BIOHERBICIDAL POTENTIAL OF ROOT EXTRACTS OF *TAGETUS MINUTA* AGAINST *PARTHENIUM HYSTEROPHORUS* L.

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ABSTRACT

The present paper highlights the bioherbicidal property of the root extracts of Tagetus minuta against the obnoxious alien weed Parthenium hysterophorus L. Root extracts of Tagetus minuta significantly inhibited seed germination, seedling length, vigour index, biomass and biochemical components of Parthenium weed. The phytotoxicity of the roots of Tagetus minuta against Parthenium shoots and seedlings was directly proportional to the concentration, immersion period and exposure period. It was also observed that phytotoxicity of the roots of Tagetus minuta collected at flowering stage was more in comparison to the roots of vegetative stage of Tagetus minuta and it may be due to the presence of potential allelochemicals in the roots of Tagetus minuta.

Keywords : Allelochemicals, biopesticide, Parthenium hysterophorus and Tagetus minuta.

INTRODUCTION

Weeds are undesirable and non - economic plants that compete with crops for natural resources like water, nutrients and sunlight. *Parthenium hysterophorus* L., an obnoxious weed has been reported as a main source of nuisance and health hazard to mankind and animals as well as threat to biodiversity and danger to environment[1].*Parthenium hysterophorus* L. popularly known as carrot weed, congress weed and feverfew, is native of North - east Mexico, probably introduced in India along with wheat grains under the PL 480 scheme and spread alarmingly like a wild blaze to almost all the states in India and established as a naturalized weed. *Parthenium* is commonly seen lavishly growing in vacant sites, rock crevices, city waste - dumped areas, roadsides, railway tracks and construction sites[2]. This plant belongs to the division : Magnoliophyta, class : Magnoliopsida, order : Asterales and family : Asteraceae [3]. *Parthenium hysterophorus* L. has been originated as a result of natural hybridization between *Parthenium confertum* and *Parthenium bipinnatifidum*[4]. *Parthenium* completes its life - cycle within 3 - 4 months and it shows three to four generations in a year which helps in quick spreading and generation of adverse impacts on the surrounding vegetation[5]. A successful establishment of *Parthenium* in any ecosystem is attributed to several reasons such as high germination ability throughout the year, an enormous seed bank, rapid spread, plasticity in physiological behaviour and extreme adaptability in a wide range of habitats[6,7]. The chemical analysis of *Parthenium hysterophorus* has indicated that all the plant parts including trichomes and pollen contain several secondary metabolites such as alkaloids, parthenin, kaempferol, p - coumaric acid and caffeic acid being high in leaves followed by inflorescence, fruit, root and stem[8]. The sesquiterpene lactones namely parthenin and coronopilin present in the trichomes of leaves and stems of *Parthenium* are responsible for causing various allergies like contact dermatitis, hay fever, asthma and bronchitis in human - beings[9]. *Parthenium hysterophorus* interferes with the growth of other species by releasing allelochemicals like phenolic acids and sesquiterpene lactones which seize the growth phenomenon of the co - existent species[8].

Plants are the natural treasure of the biologically active chemicals (secondary - metabolites) that affect the growth and population - biology of individuals of other species and they impose an environmental stress on other plants growing in their vicinity. *Tagetes minuta* L. (Asteraceae) is an annual aromatic species native to South America, although it has become widespread throughout the world[10].Thiophenes are sulphur containing

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heterocyclic - compounds derived from polyacetylene, are present in different plant parts of *Tagetus minuta* [11]. Higher concentration of thiophene was observed in roots of *Tagetus minuta* in comparison to leaves and shoots[12.] Thiophene acts as toxins against fungi, bacteria, insects and nematodes and toxicity of this compound is related with the generation of reactive oxygen species that adversely affect biological-membranes and other biochemical components of the target pest.

It has been found that the root extracts of *T. minuta* significantly inhibited seed germination, relative germination rate, vigour index, biomass and biochemical components of *Parthenium hysterophorus*. It was also observed that phytotoxicity of the roots of *Tagetus minuta* collected at flowering stage was more in comparison to the roots collected at vegetative stage of *Tagetus minuta.* The rationale behind the selection of *Tagetus minuta* plant as a biopesticide against *Parthenium* weed was due to the reason that overuse of synthetic agrochemicals causes environmental hazards, an imbalance of soil microbes, nutrient deficiency, change of physico - chemical properties of soil resulting in decrease of crop productivity. The indiscriminate use of hazardous pesticides have eroded the ecological sustainability and deleterious effect on human health. Therefore, root extracts of *Tagetus minuta* may be use as natural herbicide for the biological management of *Parthenium hysterophorus* L. It is believed that crude extract of the plant are biologically more active than isolated compounds due to synergistic effects[13].Therefore, the present investigation was carried out to study the bioherbicidal impact of root extracts of *Tagetus minuta* on *Parthenium hysterophorus* L. This study may provide baseline information for the development of future strategies for the production of herbicidal formulation for the management of obnoxious *Parthenium* weed.

MATERIALS AND METHODS

Experimental design: The experiment was conducted during November 2010 - March 2012 in Amity Institute of Biotechnology, Amity University, Noida, India. The systematic survey of the surroundings of Amity University Campus, Noida was made to study the interaction between *Parthenium hysterophorus* and *Tagetus minuta* in nature. The fresh and healthy plants of *Tagetus minuta* L. were collected at vegetative and flowering stage from surrounding areas of Amity University campus, Noida. Fresh roots of *Tagetus minuta*, both vegetative and flowering stages were separated carefully from the intact plants. The roots of *Tagetus minuta* were washed gently with tap water for 10 minutes and surface sterilized with 70% ethyl alcohol for 30 seconds then immersed in 1% NaOCl solution containing two drops of Tween-20 for 15 minutes and finally rinsed thrice with sterilized distilled water and drying with clean absorbent paper. The sterilized roots were cut into small pieces with sterilized knife and air - dried under shade for 15 days. After air drying, roots were homogenized to fine powder in a grinder and stored in airtight bottles for further bioassay tests and analysis of biochemical components.

Preparation of aqueous extracts: For preparation of aqueous root extracts, 10 g of air - dried root powder of *Tagetus minuta* was macerated with 100 ml of sterilized distilled water in a blender for 10 minutes then transferred in conical flask and boiled on slow heat for 2 h. It was then filtered through double layered muslin cloth and centrifuged at 5000 g for 10 minutes. The supernatant was filtered through Whatman No. 1 filter paper. The supernatant was collected and this procedure was repeated twice. After 6 h, the supernatant was collected at an interval of every 2 hours was pooled together and concentrated to make the final volume one - fourth of the original volume. It was then autoclaved at 121^0C temperature and at 15 lbs pressure[14]. The aqueous root extracts of both vegetative and flowering stages were preserved aseptically in amber coloured bottle at 4^0C until further use.

Seed germination bioassay test: Seed germination bioassay test was used to test the inhibitory effect of aqueous root extracts of *Tagetus minuta* on the seeds of *Parthenium hysterophorus* under laboratory conditions. Before seed germination test, empty and undeveloped seeds of *Parthenium hysterophorus* were discarded by floating in tap water. Seeds of *Parthenium hysterophorus* were thoroughly washed with tap water to remove dirt and dust for 5 minutes. To avoid possible

inhibition caused by toxins from fungi or bacteria, seeds were surface sterilized with 10 : 1 distilled water/ bleach (commercial NaOCl) solution for 5 minutes and then washed 6 - 7 times with distilled water. *Parthenium* seeds were soaked in different concentrations of aqueous root extracts of *T. minuta* for 4 hours. Two pieces of filter paper were placed in sterilized petri - dishes (20 cm diameter) and *Parthenium* seeds which were soaked in aqueous root extracts transferred into petridishes. The petridishes were covered and placed in sterilized polythene bags to prevent further loss of volatiles and kept in a Seed Germinator for 10 days under 70% relative humidity at 25 ± 2^{0}C with a 12 h photoperiod following a guidelines of ISTA[15] to test the seed germination under different concentrations of aqueous root extracts in three replicates with completely randomized block design.

Germination percentage: Total number of seeds germinated / Total number of seeds taken for germination x 100

Determination of growth parameters: Different growth characteristics such as radicle and plumule length, relative germination rate, vigour index and biomass were determined in control and treatment by the following methods :

Seedling length: After 10 days of seed sowing, radicle and plumule length of the seedlings were measured as per standard methods of ISTA[15]. Seeds were considered to be germinated with the emergence of both plumule and radicle. The radicle and plumule length were measured with a measuring scale and values were expressed in centimeters.

Vigour index: Vigour index of the seedlings was estimated according to the formula: Vigour index = Total seedling length (mm) x germination percentage[16].

Biomass estimation: Fresh weight of the seedlings of control and treatment was measured after 10 days of sowing. After that, the seedlings were oven dried at 65^{0}C for 72 hours and dry weight was also estimated. The moisture content of the tissue was calculated by the formula : (FW - DW/FW x 100).

Shoot cut bioassay: *Parthenium hysterophorus* shoots (30 days old) with one inflorescence and 15 cm in length were taken for the shoot cut bioassay test. *Parthenium* shoots were cut and washed in tap water and dipped in 1% NaOCl solution for 3 minutes. The tips of the shoots were immediately washed in sterilized distilled water to remove any residual trace of the chemical. An inclined cut was made at the tip of the shoots and the shoots were placed in test tubes containing 10 ml of 50 and 100% concentrations of aqueous root extracts of vegetative and flowering stages of *Tagetus minuta*. In control, *Parthenium hysterophorus* shoots were dipped in 10 ml of distilled water. The tubes were sealed with cotton buds and aluminium foil to make it airtight. The effect of root extracts of *Tagetus minuta* on *Parthenium* shoots was observed at regular interval of 24, 48 and 72 hours at room temperature. In shoot cut bioassay, phytotoxic damage was recorded on the basis of a rating scale of 0 - 5; where

0 = No effect

1 = Slight chlorosis / wilting of leaves

2 = Marked chlorosis and slight necrosis

3 = Acute chlorosis and marked necrosis/drooping of entire twigs

4 = Falling of petals and leaves/high necrosis and chlorosis

5 = Acute chlorosis and very high necrosis leading to death of the whole shoot.

Seedling bioassay: *Parthenium hysterophorus* seedlings were raised in plastic pots (depth 7 cm and diameter 7 cm) containing 150 gms of sterilized soil, sand and peat (1 : 1 : 1) and placed in growth chamber at 26 ± 1°C. *Parthenium* seedlings were sprayed with 5 ml of 50 and 100% concentrations of aqueous root extracts of vegetative and flowering stages of *Tagetus minuta* and irrigation was carried out for 3 days with root extracts in treatment and with distilled water in control respectively. Observations regarding the phytotoxic symptoms on the *Parthenium* seedlings were made at regular interval of 24, 48 and 72 hours at rating scale of 0 - 5; where

0 = No effect

1 = Slight chlorosis / wilting of leaves

2 = Marked chlorosis and slight necrosis

3 = Acute chlorosis and marked necrosis/drooping of entire seedling.

4 = Falling of petals and leaves/high necrosis and chlorosis

5 = Acute chlorosis and very high necrosis leading to death of the whole seedling.

Quantitative estimation of chlorophyll: The amount of chlorophyll was determined by the following method[17]**.** 10 mg of fresh *Parthenium* leaves of the shoot cut bioassay treated with 50 and 100% concentrations of aqueous root extracts of vegetative and flowering stages of *Tagetus minuta* were grounded with neutral sand and 10 ml of 80% acetone and centrifuged at 3000 rpm for 10 minutes. The volume of supernatant was recorded. Optical density was measured at 645 nm and 663 nm. For the determination of chlorophyll a, chlorophyll b and total chlorophyll following formulae were employed :

Total chlorophyll (mg/g) =

$$\frac{20.2\times OD_{645}+8.02\times OD_{663}}{1000\times W}\times V$$

Chlorophyll a (mg/g) =

$$\frac{12.7\times OD_{663}-2.69\times OD_{645}}{1000\times W}\times V$$

Chlorophyll b (mg/g) =

$$\frac{22.9\times OD_{645}-4.68\times OD_{663}}{1000\times W}\times V$$

Where, V = Volume of the supernatant in ml, W = Fresh weight of the *Parthenium* leaves in gm and OD = Optical density.

Quantitative estimation of protein: Quantitative estimation of protein was done by the following method[18]. Stock solution of the following reagents was prepared :

Reagents:

A. Alkaline sodium carbonate solution (0.2% Na_2CO_3 in 0.1 N NaOH).

B. Copper sulphate - sodium potassium tartarate solution (0.5% $CuSO_4$. $5H_2O$

1% sodium potassium tartarate).

C. Alkaline copper reagent: Mixed 50 ml of reagent A and 1 ml of reagent B.

D. Folin - Ciocalteu reagent (Diluted Folin - Ciocalteu reagent with equal volume of distilled water just before use).

E. 1 N NaOH.

Ten (10) mg fresh *Parthenium* leaves of the shoot cut bioassay of 50 and 100% concentrations of vegetative and flowering stages of aqueous root extracts were homogenized with 1ml of 1N NaOH and kept for 5 minutes at 100°C into the boiling water bath. Added 5 ml of alkaline copper reagent to it and allowed the mixture to stand at room temperature for 10 minutes. Added 0.5 ml of Folin - Ciocalteu reagent immediately and mixed the contents properly in the test tube. The absorbance of the solution was measured at 650 nm after 30 minutes. The amount of protein was calculated with reference to standard curve of lysozyme.

Statistical analysis: The treatment in all the experiments were laid out in a complete randomized block design with a three replicates and Duncans Multiple Range Test was employed to test the effect of treatment over the control[19].

RESULTS AND DISCUSSION

The aqueous root extracts of *Tagetus minuta* both at vegetative and flowering stages significantly affected the growth and physiological parameters of *Parthenium hysterophorus* L.

Seed germination bioassay: The treatment of *Parthenium* seeds with aqueous root exracts of vegetative and flowering stages of *Tagetus minuta* of 100% concentration exhibited marked variation on seed germination over control. Root extracts of flowering stage were more inhibitory than root extracts of vegetative stage of *Tagetus minuta*. Reduction in seed germination was more with roots extracts of flowering stage in comparison to vegetative stage. 34.41% reduction in seed germination was observed with 50% concentration of root extracts of *Tagetus minuta* at vegetative stage, which increased to 70.97 % in 100% concentration. The 100% concentration of root extracts of *Tagetus minuta* at flowering stage exhibited maximum 84.95% reduction and 50% concentration exhibited 59.14% reduction in

Parthenium seed germination as compared to control. Relative germination rate was also reduced with 100% concentration in comparison to 50% concentration of aqueous root extracts of *Tagetus minuta.*

Table 1. Effect of root extracts of *Tagetus minuta* on the seed germination of *Parthenium hysterophorus* L.

Concentration of root extract	Germination percentage (%)			
	Aqueous root extract of *Tagetus minuta* at vegetative stage	Relative germination rate	Aqueous root extract of *Tagetus minuta* at flowering stage	Relative germination rate
Control	93 ± 0.86	-	93 ± 0.65	-
50%	61± 0.57 (34.41)	0.66	38 ± 0.21 (59.14)	0.41
100%	27± 0.32 (70.97)	0.29	14 ± 0.22 (84.95)	0.15

Values are mean of three replicates ± sem

Figures in parentheses indicate percent inhibition over control.

Table 2. Effect of root extracts of *Tagetus minuta* on the seedling length of *Parthenium hysterophorus* L.

Concentration of root extract	Seedling length (cms)			
	Aqueous root extract of *Tagetus minuta* at vegetative stage		Aqueous root extract of *Tagetus minuta* at flowering stage	
	Radicle	Plumule	Radicle	Plumule
Control	2.8 ± 0.09	3.9 ± 0.09	2.9 ± 0.05	4.3 ± 0.09
50%	2.4 ± 0.06	3.5 ± 0.06	2.1 ± 0.03	2.5 ± 0.06
100%	1.5 ± 0.01	2.7 ± 0.07	0.9 ± 0.02	1.2 ± 0.04

Where R = radicle length and P = plumule length

Values are mean of three replicates ± sem

Figures in parentheses indicate percent inhibition over control.

Table 3. Effect of root extracts of *Tagetus minuta* on the vigour index of *Parthenium hysterophorus* L.

Concentration of root extract	Vigour index	
	Aqueous root extract of *Tagetus minuta* at vegetative stage	Aqueous root extract of *Tagetus minuta* at flowering stage
Control	6231	6696
50%	3599 (42.24)	1748 (73.89)
100%	1134 (81.80)	294 (95.61)

Values are mean of three replicates ± sem

Figures in parentheses indicate percent inhibition over control.

Table 4. Effect of root extracts of *Tagetus minuta* on the biomass of *Parthenium hysterophorus* L.

Concentration of root extract	Biomass (gm)					
	Aqueous root extract of *Tagetus minuta* at vegetative stage			Aqueous root extract of *Tagetus minuta* at flowering stage		
	Fresh weight (gm)	Dry weight (gm)	Moisture content (%)	Fresh weight (gm)	Dry weight (gm)	Moisture content (%)
Control	1.214 ± 0.09	0.9410±0.05	22.49	1.350±0.02	1.064±0.01	21.19
50%	0.9821±0.08	0.5613±0.04	42.85	0.8952±0.05	0.4816±0.02	46.20
100%	0.7639±0.06	0.5482±0.02	28.24	0.6548±0.05	0.4319±0.03	34.04

Values are mean of three replicates ± sem

Figures in parentheses indicate percent inhibition over control.

Table 5. Effect of root extracts of *Tagetus minuta* on the shoots of *Parthenium hysterophorus* L. in shoot cut bioassay.

Exposure Time (h)	Shoot cut bioassay					
	Aqueous root extract of *Tagetus minuta* at vegetative stage			Aqueous root extract of *Tagetus minuta* at flowering stage		
	C	50%	100%	C	50%	100%
24 h	0.00±0.00	0.66±0.54	2.33±0.27	0.00±0.00	3.33±0.27	3.90±0.05
48 h	0.00±0.00	1.00±0.47	3.00±0.47	0.00±0.00	3.66±0.72	4.50±0.24
72 h	0.00±0.00	1.50±0.24	4.00±0.47	0.00±0.00	3.90±0.05	5.00±0.09

Values are mean of three replicates ± sem

Phytotoxicity rating scale : 0 = no effect, 1 = slight chlorosis/wilting of leaves, 2 = marked chlorosis and slight necrosis, 3= acute chlorosis and marked necrosis/drooping of entire shoot, 4 = falling of petals and leaves/high necrosis and chlorosis, 5 = acute chlorosis and very high necrosis leading to the death of *Parthenium* shoots.

Table 6. Effect of root extracts of *Tagetus minuta* on the seedlings of *Parthenium hysterophorus* L. in seedling bioassay.

Exposure time (h)	Seedling bioassay					
	Aqueous root extract of *Tagetus minuta* at vegetative stage			Aqueous root extract of *Tagetus minuta* at flowering stage		
	C	50%	100%	C	50%	100%
24 h	0.00±0.00	0.00±0.00	0.66±0.54	0.00±0.00	2.50±0.28	3.33±0.27
48 h	0.00±0.00	0.66±0.54	1.50±0.24	0.00±0.00	2.80±0.09	3.66±0.72
72 h	0.00±0.00	1.00±0.47	3.66±0.72	0.00±0.00	3.33±0.27	4.00±0.47

Values are mean of three replicates ± sem

Phytotoxicity rating scale : 0 = no effect, 1 = slight chlorosis/wilting of leaves, 2 = marked chlorosis and slight necrosis, 3= acute chlorosis and marked necrosis/drooping of entire seedling, 4 = falling of petals and leaves/high necrosis and chlorosis, 5 = acute chlorosis and very high necrosis leading to the death of *Parthenium* seedling.

Table 7. Effect of root extracts of *Tagetus minuta* on the chlorophyll content of *Parthenium hysterophorus* L.

Concentration of root extract	Chlorophyll content (mg/g)	
	Aqueous root extract of *Tagetus minuta* at vegetative stage	Aqueous root extract of *Tagetus minuta* at flowering stage
Control	2.51± 0.19	2.51± 0.19
50%	1.99 ± 0.08 (20.72)	1.63 ± 0.05 (35.06)
100%	1.54 ± 0.06 (38.65)	0.97 ± 0.01 (61.35)

Values are mean of three replicates ± sem

Figures in parentheses indicate percent inhibition over control.

Table 8. Effect of root extracts of *Tagetus minuta* on the protein content of *Parthenium hysterophorus* L.

Concentration of root extract	Protein content (µg/ml)	
	Aqueous root extract of *Tagetus minuta* at vegetative stage	Aqueous root extract of *Tagetus minuta* at flowering stage
Control	96 ± 0.78	96 ± 0.85
50%	75 ± 0.53 (21.88)	42 ± 0.38 (56.25)
100%	48 ± 0.29 (50)	29 ± 0.21 (69.79)

Values are mean of three replicates ± sem

Figures in parentheses indicate percent inhibition over control.

Growth parameters: Radicle and plumule length of *Parthenium hysterophorus* seedlings were more in control and these were significantly reduced in different treatments. Similarly fresh and dry weight of *Parthenium* seedlings was higher in control in comparison to treatment. In 100% concentration, 81.80 and 95.61% reduction in vigour index was observed with 100% concentration of vegetative and flowering stages of aqueous root extracts of *Tagetus minuta*.

Shoot cut bioassay: The root extracts of vegetative and flowering stages of *Tagetus minuta* of 50 and 100% concentrations showed marked and varying degree of toxicity on *Parthenium* shoots. Toxicity increased with increase in exposure time and resulted into wilting, chlorosis, necrosis and blackening of *Parthenium* shoots. The root extracts of *Tagetus minuta* at flowering stage exhibited maximum toxicity i.e. acute chlorosis, necrosis and leading to the death of whole *Parthenium* shoot after 72 h exposure period. The inhibitory effect of root extracts of *Tagetus minuta* was in order of flowering stage > vegetative stage. It is evident from the data that the phytotoxicity was directly proportional to the concentration, immersion period and exposure period of *Parthenium hysterophorus* shoots to the aqueous root extracts of *Tagetus minuta*[20]. No visible symptoms were observed in control after 72 h exposure period.

Seedling bioassay: In seedling bioassay, the toxic effects of aqueous root extracts of *Tagetus minuta* on *Parthenium hysterophorus* seedlings were less than shoot cut bioassay. The spraying of root extracts of vegetative and flowering stages of *Tagetus minuta* on 15 days old *Parthenium* seedlings produced visible toxicity symptoms i.e. chlorosis, wilting and browning of the tips of the *Parthenium* leaves. The phytotoxicity was rapid and severe after 48 h with spraying of aqueous root extracts of *Tagetus minuta* collected at flowering stage. 100% concentration of root extracts of flowering stage of *Tagetus minuta* was more toxic to the *Parthenium* seedlings in comparison to 50% concentration of aqueous root extracts and adversely affected *Parthenium* leaves which could not recover from the initial effects and dried rapidly.

Chlorophyll content: In the shoot cut bioassay, aqueous root extracts of *Tagetus minuta* markedly reduced the chlorophyll contents in the *Parthenium* leaves. Reduction in total chlorophyll content was observed more with aqueous root extracts of *Tagetus minuta* at flowering stage in comparison to vegetative stage. 20.72% reduction was observed in 50% concentration of root extracts of *Tagetus minuta* at vegetative stage, which increased to 38.65% in 100% concentration. The 100% concentration of root extracts of *Tagetus minuta* collected at flowering stage exhibited maximum 61.35% and 50% concentration exhibited 35.06% reduction in total chlorophyll content as compared to control.

Protein content: In the shoot cut bioassay, 50 and 100% concentrations of aqueous root extracts of *Tagetus minuta* drastically reduced the protein contents in the *Parthenium* leaves. However, aqueous root extracts of *Tagetus minuta* at flowering stage were more inhibitory than vegetative stage. At vegetative stage, root extracts of *Tagetus minuta* showed 21.88% reduction in 50% concentration which increased to 50% in 100% concentration. At flowering stage, 100% concentration of root extracts of *Tagetus minuta* exhibited maximum 69.79% and 50% concentration of root extracts exhibited 56.25% reduction in protein content in *Parthenium hysterophorus* leaves as compared to control.

The bioactive compounds present in root - residues have been found to affect the various metabolic processes of *Parthenium* weed. Germination is the resumption of metabolic activity and growth by the seed tissues which starts with the imbibition of water and ends with the protrusion of embryonic roots. Inhibition of growth parameters might be due to inhibition in synthesis of gibberellin, auxin and other growth hormones under the influence of bioactive compounds present in the roots of *Tagetus minuta*. The growth inhibition caused by allelochemicals present in root residues of *Tagetus minuta* could be due to interference with different plant growth processes like cell division and cell enlargement[21], inhibition in nutrient uptake[22], reduction in dry matter production due to inhibition of metabolic processes such as photosynthesis[23] and respiration[24]. Allelochemicals are known to

impede the absorption of water[25] and ions[26] from the soil which may cause the loss of turgidity of cell and affect the metabolic activity of cells. Reduction in seedling length and dry weight of *Parthenium* weed might be due to inhibition of CO_2 - fixing efficiency or delaying of germination coupled with low efficiency in dry matter production[27]. The phenolic acids reduce the expansion of the leaves and reduces photosynthesis[28]. Allelochemicals present in the roots of *Tagetus minuta* reduced the chlorophyll content in the leaves of *Parthenium hysterophorus* and altered the photosynthetic rate and other metabolic processes[23, 29]. The reduction in the amount of chlorophyll might be due to inhibition of synthesis of enzymes, proteins and cofactors required for synthesis of chlorophyll[30] or excessive breakdown of chlorophyll under the influence of bioactive compounds present in roots of *Tagetus minuta***.** Carbohydrates are the cellular source of energy and starting materials for the synthesis of protein, lipid and other plant products. Inhibition of photosynthesis leading to decreased amount of photosynthates might be due to decreased biosynthesis of chlorophyll or degradation of photosynthetic pigments[31, 32, 33] or inhibition of photosynthesis by allelochemicals resulting in decreased dry matter production i.e. photosynthates[25, 34]. The bioactive compounds or allelochemicals inhibit the rate of photosynthesis due to interference with water balance and chlorophyll content. Allelochemicals inhibit electron transport in mitochondria and impaired enzyme activity as a primary target of allelopathic activity which may result in reduced ability to metabolize reserve materials[35]. Proteins play a pivotal role in biological processes and it regulates growth, development and reproduction of plant[36]. The inhibition of protein synthesis might have resulted in inhibition of biosynthesis of chlorophyll molecules, photosystem I, II, ATPase and other enzymes required for photosynthesis. The protein may serve as respiratory substrate if the supply of carbohydrates are inadequate because of decreased photosynthetic rate[37]. Reduced rate of photosynthesis caused decreased synthesis of carbohydrates, the precursors of amino acids, protein and this might have resulted in reduction in overall growth.

CONCLUSION

In conclusion, from the present study it is evident that the change in physiological processes inhibited and delayed the seed germination and growth of *Parthenium hysterophorus* under the influence of bioactive compounds present in root residues of *Tagetus minuta*. It was also observed that aqueous root extracts of *Tagetus minuta* at flowering stage were more inhibitory in comparison to vegetative stage and it may be due to the presence of potential allelochemicals in the roots of *Tagetus minuta*. The inhibition of growth parameters may decrease the number of seeds per plant and this may restrict the spread of obnoxious *Parthenium* weed. Sowing of seeds of *Tagetus minuta* in the waste land may help in controlling the spread and growth of *Parthenium* weed. Therefore, root residues of *Tagetus minuta* may be used as potential bioherbicide to control *Parthenium hysterophorus.*

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