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Allelopathic effect of aqueous extract of *Polygonum bistorta* and *Terminalia chebula* on germination and seedling growth of *Daucus carota* and *Medicago polymorpha*

Efeito alelopático do extrato aquoso de *Polygonum bistorta* e *Terminalia chebula* na germinação e crescimento de plântulas de *Daucus carota* e *Medicago polymorpha*

Rizwan Maqbool[1]; Bilal Ahmad Khan[2]*; Muhammad Ather Nadeem[3]; Rabia Azam[4]; Ali Raza[5]; Aneela Nijabat[6]; Tasawer Abbas[7]; Talou- E-Islam Inqalabi[8] ; Muhammad Ikram[9]; Muhammad Mohsin Amin[10]

Highlights

Weeds in crop fields compete for nutrients and cause yield and quality loss.
Weed management is essential for sustainable crop production.
The use of herbicides is causing herbicide-resistant in weeds.

Abstract

Weed management is essential for sustainable crop production in all cropping systems. The use of herbicides is causing the problems of herbicide resistant weeds and environmental pollution. Plant-

[1] Associate Professor in Department of Agronomy, Agriculture Univerisity Faisalabad-38000, Pakistan. E-mail: rizwan.maqbool@uaf.edu.pk
[2] PhD Scholar in Department of Agronomy, College of Agriculture, Univerisity of Sargodha-40100, Pakistan. E-mail: bilalahmadkhan678@gmail.com
[3] Professor in Department of Agronomy, College of Agriculture, Univerisity of Sargodha-40100, Pakistan. E-mail: ather.nadeem@uos.edu.pk
[4] Scholar in Department of Botany, Agriculture Univerisity Faisalabad-38000, Pakistan. E-mail: rizawn.maqbool@uaf.edu.pk
[5] PhD Scholar Agriculture Univerisity Faisalabad-38000, Pakistan. E-mail: alisialali@gmail.com
[6] Dr. Aneela Nijabat; Department of botany, Ghazi Univerisity Dera Ghazi Khan, Pakistan. E-mail; aneelanijabat49@gmail.com
[7] Instructor In-Service Agricultural Training Institute, Sargodha, Pakistan. E-mail; tgondaluaf@gmail.com
[8] Department of Botany, Univerisity of Education, Lahore, Pakistan. E-mail: razaa5026@gmail.com
[9] PhD Scholar; Department of Botany, PMAS, Arid Agriculture, Univerisity Rawalpindi, Pakistan. E-mail; Ikramgondal464@gmail.com
[10] Scholar in Department of Agronomy, College of Agriculture, Univerisity of Sargodha-40100, Pakistan. E-mail: mohsinamin123@gmail.com
* Author for correspondence

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released phytotoxins have ability to promote or inhibit the germination and seedling growth of surrounding crops and weeds. Therefore, it is imperative to identify plant species having the herbicidal potential to use as more safe and sustainable weed management approach. The present study was planned in 2018 to investigate the germination and seedling growth of *Daucus carota* and *Medicago polymorpha* influenced by the root extract of *Polygonum bistorta* and fruit extract of *Terminalia chebula.* Different concentrations of aqueous extract (0, 2.5, 5, 10, 20, 40 and 80%) significantly reduced the germination and seedling growth of target species. Results of these experiments revealed that thefruit extract of *T. chebula* was more phytotoxic as compared to root extract of *P. bistorta* regarding seedling growth of weed. However, fruit extract of *T. chebula* having 80% concentration fully inhibit the germination of *D. carota* and *M. polymorpha.* One the bases of these finding it was concluded that the fruit extract of *T. chebula* and root extract of *P. bistorta* have comparatively more active substances with ability to suppress germination and growth of plants which could be exploited as prospective source of bio-herbicides.
Key words: Allelopathy. Extract. Germination. Seedling growth and management.

Resumo

O manejo de ervas daninhas é essencial para a produção agrícola sustentável em todos os sistemas de cultivo. O uso de herbicidas está causando problemas de ervas daninhas resistentes a herbicidas e poluição ambiental. As fitotoxinas liberadas pelas plantas têm a capacidade de promover ou inibir a germinação e o crescimento das mudas de culturas e ervas daninhas circundantes. Portanto, é imperativo identificar espécies de plantas com potencial herbicida para uso como abordagem de manejo de ervas daninhas mais segura e sustentável. O presente estudo teve como objetivo investigar a germinação e o crescimento de plântulas de *Daucus carrota* e *Medicago polymorphaas* influenciados pelo extrato de raiz de Polygonum bistorta e extrato de fruto de *Terminalia chebula.* Diferentes concentrações de extrato aquoso (0, 2, 5, 5, 10, 20, 40 e 80%) reduziram significativamente a germinação e o crescimento de plântulas das espécies-alvo. Os resultados desses experimentos revelaram que o extrato do fruto de *T. chebula* foi mais fitotóxico do que o extrato da raiz de *P. bistorta* em relação ao crescimento de mudas de plantas daninhas. No entanto, o extrato de frutos de *T. chebula* com concentração de 80% inibe totalmente a germinação de *D. carrota* e *M. polymorpha.* Uma das bases deste achado concluiu-se que o extrato do fruto de *T. chebula* e o extrato da raiz de *P. bistorta* possuem comparativamente mais substâncias ativas com capacidade de suprimir a germinação e o crescimento das plantas, o que poderia ser explorado como fonte potencial de bio-herbicidas.
Palavras-chave: Alelopatia. Extrair. Germinação. Crescimento e manejo de mudas.

Introduction

Weeds in crop fields compete for nutrients and cause yield and quality loss (Nadeem et al., 2020a; Maqbool et al., 2021a; Javaid et al., 2022). Further 30% once a year yield wounded due to weeds that cause severe consequences on the growth of agricultural crops (Arooj et al., 2021; Maqbool et al., 2021b; Nadeem et al., 2020b). Currently, weeds are controlled by chemical herbicides in agricultural production that have imparted harmful effects on the environment (Tariq et al., 2022; Nadeem et al., 2020c,d; Maqbool et al., 2021c, 2022a). Herbicides have helped farmers by enhancing crops yield while reducing the labor and time input. Indeed, without herbicides, labors have utmost outlay of crop invention in developed countries (Javaid et al., 2022; Arooj et al., 2021). However, arbitrary exploit of herbicides against weeds have resulted in herbicide resistance (Nadeem et al 2021a; Maqbool et al., 2022b). Hence, alternate sustainable weed management approaches are need of time to sustain crop production worldwide. Usage of plants extracts for weed control and increase crop yield has been reported (Nadeem et al, 2021b; Maqbool et al., 2022b). Allelopathic plants improve crop yields by controlling weeds and reducing competition for resources (Nadeem et al., 2021c; Farooq et al., 2013; Ali et al., 2021). Allelopathic plants are considered as alternate choice to control the growth of weeds without using the chemical herbicides (Nadeem et al., 2020a,b; Maqbool et al., 2022c).

Polygnum bistorta L. (Anjbar) is most important herb in the genius of Polygonum which belongs to family Polygonaceae. This herb is dispersed in excess of all over the world, it is also found in north temperate zones. Externally, its root-stock is brown-black, but internally it's colored red and is rich in tannic and gallic acids. Water extracts of allelopathic crops have the ability to promote or inhibit the growth of different species (Nadeem et al., 2020a,b; Teasdale & Pillai, 2005). Allelochemicals are liberated into the environment by evaporation, leakage, root emission and by decaying plant debris, which are found in all plant tissues as well as leaves, stems, flowers, roots and pollens (Maqbool et al., 2022c).

Terminalia chebula, (Harer) commonly known as "King of medicines" is an inhabitant to South Asia from India to Sri Lanka. This is widely cultivated in Taiwan because of its extraordinary healing powers. This is actually dry fruit, furthermore known as Harer fruit (Edison et al., 2016). *T. chebula* (Harer) place to own family Combretacea, and introduced in all over India mainly in caducous plantations and regions of low precipitation (Naik et al., 2004). Gallic acid known as chebulin and other chemicals like favonol glycosides, triterpenoids and phenolic compounds were also derived from *T. chebula.* (Edison et al., 2016). From the fruit of *T. chebulain* addition, ethyl gallate and luteolin were also remote and play a significant role in plant growth (Edison et al., 2016). It was also included nutrients and minerals (Mahesh et al., 2007). Bhatt et al. (2021) demonstrated significantly low germination and furthermore roots expansion of the finger millet, mustard, barley and soybean by rhizosphere soil layer and dry plant parts watery extracts of *T. chebula* and *P. bistorta.* Germination and root adjunct of every test crop had also remarkably reduced via exposure to water extracts of leaves and fruit pulp of *T. chebula.*

The carrot (*Daucus carota*) is native to Iran and firstly was cultivated for its vegetative organs. The carrot is a biennial plan that belongs to the Apiaceae family. Carrot is a good source alpha and beta carotene compounds, fiber, vitamin K and antioxidant. It is a biennial vegetable, grown for its edible, fleshy taproots. Its flower stalk starts from the crown and grows up to a height of 60 to 90 cm. Swollen taproot can reach a length of 20 to 25 cm but its shape, colour and size depend upon the cultivar grown. Roots may be yellow, red dark, blue and seeds are small and prominently ribbed. *Medicago polymorpha* (Maina) is annual plant growing up to 0.6 m (2ft), and it belongs to family Fabaceae. It is inhabitant of Mediterranean basin but is present all over the world. The first true leaf rounded. Later leaves are tripartite, with clover like shape. Weeds can reduce the yield of crop. Knowledge about the weeds occurring, their distribution within the fields and their effects on crop production is significant to achieve effective weed management. Allelochemical-environmental interactions must be measured in efforts to benefit from allelopathy. Therefore, the present study was planned to investigate the allelopathic effect of aqueous extract of *P. bistorta* and *T. chebula* on germination and seedling growth of *D. carota* and *M. polymorpho.*

Materials and Methods

To investigate the allelopathic potential of aqueous extract of root of *Polygonum bistorta* (Anjbar) and fruit of *Terminalia chebula* (Harer) on *Medicago polymorpha* (Mena) and *Daucus carrota* (carrot), a series of experiments were organized during 2018. Laboratory experiments were conducted during winter season 2018 at University of Agriculture, Faisalabad in the weed science laboratory of the Agronomy department.

Collections of seeds

Daucus carota (T-29) and *Medicago polymorpha* (wild) seeds were collected from Ayub Agricultural Research Institute and weed science laboratory of Agronomy department, University of Agriculture, Faisalabad.

Collections of plant materials for extract

The dry roots of *P. bistorta* and dry fruit of *T. chebula* were collected from a local medicinal plant shop. By using manual chopper roots were separated and chop into small pieces and preserved at room temperature for further exposition.

Preparation of plants aqueous extracts

For preparing the aqueous extract of roots of *P. bistorta* and fruit of *T. chebula* the chopped sample of both root and fruit were soaked separately at a ratio 1:80 m/v in water for 48 hours. The decanted material was passed through a cotton cloth to obtain water extract of different parts of plants. The extract was diluted as per treatment as 0, 0.25, 0.5, 1, 2, 4, 8%. The water extracts of *P. bistorta* and *T. chebula* were applied to *D. carota* in the first experiment and *M. polymorpha* in second experiment.

For these experiments, 10 seeds of each target weed seed were placed in each Petri plate having double layer of filter paper. Then the prepared different seven dilutions of root extract of *P. bistorta* and fruit extract of *T. chebula* were applied on the *D. carota* and *M. polymorpha* seeds and all the Petri plates were wrapped and replicated four times. Data was recorded on daily basis for a period of 15 days.

Principal component analysis

To explain the variation and correlation among germination and growth response of targeted species principal component analysis was performed.

Experimental layout

The experiment contained two tested plants and two applied plants. These experiments were laid out as a single factor completely randomized design (CRD) and having four replicates.

The following parameters were noticed during laboratory experiments using the standard procedures: Seedling shoot length (cm), Seedling root length (cm), Seedling fresh weight (mg), Mean germination time (MGT), Germination index, Time to 50% germination and Seed germination (%).

Mean germination time (MGT)

Mean germination time as measures the time and rate of germination spread. According to Dezfuli et al. (2008) mean germination was recorded by using this equation.

$$\text{MET} = \sum \frac{(\text{Dn})}{\sum \text{n}}$$

D= counted overall days from germination of seeds start. While n is the num of seeds emerge on D days were counted.

Germination index (GI)

According to process of Association of official seed analysis (1990) germination index was calculated. Germination index of seeds was calculated by following equation.

$$\text{GI} = \frac{No.of\ germinated\ seeds}{Days\ of\ first\ count} + \text{-----------} + \frac{No.of\ germinated\ seeds}{Days\ of\ final\ count}$$

Time to 50% germination (Days)

By using the modified equation of Farooq et al. (2004) for time to 50% germination was calculated.

$$\text{T}_{50} = \text{ti}\left[\frac{\frac{N}{2} - ni}{nj - ni}\right](tj - ti)$$

N is the number of whole germinated seeds. Whereas ni and nj are the collective no. of germinate seeds count at the time of tj and ti respectively.

Seed germination percentage (%)

When seedling length was up to 1-2 cm seeds were counted as germinated. By using fallowing formula, percentage of germinated seed for each replication of treatment after two weeks germination of seedlings was calculated.

$$GP/EP = \frac{\text{Germinated/emerged seeds}}{\text{Total seeds}} \times 100$$

Total phenolic contents

Phenolic content were determined by using HPLC (Gradient, reverse phase mode from Shimadzu Japan; detector SPD-10AV pump LC-10AT). 10-gram powder of *P. bistorta* and *T. cheblua* were taken in 90% methanol. Then samples were placed in beakers and cover the beaker with orifice aluminum foil for 10 days. After 10 days the material was dried and 5 mg weighs were taken out for phenolic analysis.

Statistically analysis

Data was analyzed using Fisher's Analysis of Variance (ANOVA). The differences among treatments were separated using the least significant difference test (LSD).

Results and Discussions

Allelopathic effect of aqueous extract of Polygonum bistorta and Terminalia chebula on Germination of Daucus carota and Medicago polymorpha

Allelopathic weeds extract and different dose levels significantly affected the germination response of two targeted species. Variation between targeted species was significant ($P \leq 0.05$) for germination time and germination index. Data presented in figure 1 clearly indicates that more time was taken for 50% seed germination (T_{50}) in *M. polymorpha* than *D. carrota.* This variation in germination response of two targeted species is attributed to their genetic variation (Bolton & Simon, 2019a; Colque-Little et al., 2021), quantity of stored food material in seed (A. Bhatt et al., 2021; Mokotjomela et al., 2021), and endogenous production of various enzymes like amylase that affect the germination process (Adetunji et al., 2021; Aguieiras et al., 2021). Furthermore, an aqueous extract of *T. chebula* did not affected the time taken for 50% seed germination of both targeted species at every tested dose level as compared to control but no seed germination was observed at application of 80% pure extract. This complete inhibition of seed germination of targeted species in response to 80% pure extract of *T. chebula* confirms the presence of high concentration of allelopathic compounds in extract. On the other hand, an aqueous extract of *P. bistorta* moderately affected the time taken to 50% seed germination of both species at various dose levels as compared to control. Time taken for 50% seed germination was reduced than control in both targeted species in response to 2.5% of aqueous extract of *Polygonum bistorta* and moderately increased in response to 80% extract of *P. bistorta.* Time reduction in completion of 50% seed germination in response to 2.5% extract of P. bistorta suggest that diluted extract contain less quantity of allelopathic compounds that favors the germination process (Galon et al., 2021; Nadeem et al., 2020b), while concentrated extract contains higher concentration of allelopathic compounds that inhibits the seed germination (Nadeem et al., 2020a; Maqbool et al., 2021a; Nadeem et al., 2021a; Maqbool et al., 2022a).

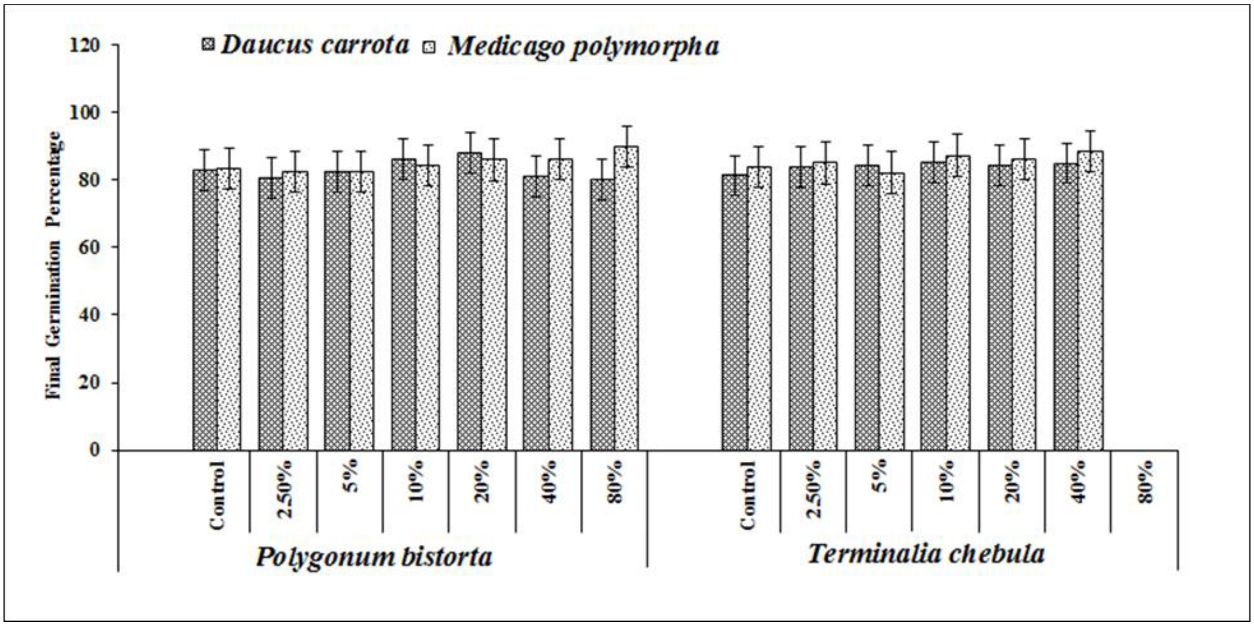

Figure 1. Mean germination time of *Daucus carota* and *Medicago polymorpha* as influence by allelopathic effect of root of *Polygonum bistorta* and fruit of *Terminalia chebula.*
The meaning of bar is standar error.

Mean germination time was non-significantly varied between two target species in response to various doses of aqueous extract of *P. bistorta.* About 8 to 9 days were recorded as mean germination time for *D. carota* and *M. polymorpha* in response to each dose of *P. bistorta*. Interestingly, the mean germination time of *D. carota* seeds was not affected in response to the allelopathic extract of *T. chebula* but no germination was observed when 80% extract was applied. In the case of *M. polymorpha,* mean germination time was reduced in response to 2.5%, and 10%, but time taken for germination gradually increased with an increase in the concentration of extract from 10% to 40% and 80% aqueous extract of *T. chebula* fruit completely inhibited the seed germination (Figure 2). Similar findings were reported in previous studies regarding allelopathic impacts of different weed species on seed germination of various crops (Nadeem et al., 2020b; Galon et al., 2021; Nadeem et al., 2021b; Maqbool et al., 2021b, 2022b).

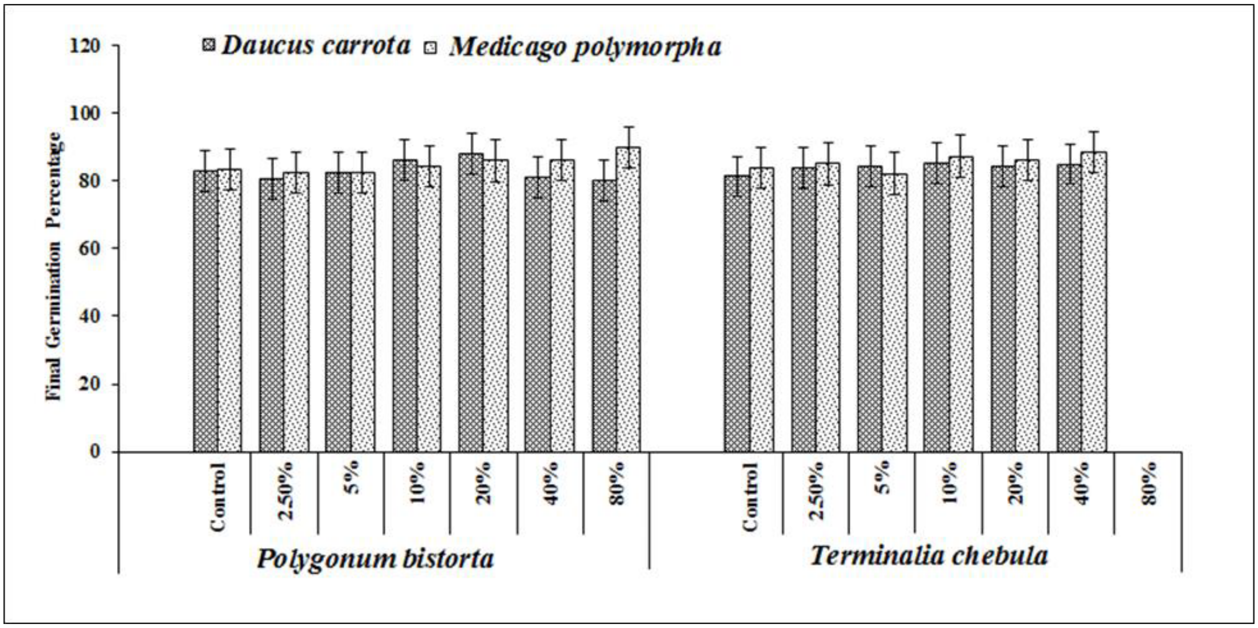

Figure 2. Final germination (%) of *Daucus carota* and *Medicago polymorpha* as influence by allelopathic effect of root of *Polygonum bistorta* and fruit of *Terminalia chebula.*

Final germination percentage of *D. carota* and *M. polymorpha* in response to different doses of aqueous extract of *P. bistorta* and *T. chebula* exhibited non-significant variation. Final germination percent *D. carota* ranged from 78% to 84% and 81% to 85% in response to root and fruit extract of *P. bistorta* and *T. chebula*, respectively, and similar trend was observed for the final germination percent of *M. polymorpha* seeds. While comparing the variation for final germination percentage of both target species among different doses of aqueous extract, minimum germination was recorded in response to 80% extract of *P. bistorta* and complete germination inhibition in response to 80% of *T. chebula* (Figure 3). Germination index of carrot seeds exhibited no variation in response to aqueous extract of *P. bistorta* but germination index of *M. polymorpha* was gradually reduced with increase in concentration of aqueous of *P. bistorta.* On the other hand, germination index was increased initially at lower concentrations of aqueous extract of *T. chebula* as compared to control but later on it was decreased at 40% concentrated extract and complete inhibition at 80% concentrated extract (Figure 4). This complete or partial inhibition of seed germination in response to concentrated extract of *T. chebula* is attributed to presence of higher concentration of different allelopathic compounds including water soluble phenolics, gallic acid and caffic acid. Presence of more compounds in aqueous extract of *T. chebula* in higher concentration than aqueous extract of *P. bistorta* (Table 1) confirms its more allelopathic impacts on target species. Previous studies also reported that more concentration of compounds in aqueous extract of weeds poses strong inhibitory impacts on germination response of target species (Nadeem et al., 2020b,c; Galon et al., 2021; Nadeem et al., 2021c,d; Maqbool et al, 2022c).

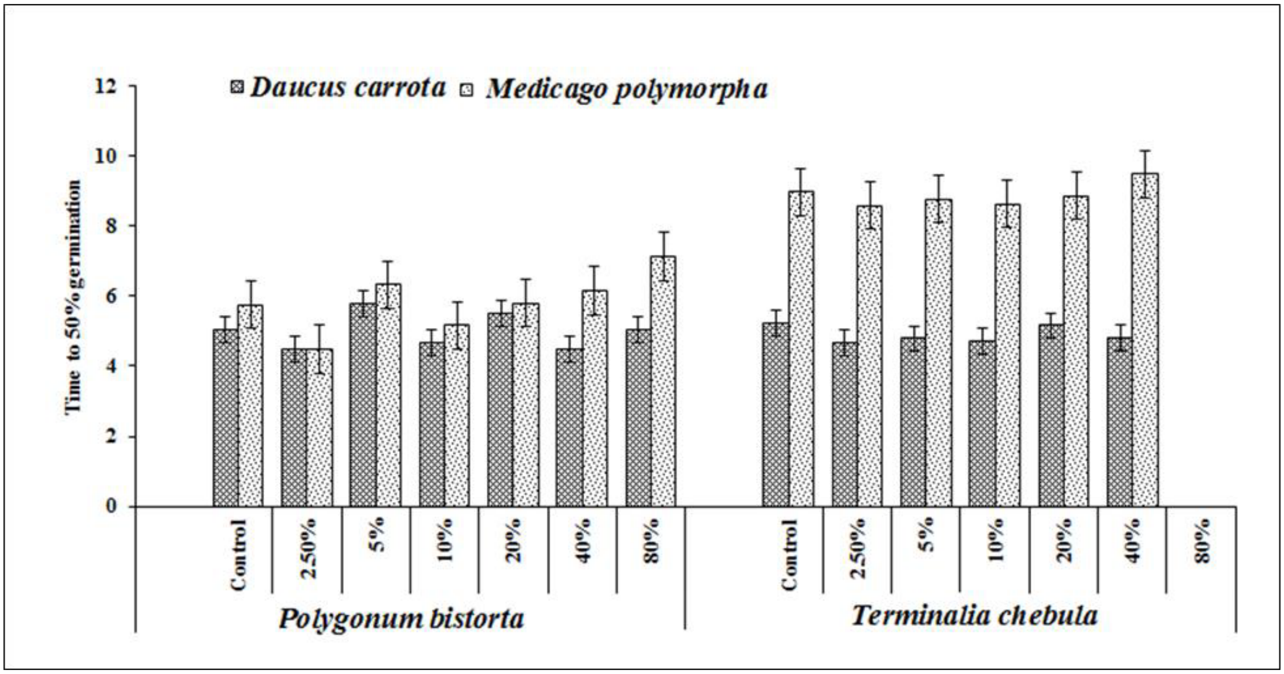

Figure 3. Time to 50% germination of *Daucus carota* and *Medicago polymorpha* as influenced by the allelopathic effect of root of *Polygonum bistorta* and fruit of *Terminalia chebula.*
The meaning of the bar is standard error.

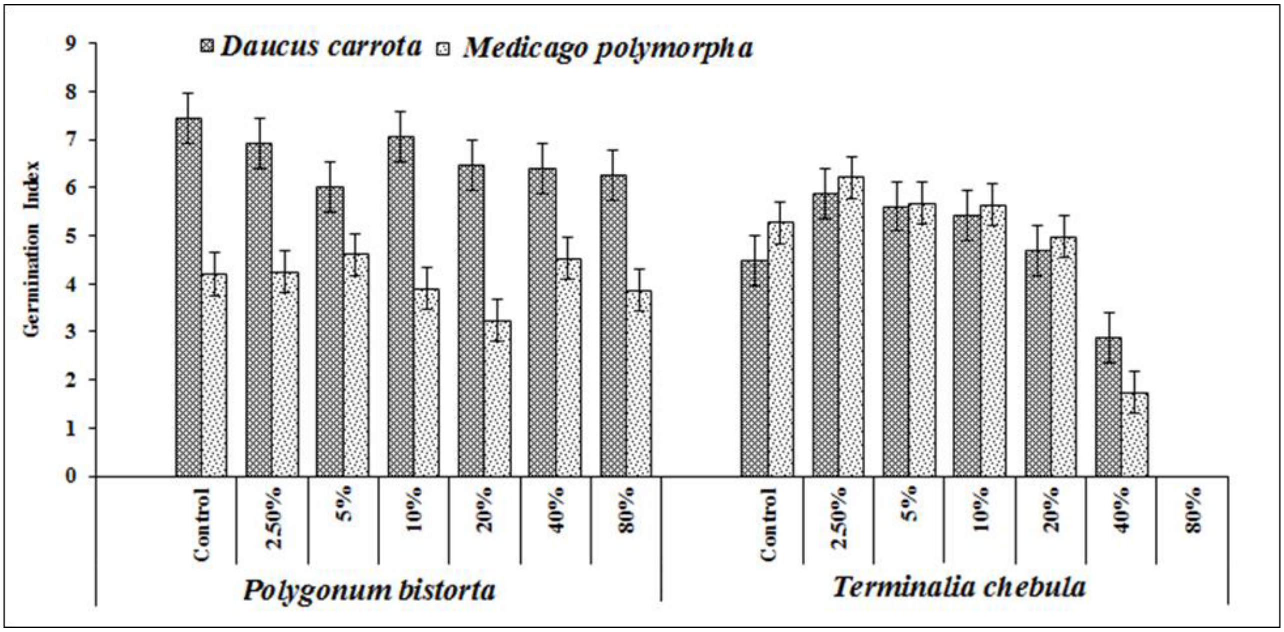

Figure 4. Germination index (%) of *Daucus carota* and *Medicago polymorpha* as influence by allelopathic effect of root of *Polygonum bistorta* and fruit of *Terminalia chebula.*
The meaning of bar is standar error.

Table 1
Allelopathic compound detected in aqueous extract of two weeds.

Compound	*Terminalia chebula* (ppm)	*Polygonum bistorta* (ppm)
Gallic acid	33.45	-
Caffic acid	6.51	6.56
Vanillic	4.69	0.58
Benzoic	21.61	-
Syringic	3.85	-
P-coumeric	1.98	-
M-coumeric	1.81	-
Femlic acid	6.66	-
Cinamic	3.26	-
Sinapic	4.13	-
Querctin	26.92	21.55

Allelopathic effect of aqueous extract of Polygonum bistorta and Terminalia chebula on seedling growth of Daucus carota and Medicago polymorpha

The allelopathic effect of two weeds *P. bistorta* and *T. chebula* on root and shoot growth and biomass accumulation of target species *D. carota* and *M. polymorpha* was evaluated in the present study (Table 2). Allelopathic weeds extract and different dose levels significantly affected the germination response of two targeted species i.e. *D. carota* and *M. polymorpha.* Variation between targeted species was significant for growth ($P \leq 0.01$) and biomass accumulation ($P \leq 0.05$). A differential response was observed for root length and shoot length of *D. carota* and *M. polymorpha* seedlings towards different concentration levels of aqueous extract of both weeds. Minimum reduction in root length, shoot length, root biomass accumulation and shoot biomass accumulation were measured in *D. carota* in response to 2.5% and 5% of extract ,and maximum reduction in response to 40% and 80% of *P. bistorta* (figure 5,6,7 8). Similar trend was observed for both target species in response to the aqueous extract of *T. chebula.* Lower concentrations of an extract with less quantity of allelochemicals stimulated the negative growth regulating processes while high concentrations of extract proved to be growth inhibitory due to more quantity of allelochemicals. These allelochemicals affect the primary and secondary metabolic pathways directly or indirectly. They interrupt physiological processes like absorption of nutrients, signal transduction, photosynthesis and transpiration, cell division, elongation and differentiation, reactive-oxygen species production (Maqbool et al., 2021a,b; Nadeem et al., 2020d; Hussain et al., 2020; Ma et al., 2020; Marchiosi et al., 2020). It has also been reported in previously that effects of allelochemicals on target species depends on chemical nature of compound, concentration in allelopathic source, level of exudation, translocation in soil and sensitivity of target species (Hussain et al., 2020). Present study highlighted that *D. carota* was less sensitive to

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tested weeds than *M. polymorpha.* Moreover, *T. chebula* was more allelopathic than *P. bistorta* due to presence of more allelochemicals with higher concentrations.

Table 2
Mean squares from three-factorial (Allelopathic weed extract: AWE, extract dose: ED, and targeted species: TSp) ANOVA for germination and seedling growth.

SOV	*DF*	*Time to 50% Germination*	Mean Germination Time	Final Germination Percent	Germination Index
AWE	1	17.6*	38.1*	29.3**	12.5**
ED	6	54.23NS	73.1NS	56.2*	79.4**
TSP	1	6.7*	2.34*	11.6 NS	9.4*
AWE × ED × TSp	6	29.3*	63.5*	78.2*	63.4*
Error	15	0.6	1.7	0.3	1.93
SOV	DF	Root Length	Shoot Length	Root Fresh Weight	Shoot Fresh Weight
AWE	1	62.9***	38.1***	63.2***	84.1**
ED	6	106.7**	87.0**	25.27**	31.0*
TSP	1	19.53**	33.9**	71.29*	98.8*
AWE × ED × TSp	6	81.1**	65.0**	43.05*	70.0*
Error	15	2.6	3.12	3.27	4.16

SOV: Source of variation, DF: Degree of freedom, ***: Significant P ≤ 0.0001, **: Significant P ≤ 0.001, *: Significant P ≤ 0.05,AWE:Allelopathic weed extract, ED: Extract dose, TSp: Targeted species.

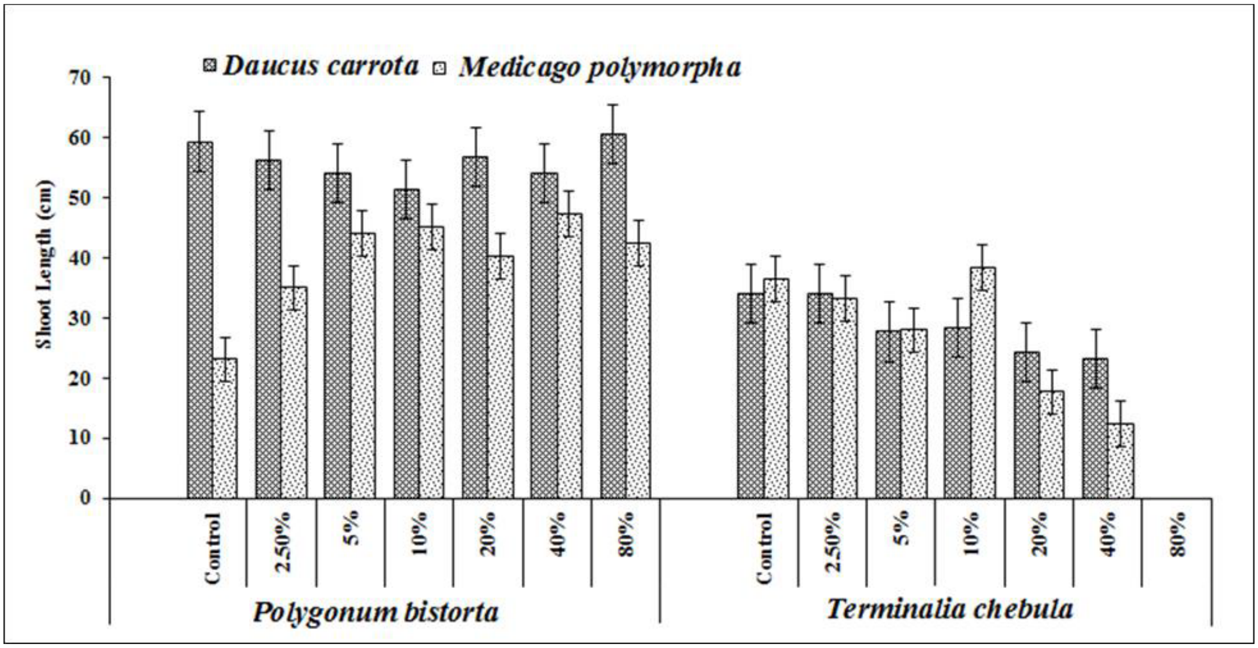

Figure 5. Shoot length (cm) of *Daucus carrota* and *Medicago polymorpha* as influence by allelopathic effect of root of *Polygonum bistorta* and fruit of *Terminalia chebula.*
The meaning of bar is standar error.

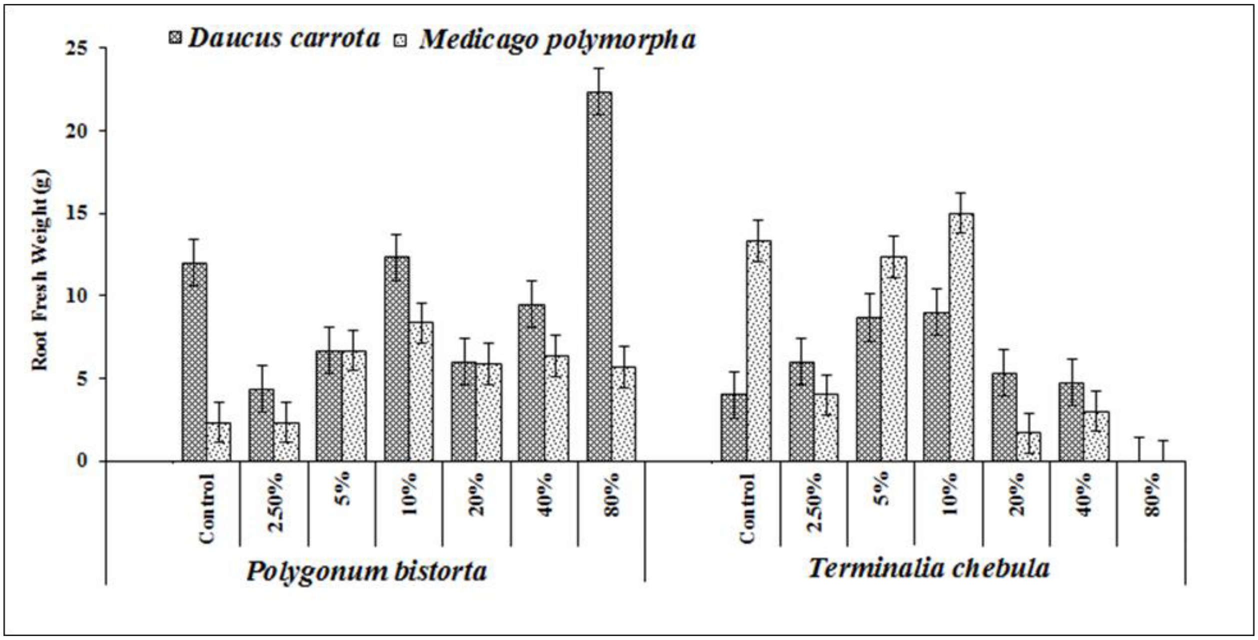

Figure 6. Root Fresh weight (g) of *Daucus carrota* and *Medicago polymorpha* as influence by allelopathic effect of root of *Polygonum bistorta* and fruit of *Terminalia chebula.*
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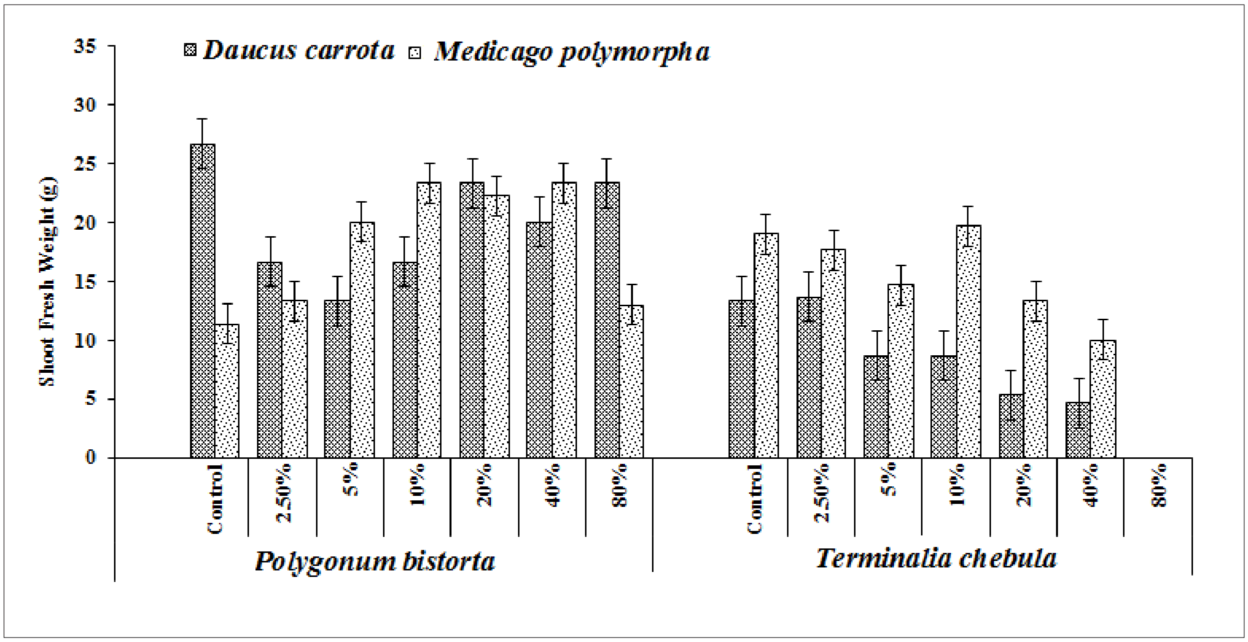

Figure 7. Shoot fresh weight (g) of *Daucus carrota* and *Medicago polymorpha* as influence by allelopathic effect of root of *Polygonum bistorta* and fruit of *Terminalia chebula.*
The meaning of bar is standar error.

Generally, it was reported that these allelochemicals like organic acids, phenolic compounds and flavonoids identified in the present study are water soluble and potentially toxic to plant species. They induce oxidative stress when interact with plant cell and retard the plant growth and development in response to cell injury and membrane disintegration (Nijabat et al., 2020), disruption of photosynthetic apparatus (Kabashnikova et al., 2020; Parrotta et al., 2020), dysfunctioning of enzymes, proteins denaturation and DNA damages (Jmii et al., 2020; Li et al., 2020; Nijabat et al., 2020).

Quantity of allelochemicals in aqueous extract of Terminalia chebula and Polygonum bistorta

In presents study emphasized on determination of allelopathic constituents of two weeds (*Polygonum bistorta* and *Terminalia chebula*) their impacts on seed germination and biomass accumulation of two target species: *D. carota* and *M. polymorpha.* More allelopathic compounds including soluble phenolics, gallic acid, caffiec acid, vanillic acid, benzoic acid, syringic acid, P-Coumeric acid, M-Coumeric ferulic acid, cinamic acid, sinapic acid, and querctin were found in aqueous extract of *T. chebula.* While less allelochemicals were found in aqueous extract of *P. bistorta.* Quantity of these allelochemicals were also higher in aqueous extract of *T. chebula* than *P. bistorta* (Table 1). Weeds are among one of the most drastic biotic factors that hinders the growth and biomass accumulation of other plants due to their allelopathic exudations (Ma et al., 2020; Maqbool et al, 2022c; Nadeem et al., 2020c). These allelochemicals directly or indirectly act with on target species and negatively affect their life cycle from seed germination till maturity (Hussain et al., 2020).

Variation and correlation among germination and growth response of targeted species

A total of 25.9% variation was explained by the first principal component (PC1) and 31.2% by second principal component (PC2) under the influence of allelopathic aqueous extract of two weeds for germination and biomass accumulation in target species (Figure 9). Root length, shoot length, root fresh weight and germination index showed highly significant and positive correlation among themselves and 10% (T_3) application of an aqueous extract of *P. bistorta* while a strong negative relation with 40% and 80% (T_5 and T_6) aqueous extract of *T. chebula.* Mean germination time and shoot fresh weight were in strong correlation with T_0 (Control), T_1 (2.5% aqueous extract) and T_2 (5% aqueous extract) while the time taken for 50% germination and final germination percent showed closer to T_4 (20% aqueous extract). All traits of both target species showed a strong but negative association with T_5 and T_6 of T. chebula. Significant variation was observed in the response the target species towards the allelopathic nature of both weeds. Data shows that *D. carota* was less sensitive than *M. polymorpha.*

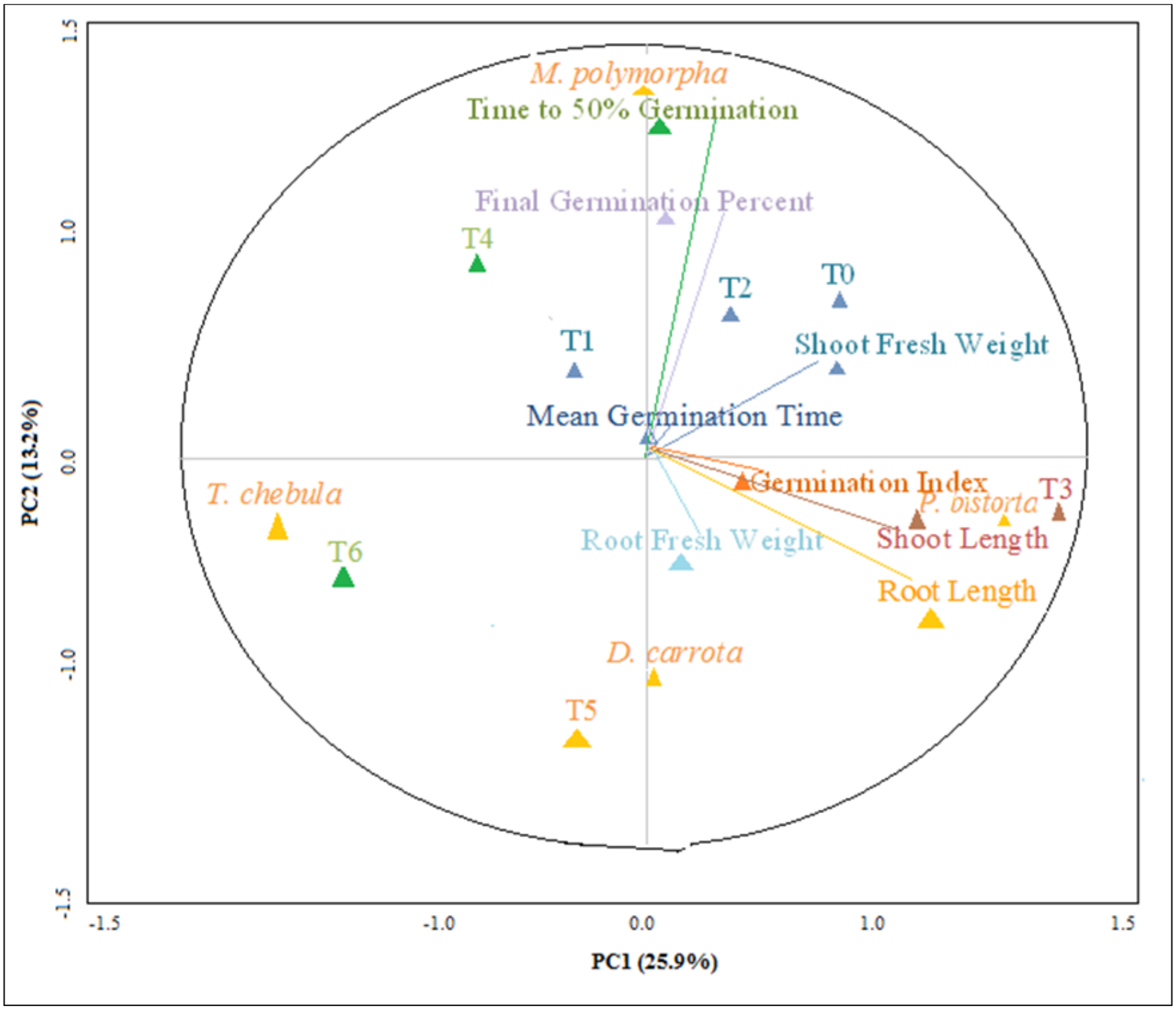

Figure 8. Biplot between PC1 and PC2 displaying contribution of various treatments of aqueous extract of allelopathic weeds on various traits of targeted species.

Conclusion

It was concluded that germination and seedling growth of target weeds (*D. Carota* and *M. polymorpha*) were significantly reduced by the influence of root extract of *P. bistorta* and fruit extract of *T chebula* while *T. chebula* proved to be more toxic and its toxicity increased by the increase of concentration. Therefore, root extract of *P. bistorta* and fruit extract of *T chebula* can be use as ecofriendly management weeds in crops.

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