Phytotoxic Effects of Leaf Leachates of Invasive Weeds *Cosmos sulphureus* and *Xanthium strumarium* on Agricultural Crops

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Many invasive weeds are known to create their deleterious effects on biological ecosystems and also rhizosphere soils. Weeds such as *Cosmos sulphureus*Cav. and *Xanthium strumarium*L. have their existence near agricultural cropfields. Such weeds grow in abundance, releasing specific allelochemicals which have adverse effect on germination rate, physiological patterns and reproduction of crop plants. In present work, allelopathic effects of leaf leachates of *Cosmos* and *Xanthium* were observed on seed germination and seedling growth of *Triticumaestivum*, *Vignaradiata* and *Trigonellafoenum-graceum*like crops. Seed germination was inhibited at higher concentration at 6% while lower concentrations showed stimulatory effect on *Vignaradiata* and *Trigonellafoenum-graceum*from 1%-4% concentrations. But seed germination percentage of *Vigna* and *Trigonella*showed 70% and 60% growth in response to leaf leachates of *Cosmos* at 6% concentration. *Triticum*showed total inhibition of 40% to both leaf leachates. The qualitative phytochemical analyses showed presence of alkaloids,phytosterols, phenols, tannins and flavonoids. GCMS and IR studies revealed presence of major constituents such as esters, ethers, anhydride and polyalcohols. *Cosmos* and *Xanthium* showed the characteristic FTIR fingerprinting regions of various functional groups such as –OH, carbonyl, anhydride, ester and amides. The variations in phytochemicals of these invasive might be attributed to response of the plants to different environmental stresses.The rate of growth of these invasive weeds and their over-dominance is very difficult to control but their huge biomass can be used in sustainable development.

Keywords: Allelopathy,Asteraceae, GC-MS, IR Spectroscopy,Phytochemicals.

Allelopathy majorly is described as any direct or indirect effect of one plant on another plants, animals or microorganismsthrough the production of chemical compounds that escape into the environment and influence the growth and development(Bezuidenhout; 2012, Sangeetha and Bhaskar; 2015, Riceand Pancholy; 1974). This phenomenon encompasses many aspects of physiology, biochemistry, observations and interactions occurring in nature. The effect of any alien or exotic species can cause loss of ecosystem balance, affecting its functioning incurring the loss of biodiversity through release of allelochemicals (Inderjit, 2005). Such plants can have stimulatory

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or inhibitory effectson crops. The secondary metabolites from such plants that are considered as allelochemicals do not have much physiological function in metabolism of plants. Theyserve only as agents of plant-plant competition during establishment in new ecosystem along with plant-microbe symbioses.

Exotic invasive weeds are non-natives due to their occurrence in different geographical regions. These invasive plants species have several motives for their successful establishment such as faster rate of reproduction, dispersal mechanisms, potency to establish large populations in short period of time with adaptive nature resulting in resource depletion. Such exotic plants show faster rate of establishment without any human assistance. Many recent investigations have shown that invasive plants have changed several community attributes like species diversity, richness, species composition and abundance (Keblawy*et al.* 2006). Dominance, higher growth rate throughout the invaded area, high tolerance towards abiotic environmental factors and also the allelopathic potential are probable favourable factors for their sustainability.The researches have indicated that some plant invaders become much more dominant in their invaded range than their native range. Frankel (1999) showed that alien plants displaced local flora, changed ecosystems and disturbed the biodiversity. The dominant plants by forming pure stands/ monothickets through interactions exhibit allelopathy and such plants generally are free of predators, parasites and diseases. They have influence on the growth and development of agricultural and biological ecosystem (Sangeetha and Bhaskar, 2015) through release of various allelochemicals or ecochemicals.

Allelochemicals are produced in all plant parts in different concentrations and released in the environment through leaching, volatilization, root exudation, decomposition etc.Their concentration depends on maturity stage of plant as younger plants are more toxic. Soil and soil factors as biotic and abiotic components are important in terms of determining the quality, quantity and availability of allelochemicals in the vicinity of neighbouring species (Inderjit, 2005). Concentration of allelochemicals is density-dependent factor (Inderjit 2005) present in soil, which shows changes in the soil characteristics, but to detect the exact concentrations of allelochemicals in field is difficult. It has been reported that even low concentrations of chemicals can cause significant effect on growth and germination of other plants. Therefore isolation and identification of chemicals is most significant part in demonstrating allelopathy (Inderjit and Dakshini,1994, Inderjit*et al.* 2005). According to (Rice, 1984) and Putnam and Tang (1986) the most widely used standard bioassay test is the influence of allelochemicals on germination parameters such as seed germination, seedling growth, root length and shoot length etc. under controlled laboratory conditions, which is undertaken in the present investigation as well. So also to establish phenomenon of allelobiogenesis, the assessment of allelochemicals by qualitative phytochemical analysis, GC-MS and IR methods was taken on.

Khadakwasla, Mulshi and Paud-Pirangutareas (18^{o}30’42.30" N, 73^{o}40’49.28" E) are agricultural fields on Lavasa road. According to field observations *Cosmos* has enormously invaded from BhugaonuptoMulshi(about 30kms) covering the whole area along road side edges and rice crops. *Xanthium*also shows widespread expansion and has been observed near agricultural crop fields (Patil 2009). These areas are with moderate rainfall and have sandy and loamy soil which is favourable for growth of these two invasive speciesandcompete with the kharif crops (Patil 2009).*Cosmos* and *Xanthium* both are considered toxic and non-palatable alien weeds unsuitable as fodder with no certain methods of eradications. Katrajghat ranges (18^{0}27’27.12"N, 73^{0}52’3.89"E) was another area selected for the study and found to be invaded greatly by the same two weeds *Cosmos* and *Xanthium.*

Cosmos sulphureus Cav. (Asteraceae) a native of Mexico, was accidentally introduced to Indian sub-continent. This annual, herbaceous, invasive weed, with non-fragrant flowers, is found gregariously growing on fields and hedges of crops, wastelands and marshy places. It can grow upto height of 8-10 feet, that can create shadow for short heighted crops inhibiting their light harvesting mechanism.It is a moderate reseeder, growing onsandy and loamy soils favor its profuse seed germination.

Xanthium strumarium L. (Asteraceae),a perennial shrub, native of North America and

Eurasia. It germinates by reseeding itself. The plant seeds can sustain very harsh environmental conditions, by remaining dormant for about 2-10 years. Young seedlings of *Xanthium* release toxic chemicals that can inhibit germination and prove fatal to neighbouring plants. Specific environmental conditions such as optimum oxygen, high moisture and direct sunlight with moist, loamy or sandy soil are necessary requirements for its successful growth. Seeds and seedlings are highly toxic to mammalian herbivores.

Mungbean (*Vignaradiata* L) and Fenugreek/ Methi (*Trigonellafoenum-graceum*L.)— (Both Members of Fabaceae), cultivated crops throughout India and also Wheat (*Triticumaestivum* L)—Wheat (Poaceae), a major cultivated staple food crop were used for standard seed germination bioassay studies.

MATERIALS AND METHODS

Collection of plant materials

Fresh leaves of *Cosmos* and *Xanthium* at maturity stage were collected during monsoon period (July-October2016, 2017) along agricultural fields from selected ruralareas like Khadakwasla, Mulshi and Paud-Pirangutareas, Lavasa area and Katrajghat area.

Preparation of leaf leachates

Fresh leaves were air shade dried, ground in a mixer to form a homogeneous powder and stored in air tight bottles. 10 g. of dried powders of *Cosmos* and *Xanthium* were mixed with 100 ml water in 250 ml beakers separately and kept in dark for 24 hours at room temperature. They were filtered through muslin cloth (size 2mm) to obtain aqueous leachates (10%). From this stock solution different concentrations were prepared (1% - 6% v/v) by making dilutions with distilled waterand were usedfor seed germination bioassays.

The seeds of three crop plants *Triticum, Trigonella, Vigna*were procured from authentic source. Healthy seeds of wheat, fenugreek (methi) and mungbean were surface sterilized with 0.02% aqueous $Hgcl_2$ (Mercuric chloride) for two minutes. Then the seeds were thoroughly washed with distilled water. Petri-plates(9 cm dia.) were sterilized with 70% alcohol and lined with germination paper moistened with different concentrations of leaf leachates of *Cosmos* and *Xanthium*. The seeds were placed in these petri-plates for germination. Experiments were carried out twicein laboratory conditions in triplicates with control of distilled water. Petri-plates were monitored daily and germination papers were moistened after 2-3 days with solutions of respective concentrations. After 8 days germination parameters such as number of seeds germinated (germination %), root length, shoot length, root **:** shoot ratio, vigour index were studied (Gupta *et al*; 1996).

Solventextraction method for phytochemicals analyses

Powdered dried leaf materials of *Cosmos* and *Xanthium* weighing about 5mg was mixed with Ethanol solvent and allowed to homogenize for 3-4 hrs approximately. The mixtures were filtered through muslin cloth and filtrate was further tested for phytochemical analysis.

Detection of allelochemicals by GC-MS methods

Working procedure for test sample was as follows - 10 ppm solution was prepared in ethanol for *Cosmos* and *Xanthium* dry leaf powders separately. The solutions were filtered through 0.2µ nylon filter and filtrate sample was injected. GC-MS method- Make model: Agilent Technologies 7000 GC/MS Triple Quad The 7000 Series Triple Quad GC/MS is a standalone capillary GC detector for use with the Agilent 7890A Series gas chromatograph. Inbuilt Software installed for qualitative analysis of MS is Mass Hunter.

Detection of allelochemicals by IR method

Jasco FTIR (V-5300) model: Dry leaf powders of *Cosmos* and *Xanthium* were homogenised in mortar – pestle in a palette along with Potassium bromide powder (KBr) and about 1% of mixture was filled in cuvette. Control or Blank was run with Pottassium bromide (KBr) only followed with samples respectively. Wavelength from 400-4000 nm is generally an installed range for the detection of compound. Graph is overlay with API (Active Pharmacopeia Ingredients) standards to identify the peaks.

Statistical analysis

Calculations of germination parameters were carried out as average of three determinants along with standard deviation. Analysis of data results were subjected to correlation to find out overall effect of increasing concentrations on germination % and graphs were performed in SPPS software.

RESULTS AND DISCUSSION

Cosmos and *Xanthium* are invasive weeds, mainly found in the areas which are altered due to great human disturbances such as changes in land-use activities like agriculture, construction of road etc. Previous studies have revealed that these invasive weeds have deleterious effects on natural habitats like changes in soil composition due to release of allelochemicals which makes it unsuitable for growth of indigenous plants / crops. Different cropplants show different levels of tolerance towards allelochemicals which may be either beneficial or deleterious. Therefore to evaluate the effects of allelochemicals on germination and growth of cropseeds, standard laboratory germination bioassay method was

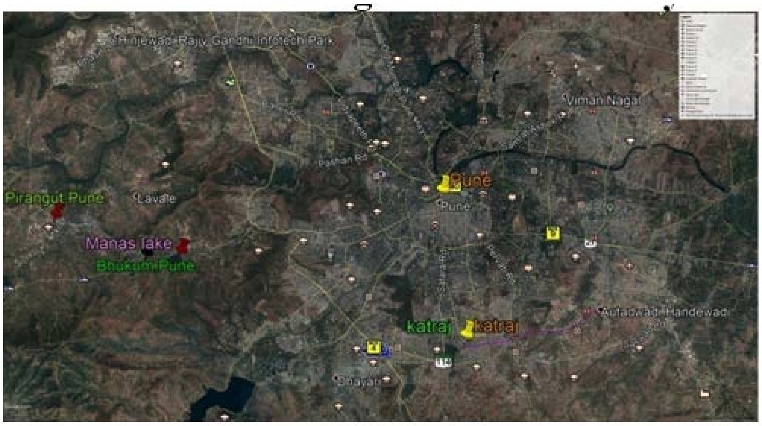

Plate 1. Satellite image of selected study sites

Plate 2. Seed germination bioassay by petriplate method

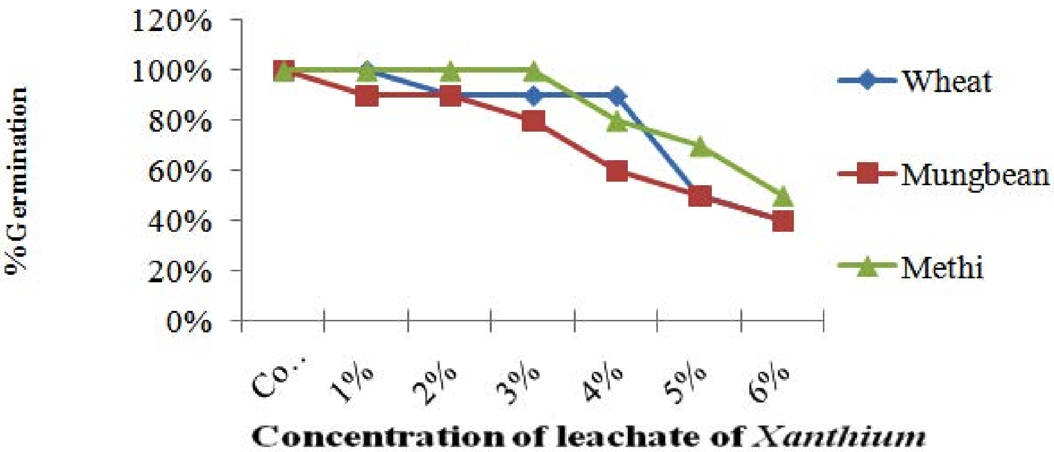

Fig. 1. Effect of leaf leachates of *Xanthium* on seed germination of Wheat, Mungbean and Fenugreek (Methi)

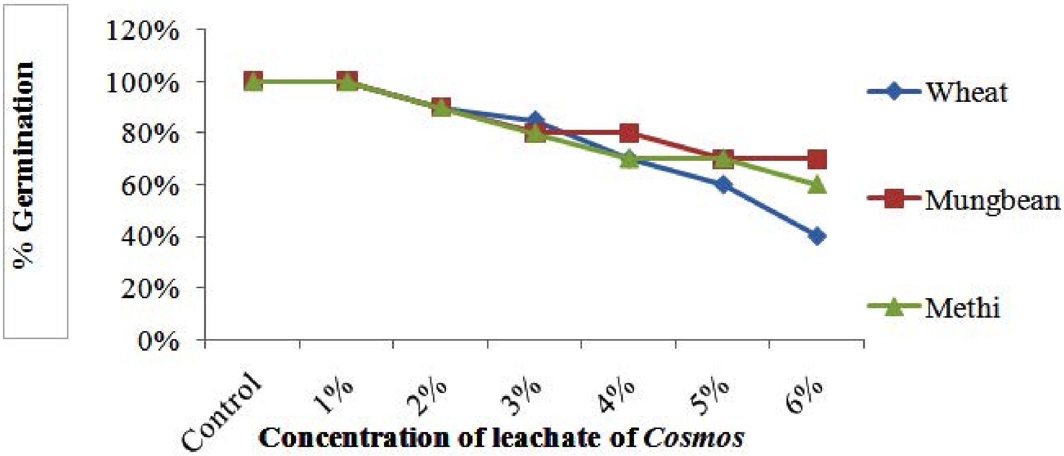

Fig. 2. Effect of leaf leachates of *Cosmos* on seed germination of Wheat, Mungbean and Fenugreek(Methi)

considered as most important part of this study (Friedman 1995; Gogoi*et al.* 2002)

Seed germination and seedling growth are two focal parameters used in allelopathic bioassays (Rice, 1984), since the plant is considered to be most susceptible in its growth stage. The latter shows effective response to presence of allelochemicals as elongation of root. It is natural deciding factor that shows positive or negative effect on rate of germination.

Seed germination studies

Germination percentage

Leaf leachates of *Cosmos* and *Xanthium* inhibited the seed germination with increased concentrations in all selected crops with varying degree of response. For *Xanthium*5% and 6% concentrations showed significant changes in seed germination for all 3 crops, Wheat, Mungbean and Fenugreek whereas at lower concentrations (1%) seed germination was at par with control for*Xanthium*. *Cosmos* in comparison with *Xanthium* showed inhibition at 5% to Wheat whereas for Mungbean and Fenugreek LC_{50} was observed at 6% concentration. The graphs (Figures 1 and 2) showed a gradual decrease in germination for *Cosmos* as compared to *Xanthium*. Changing response of different plants may be due to concentration of inhibitory chemicals and mechanisms of inhibitory effects. The wheat seeds were found to be more receptive with respect to inhibition of germination than mungbean and Fenugreek cropseeds.

Effects of *Cosmos* and *Xanthium* leaf leachates on seed germination of *Triticumaestivum*(Wheat)

Triticum showed minimum i.e. (40%) germination at 6% concentration for both *Xanthium* and *Cosmos*. At 1% concentration effect was at par with control. The inhibitory effects were observed in all germination parameters with increasing concentrations of leachates. The effect of leachates on root length was similar to shoot length for both *Cosmos* and *Xanthium* with minimal differences. Shoot length was inhibited (9.2 ± 0.60) over control (12.2 ± 0.92) to *Cosmos* leachate at 1%concentration whereas effect of Xanthium leachates at 1% on germination was similar with that of control and it reduced to 90% of germination at 2% to 4% of leachates concentrations. Root: shoot ratio remained consistent for concentration upto 6% for *Cosmos* but it showed increasing trend with *Xanthium*. Vigor index showed drastic reduction with increasing concentrations as shown in both the Tables1 and 2.

Effects of *Cosmos* and *Xanthium* leaf leachates on seed germination of *Vignaradiata*(Mungbean)

Germination was observed to be inhibitory at 6% for *Xanthium* (40%) but it remained upto (70%) for 6% concentration for *Cosmos*. Leaf leachates of *Cosmos* probably had stimulatory effect on germination percentage, root and shoot length at all concentrations as concentrations increased (Table 3). Shoot length was increased at 1% concentration (11.03cm) than that of control

(9.6 cm) in response to leachate of *Cosmos*. Though *Xanthium* showed ~~similar~~little increase in shoot length at 1% (8.07cm) as compared with control (8.17cm), but as concentration increases there was reduction in shoot length. Root: shoot ratio showed a constant trend of increase from control to 6% concentration for *Xanthium* but a reduced trend in case of *Cosmos*. Root: Shoot ratio is a highly dependent factor on length of root and shoot of plant and also dependent on concentrations of leachates. Vigour index showed gradual decrease over control as affected by leachates of *Xanthium* and *Cosmos*.

Effects of *Cosmos* and *Xanthium* leaf leachates on seed germination of *Trigonellafoenum-graceum*(Methi)

Both the weeds*Cosmos* and *Xanthium* showed inhibitory effects on germination percentage for Fenugreek 60% and 50% respectively over control. Minimum effect was observed for leachate concentrations of *Cosmos* on germination. LC_{50} for *Xanthium* was attained at 6% concentration only. Emergence of shoot length was drastically inhibited at 5% (2.23cm) and 6% (1.07cm) concentrations of leaf leachates of *Xanthium* as compared to *Cosmos*. *Cosmos* showed minimal inhibitory effect

Table 1. Effect of leaf leachates of *Cosmos* on seed germination of *Triticumaestivum*

Conc. Of leachates	Germination %	Root length	Shoot length	R:Sratio	Vigor index
Control	100%±0.07	14.2±1.06	12.2±0.92	1.16±0.09	1220±91.50
1%	100%±0.06	7.2±0.47	9.2±0.60	0.78±0.05	920±59.80
2%	90%±0.05	7.06±0.39	7.7±0.42	0.91±0.05	693±29.64
3%	85%±0.08	6.6±0.63	8.4±0.80	0.78±0.07	714±55.86
4%	70%±0.07	6.73±0.67	7.8±0.78	0.86±0.09	546±54.60
5%	60%±0.05	3.1±0.25	3.3±0.26	0.93±0.07	198±15.84
6%	40%±0.03	2.8±0.18	2.7±0.18	1.03±0.07	108±91.50

Table 2. Effect of leaf leachates of Xanthium on seed germination of *Triticumaestivum*

Conc. Of leachates	Germination %	Root length	Shoot length	R:Sratio	Vigor index
Control	100% ±0.07	12.17 ±0.91	12.17 ±0.91	1 ± 0.07	1217 ±91.27
1%	100% ±0.06	10.83 ±0.70	11.03 ±0.72	0.98 ±0.06	1103 ±71.69
2%	90% ±0.05	9.77 ±0.54	10.23 ±0.56	0.95 ±0.05	920.7 ±50.64
3%	90% ±0.09	9.4 ± 0.89	8.87 ±0.84	1.05 ±0.10	798.3 ±75.84
4%	90% ±0.09	8.83 ±0.88	8.67 ±0.87	1.01 ±0.10	780.3 ±78.03
5%	50% ±0.04	5.07 ± 0.41	3.73 ±0.30	1.35 ±0.11	186.5 ±14.92
6%	40% ±0.03	3.67 ±0.24	1.63 ±0.11	2.25 ±0.15	65.2 ±5.30

Table 3. Effect of leaf leachates of Cosmos on seed germination of *Vignaradiata*

Conc. Of leachates	Germination %	Root length	Shoot length	R:Sratio	Vigor index
Control	100% ±0.07	7.1 ±0.53	9.6 ±0.72	0.73 ±0.05	960 ±72.00
1%	100% ±0.06	6.8 ±0.44	11.03 ±0.72	0.61 ±0.04	1103 ±71.69
2%	90% ±0.05	5.33 ±0.29	7.4 ±0.41	0.72 ±0.04	666 ±36.63
3%	80% ±0.08	5.2 ±0.49	7.06 ±0.67	0.73 ±0.07	564.8 ±53.66
4%	80% ±0.08	4.7 ±0.47	6.9 ±0.69	0.68 ±0.07	552 ±55.20
5%	70% ±0.06	3.5 ±0.28	5.3 ±0.42	0.66 ±0.05	371 ±29.12
6%	70% ±0.05	3.46 ±0.22	5.2 ±0.34	0.66 ±0.04	364 ±23.66

on all measurable parameters. Root: shoot ratio showed reduction for *Cosmos* over control to 6% concentration. But *Xanthium*showed increase in root length though shoot growth was hampered. Reduction in vigor index upto70% from control to 5% and 6% for *Cosmos* was observedbut in *Xanthium* reduction of vigor index was concentration dependent from 5% to 6%. Values are mentioned in tables5 and 6 respectively.

DISCUSSION

Allelopathic plants are described as alien, invasive weeds from different geographical areas. They establish successfully through release of various allelochemicals, according to the contiguous environmental stress conditions. Dominance, higher growth rate and adaptability to existing environments are some of the attributes

Table 4. Effect of leaf leachates of Xanthium on seed germination of Vignaradiata

Conc. Of leachates	Germination %	Root length	Shoot length	R:Sratio	Vigor index
Control	100% ±0.07	4.57 ±0.34	8.17 ±0.61	0.57 ±0.04	817 ±60.53
1%	90% ±0.06	7.93 ±0.52	8.07 ±0.52	0.98 ±0.06	726.3 ±47.21
2%	90% ±0.05	7.57 ±0.42	5.63 ±0.31	1.34 ±0.07	506.7 ±27.87
3%	80% ±0.08	7.3 ±0.69	5.2 ±0.49	1.4 ±0.13	416 ±39.52
4%	60% ±0.06	2.93 ±0.29	4.07 ±0.41	0.71 ±0.07	244.2 ±24.42
5%	50% ±0.04	0.97 ±0.08	3.53 ±0.28	0.27 ±0.02	176.5 ±14.12
6%	40% ±0.03	0.93 ±0.06	1.73 ±0.11	0.53 ±0.03	69.2 ±60.53

Table 5. Effect of leaf leachates of *Cosmos* on seed germination of *Trigonellafoenum-graceum*

Conc. Of leachates	Germination %	Root length	Shoot length	R:Sratio	Vigor index
Control	100% ±0.07	5.6 ±0.42	6.3 ±0.47	0.88 ±0.07	630 ±47.25
1%	100% ±0.06	3.7 ±0.24	5.1 ±0.33	0.72 ±0.05	510 ±26.52
2%	90% ±0.05	3.26 ±0.18	4.8 ±0.26	0.67 ±0.04	432 ±21.12
3%	80% ±0.08	2.9 ±0.28	4.66 ±0.44	0.63 ±0.06	372.8 ±35.43
4%	70% ±0.07	2.4 ±0.24	4.1 ±0.41	0.58 ±0.06	287 ±28.90
5%	70% ±0.06	2.6 ±0.21	4.2 ±0.34	0.61 ±0.05	294 ±23.84
6%	60% ±0.04	2.03 ±0.13	3.86 ±0.25	0.41 ±0.03	231.6 ±15.05

Table 6. Effect of leaf leachates of *Xanthium* on seed germination of *Trigonellafoenum-graceum*

Conc. Of leachates	Germination %	Root length	Shoot length	R:Sratio	Vigor index
Control	100% ±0.07	3.33 ±0.25	5.4 ±0.41	0.61 ±0.05	540 ±40.50
1%	100% ±0.06	4.8 ±0.31	5.17 ±0.34	0.92 ±0.06	517 ±33.60
2%	100% ±0.06	4.03 ±0.22	4.6 ±0.25	0.87 ±0.05	460 ±25.30
3%	100% ±0.09	3.97 ±0.38	4.07 ±0.39	0.97 ±0.09	407 ±38.66
4%	80% ±0.08	3.6 ±0.36	3.9 ±0.39	0.92 ±0.09	312 ±31.20
5%	70% ±0.06	3.27 ±0.26	2.23 ±0.18	1.46 ±0.12	156.1 ±12.49
6%	50% ±0.03	2.47 ±0.16	1.07 ±0.07	2.3 ±0.15	53.5 ±3.48

for their successful establishment. Most of the allelochemicals are secondary metabolites of plants playing a significant role as stress tolerant compounds. Allelo/ ecochemicals present in several parts of such plants havetheir own mechanisms of release into environment. They may have inhibitory or stimulatory effect on growth and germination of neighboring plants or crops. This effect can be

Table 7. Functional group peaks from GC-MS study of *Cosmos sulphureus*

Sr. No	Retention Time	IUPAC	Molecular formula	Molecular Weight
1.	26.334-27.049	Carnegine	$C_{13}H_{19}NO_2$	221
2.	17.559-18.553	Malonic acid,2-formamido-2{4-(4-hydroxy-3-methyl-2-butenyl)indol-3}methyl-dimethyl ester	$C_{20}H_{24}N_2O_6$	388
		N-{5-(3-hydroxy-2-methylpropenyl)-1,3,4,5-tetrahydrobenzo(cd) indol-3-yl}-N-methylacetamide	$C_{18}H_{22}N_2O_2$	298
3.	26.250-27.001	Carnegine	$C_{13}H_{19}NO_2$	221
		Hexanal,(2,4-dinitrophenyl) hydrazone	$C_{12}H_{16}N_4O_4$	280
		(2-Methyl-3-nitrophenyl) methanol, dimethylpentaflu-orophenylsiyl ether	$C_{16}H_{14}F_5NO_3Si$	391

Table 8. Functional group peaks from GC-MS study of *Xanthium strumarium*

Sr. No	Retention Time	IUPAC	Molecular formula	Molecular weight
1.	8.921	Salmeterol	$C_{25}H_{37}NO_4$	415
2.	26.581	Tricylotetradecan-6-one,4-ethenyl-3-hydroxy-2,4,7,14-tetramethyl	$C_{20}H_{32}O_2$	304
3.	8.860	4-Acetyloxyimino-6-6-dimethyl-3-methylsulfanyl-4,5,6,7-tetrahydro-benzo(c)thiophene-1-carboxylic acid methyl ester	$C_{15}H_{19}NO_4S_2$	341
	8.980	Brnzene,2,3,4,5-tetramethyl-1-(2,3,4,5-tetarmethyl benzyl)	$C_{21}H_{28}$	280
		Corynan-17-ol,18,19-didehydro-10-methoxy	$C_{20}H_{26}NO_2$	326
4.	13.996-14.241	Carda-5-20(22)-dienolide,3-(6-deoxy-alpha-L-mannopyranosy)oxy)-14-hydroxy-(3beta)	$C_{29}H_{42}O_8$	518
		11-beta,19-Cyclopregn-5-ene-3,20-dione,11-hydroxy-cyclic bis (ethylene acetal)	$C_{25}H_{36}O_5$	416
		Carnegine	$C_{13}H_{19}NO_2$	227
5.	15.801-16.681	Malonic acid,2-formamido-2{4-(4-hydroxy-3-methyl-2-butenyl)indol-3-y}methyl-dimethyl ester	$C_{20}H_{24}N_2O_6$	388
		4a-Methyl-1-methylene-1,2,3,4,4a,9,10,10a-octahydrophenanthrene	$C_{16}H_{20}$	212
		Benzeneethanamine,2-fluoro-beta,3,4-trihydroxy-N-isopropyl	$C_{11}H_{16}FNO_3$	229
6.	17.524-18.835	Malonic acid,2-formamido-2{4-(4-hydroxy-3-methyl-2-butenyl)indol-3-yl) methyl-dimethylester	$C_{20}H_{24}N_2O_6$	388
		Benzenamine,4-(1-methylethyl)-N-phenyl	$C_{15}H_{17}N$	211
		1,7-di-iso-propylnapthalene	$C_{16}H_2O$	212
7.	20.200-20.425	Carnegine	$C_{13}H_{19}NO$	221
		9,12,15-Octadecatrienoic acid,2,3-bis{(trimethylsily)oxy} propyl ester	$C_{27}H_{52}O_4Si_2$	496
		2-Oxo-4-6-diphenyl-3-(4-tolyl)-1,2,3,4-tetrahydropyrimidine	$C_{23}H_{20}N_2O$	340

monitoredLeather and Einhellig (2005) through seed germination bioassay studies by considering parameters such as germination %, root length and shoot length. According to Inderjit and Nilsen (2003) laboratory bioassays and field studies are fundamental part of allelopathy research, because they are fast and repeatable tools for exploring the potential for different types of interactions.

The study was to evaluate the phytotoxic effect of such invasive weeds on physiological growth of cereals and pulses.The allelopathic impact of leachates or extracts is more harmful to radicle (Friedman; 1995). The phytotoxicity was directly proportional to the increasing concentrations of leachates.Many researchers have shown such inhibition of seed germination as affected by leaf leachates of various invasive weeds. 10% of aqueous leaf extract of *Partheniumhysterophorus* has shown total inhibition of *Triticumaestivum* (SeerjanaMaharajan *et al.*, 2007). Aqueous extract and leachate may have different response towards the germination of Wheat. Preliminary studies had revealed significant inhibitory effect on seed germination and seedling growth of selected crops from different geographical regions. The previous studies showed that aqueous extracts of hearleaf cocklebur (*Xanthium*) was effective on crops such as Wheat and Barley even with low density of presence of weeds

Table 9. FTIR spectral peak values and functional groups for the leaf extract of *Xanthium strumarium*

Peak Values Wave number ($cm^{-}1$)	Functional groups
3517.52	Free -OH
2923.56	-CHO (C-H Stretch)
1895.68	C=O of anhydride, ester
1746.23	$=CH_2$, C=O of acid anhydride
1541.81	Aromatic region, N-H for prim and sec amide
1407.78	Aromatic region
1041.37	C-O-C
437.439	C-N,C-C,C-O
440.665	C-N,C-C,C-O
632.537	Aromatic
525.507	Aromatic
466.689	C-N,C-C,C-O, aliphatic C-I,
422.334	C-N,C-C,C-O
412.692	C-N,C-C,C-O, aliphatic C-I,
327.194	Bending vibration depends on substitution Pattern
308.555	Bending vibration depends on substitution Pattern
212.131	Bending vibration depends on substitution Pattern
159.099	Bending vibration depends on substitution Pattern
95.4591	Bending vibration depends on substitution Pattern

Table 10. FTIR spectral peak values and functional groupsfor the leaf extract of *Cosmos sulphureus*

Peak Values Wave number ($cm^{-}1$)	Functional groups
3639.02	Free -OH
2917.77	-CHO (C-H Streach)
1656.55	C=C in alkens or Aromatic region,
1425.14	Aromatic region
1014.37	C-O-C(Ethers)
782.958	=C-H(m),CH=CH,
476.331	Aliphatic C-I region.
445.476	Bending vibration depends on substitution Pattern
431.012	Bending vibration depends on substitution Pattern
415.585	Bending vibration depends on substitution Pattern
820.563	=C-H
538.042	-C-X,
501.401	-C-X,
466.689	-C-X,
422.334	Bending vibration depends on substitution Pattern
411.728	Bending vibration depends on substitution Pattern
372.194	Bending vibration depends on substitution Pattern
295.055	Bending vibration depends on substitution Pattern
282.52	Bending vibration depends on substitution Pattern
223.702	Bending vibration depends on substitution Pattern
194.775	Bending vibration depends on substitution Pattern
159.099	Bending vibration depends on substitution Pattern
123.422	Bending vibration depends on substitution Pattern
95.4591	Bending vibration depends on substitution Pattern

(IzzetKadioglu,2004). Cutler and Cole reported that potassium carboxyactractyloside, a glycoside isolated from the residues of *Xanthium strumarium* L. strongly inhibiting coleoptiles growth of Wheat (Benyas *et al*, 2010).

Similar inhibitory effects of aqueous extracts of *Ranunculus arvensis, Sinapisarvensis, Smilaxaspera* were observed with wheat germination percentage and rate as compared to inhibitory effects of *Cosmos* and *Xanthium* leaf leachates. The effect of aqueous extracts on root growth was more than shoot growth(Qasem, 2017). Different plants showed variable effects on target species and their responses.

*Clerodendruminfortunatum*showed highest inhibitory effect on *Vignaradiata*seeds at 100% concentration. And for*Trigonellafoenum-graceum* maximum inhibitory effect on germination was at 100% concentration and in case of *Triticumaestivum* and *Brassica campestris* highest inhibitory effect started from 75% and 50% concentration respectively (GopalDebnath*et al*., 2016). The results of present study showed partial stimulatory effects in comparison with studies conducted on *Clerodendruminfortunatum*in Tripura region. Similar supporting results were observed with ethanolic leaf extract of *Solanumnigrum* (Girija,2015) on seed germination, radical length and protein content of *Trigonellafoenum-graceum*. Higher concentrations showed inhibitory effect whereas lower concentrations had no effect.

*Vignaradiata*indicated both stimulatory and inhibitory response effect to some extent to leachate concentration of *Cosmos* and *Xanthium* respectively. Reduction in net yield of fallow-mungbean was reported due to allelochemicals released by Sunflower (Vishwajit, 2017). Sunflower showed significant allelopathic effect on receptor plant Mungbean in sunflower-mungbean crop rotation. *Cosmos* and *Xanthium* though are not cultivated plants but show inhibitory effects due to release of various allelochemicals. The root length of Mungbean was inhibited at 91.91% and seedling growth as inhibited at 52.05% at 50mg/ml concentration of leaf extracts of *Diospyros kaki* (Cui, 2017) similar to results of effects of *Cosmos* and *Xanthium* leaf leachates.

Correlation graph and standard deviation calculations and observations resulted in significant inhibition of hypocotyls and coleoptiles growth of all three selected crops with difference in variation to response to the allelochemials. Stimulatory effect could be observed at 1% and 2% concentration at par with control. Inhibition concentration was observed at 6% concentration of leaf leachates of *Xanthium* and *Cosmos*.

Increased concentrations of allelo chemicals suppress the mitotic activity of young cells or embryo resulting in inhibition of seed germination. Bioassay conditions play a major direct role in seed germination such as light conditions and humidity (Chen Feng*et al.* 2017, NEERI REPORT, 2000). There was direct effect of temperature on emergence of hypocotyl and coleoptile throughout the experiment.

Phytochemical analysis

Phytochemicals revealed that alkaloids, saponins, phenols, tannin, flavonoids and proteins are present in the extract.

RESULTS

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which may probably show medicinal bioactivities. Analysis of the plant extracts of *Cosmos* revealed the presence of phytochemicals such as phenols, phytosterols, tannins, flavonoids and alkaloids with absence of saponins, carbohydrates and proteins. Whereas phytochemical analysis results for *Xanthium* plant extract revealed the presence of alkaloids, tannins and flavonoids but absence of saponins and phenols.

DISCUSSION

Biologically active molecules present in plants are phytochemicals. Phytochemical analysis is considered as major analytical part for detection of various compounds.These compounds are useful to understand the ecology and stress conditions of plants.Phytochemical analysis conducted on *Xanthium strumarium* (FarooqUmer, 2014) revealed the presence of similar secondary metabolites such as alkaloids, phenols, flavonoids tanninsand terpenoids but with variations in saponins, glycosides, steroids.

Phytochemical screening tests on *Cosmos sulphureus* plant extract (Jadav, 2017) revealed the presence of proteins, flavonoids, phenols, alkaloids, tannins, saponins which are similar to the results obtained from tests conducted on *Cosmos* from Pune region. Similarities and differences in results suggest different responses to environmental stress conditions from two different geographical areas.

GC – MS results

The allelopathic potential exhibited by both the weeds might be due to different types of allelochemicals existing in them. The dominance of these two weeds and their inhibitory activity on the other plants and also the crops can be attributed to the presence of different types of ecochemicals existing in them which are detected with GC-MS. GC-MS analysis of ethyl acetate extract of both samples revealed the presence of some similar compounds. The major constituents were esters, ethers, anhydride and polyalcohols.

IR spectroscopy results

FTIR analysis of *Cosmos* and *Xanthium* in the range 4000-400 cm^{-1} showed the characteristic fingerprinting regions of various functional groups such as –OH, carbonyl, anhydride, ester, amide. For example 3517 $cm^{-1}$for free –OH group, 2323 $cm^{-1}$and 2917$cm^{-1}$for –C-H stretching. FTIR data suggested the probability of presence of compounds containing these functional groups. GC-MS analysis of both samples ethyl acetate plant extract, revealed the presence of similar compounds. The major constituents were esters, ethers, anhydride and polyalcohols. (Tables 9 and 10)

DISCUSSION

Isolation, detection and identification of functional groups is important part of allelopathic studies that helps to recognize nature of allelochemicals released by plant in response to fluctuating environmental conditions. These chemicals are from different groups as phenolic acids such as ferulic, p-coumaric acids, p-hydroxy benzoic acids, cyanogenic glycosides, tannins etc. Studies reveal that allelopathy is result of compounds having different actions as antagonistic, synergistic on plants in the vicinity. Ghayal*et al.* (2007) and RuBai*et al.* (2009) have carried out similar studies on phytotoxic effects of invasive weeds and their GC-MS analysis.

CONCLUSION

Though we are trying for increasing the sustainable organic agriculture, the over dominating herbaceous invasive species near agricultural land are posing great problems. This occurs through allelobiogenesis whose mechanism needs to be clearly understood for overcoming such problems. In the experiments conducted it was mainly observed that *Xanthium* had significant effect on crops than *Cosmos*. The effects are directly related to expanse or density of weed and age of plant. In spite of both the invasive species being members of Asteraceae, the impact was different, which may be probably due to the different combinations of allelochemicals. Hence to know the nature of allelochemicals, first the qualitative analysis was carried out which revealed the presence of phenols, phytosterols, tannins, flavonoids and alkaloids in both the aliens. Further GC-MS analyses helped to characterize the ecochemicals which might be having allelopathic activity. Detail quantification and observation of individual chemical effect could be further line of research.

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