a positive effect on its growth, shoot and root length, fresh and dry biomass. The seedlings of medicinal plants fully germinated and had a healthy growth with proper amount of nutrients which were found in the chemical analysis. The extracts exerted positive effect on the growth of medicinal plants, therefore should be commercialized for production of bio-fertilizer. The extracts of various trees found with nearby medicinal plants in their natural zone should be assessed on medicinal plant of that area.

CONFLICT OF INTEREST

The authors of the manuscript have no conflict of interest.

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