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In vitro and *in vivo* Effect of Weeds (Root) Extracts on Soil Borne Fungal Phytopathogens and Fungal Infected Legume Crop Bengal Gram (*Cicer arietinum*)

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ABSTRACT

Background: Weed management during growing season has been a serious problem for many years. Worldwide, a 10% loss of agricultural products can be attributed to the competitive effect of weeds, despite their intensive control. *Dactyloctenium aegyptium* and *Chenopodium album* L. is an annual weed of cultivated fields.

Methods: This investigation was done in 2020-2021. Root parts of both weeds *Dactyloctenium aegyptium* and *Chenopodium album* were collected from a nearby area, Sardar Vallabh Bhai Patel University of Agriculture and Technology, Meerut. To determine the antifungal potentials of both weeds *Dactyloctenium aegyptium* and *Chenopodium album* root extracts were determined by the food poisoning method against fungal phytopathogens. Methane, Ethyl acetate, Butyl alcohol, Benzene and Water-soluble root extracts of both weeds at different concentrations (5%, 10% and 15%) were used against fungal phytopathogens growth in laboratory conditions and also used on fungal infected related attributes of Bengal gram in a pot culture experiment in 1:10 ratio. Antioxidants of both weed extracts were done by DPPH, FRAP and ABTS methods.

Result: Findings showed that application of 15% concentrated extracts has maximum antifungal effects against *Fusarium oxysporum* and *Sclerotium rolfsi* (70-80%). In the pot experiment, we find that at increased concentrations of methanolic extract (15%) infected Bengal gram crops show high effectiveness against fungal phytopathogens and improve different parameters such as germination, plant physiology and morphological characteristics. However, higher concentrations of methanol extract promoted these parameters and reduced fungal growth in both *in vivo* and *vitro* conditions Results of this study reported highly significant suppressive effects of higher concentrations (10% and 15%).

Key words: Antifungal, Antioxidant, *Chenopodium album*, *Dactyloctenium aegyptium.*

INTRODUCTION

Plants possess various naturally occurring chemicals in the form of secondary metabolites which may leach out from their various parts to the surrounding rhizosphere either as exudates or rain residues that may directly or indirectly influence germination, growth and other developmental processes of nearby plants (Sajjad *et al*., 2007; Iqbal *et al*., 2010). Weeds from when humans replaced native vegetation (natural) with agricultural ecosystems were accompanied by stability with crops and influenced widely ecosystems with different mechanisms to increase their biological range (Turk and Tawaha, 2002). Weed management during the growing season has been a serious problem for many years. Worldwide, a 10% loss of agricultural products can be attributed to the competitive effect of weeds, despite their intensive control. Potential yield reductions caused by uncontrolled weed growth throughout the growing season have been estimated to be 45 to 95%, depending on crop species and ecological and climatic conditions (Ampong and Datta, 1991). Therefore, weed management is a key element of most agricultural systems. The application of herbicides has been a major factor enabling the intensification of agriculture in past

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decades. Indeed, three million tonnes of herbicides per year are used in most agricultural systems (Stephenson, 2000). Many weed species have been studied in vitro for their allelopathic potential on various field crop species such as *Chenopodium album* (Stephenson, 2000), *Nepeta meyeri* (Romel *et al*., 2007), *Brassica nigra* (Turk and Tawaha, 2002).

Dactyloctenium aegyptium is a multipurpose grass. It is mainly used as fodder and relished by all classes of ruminants. In semi-arid areas, it makes valuable annual pastures as well as excellent hay. It is also suitable for silage. The phytochemical analysis of *Dactyloctenium aegyptium* showed that the plant contained carbohydrates, proteins, amino acids, terpenoids, alkaloids, saponins, tannins, flavonoids, steroids fixed oils and phenols.

The pharmacological investigations revealed that *Dactyloctenium aegyptium* possessed antimicrobial, antioxidant, reproductive, cytooxic, antidiabetic and gastrointestinal effects (Ali Esmail Al-Snafi, 2017). *Dactyloctenium aegyptium* aerial parts were investigated fungal strains [*Aspergillus fumigates* (RCMB 02568) and *Candida albicans* (RCMB 05031)]. Ethanolic extract of *Dactyloctenium aegyptium* were examined for antimicrobial potential against three standard bacteria (*Escherichia coli, Klebsiella Pneumonia, Staphylococci*) and one standard fungus (*Aspergillus niger*) (Kumar *et al*., 2015). *Dactyloctenium aegyptium* grass species were studied for endophytic fungi by Potato dextrose agar (PDA) and Malt extract agar (MEA) methods (Rekha and Shivanna, 2014). The ethanolic extract of *Dactyloctenium aegyptium* shows the maximum antifungal activity compared to Griseoflavin. (Veeresh *et al*., 2015). The antifungal potential of different parts of *Chenopodium album* L. was examined against *Fusarium oxysporum* the effect of different concentrations (0.5, 1.0, ... 3.0%) of methanolic leaf, stem, root and inflorescence extracts of *C. album* was investigated. Different concentrations of this extract suppressed fungal growth by 24-80%. Methanolic inflorescence extract was successively extracted with n-hexane, chloroform, ethyl acetate and n-butanol. The highest antifungal activity was shown by ethyl acetate fraction resulting in a 68-100% reduction in fungal biomass (Saima Rauf and Arshad Javaid, 2013). *Sclerotium rolfsi* a soil-borne pathogen of over 500 plant species, causes collar rot disease in chickpea plants and reduces its survival rate, growth and yield. *S. rolfsi* significantly reduced the dry biomass of shoot, root and grains of chickpea by 21, 36 and 50%, respectively, as compared to negative control (without fungus) (Javaid, 2020).

This study was undertaken to evaluate different concentrations of fresh organic solvent (methanol, ethyl acetate, butyl alcohol, benzene, water) root extracts of *C. album* and *D. aegyptium* for their activity against on fungal phytopathogens (*Fusarium oxysporum, Rhizoctonia solani* and *Sclerotium rolfsi*) and these fungi (*Fusarium oxysporum, Rhizoctonia solani* and *Sclerotium rolfsi*) also infect Bengal gram crop in pot-culture experiment.

MATERIALS AND METHODS

Sample collection

The samples were collected from a nearby area of agricultural land *i.e.* Sardar Vallabhai Patel University of Agriculture And Technology, Meerut in the year 2020. We use only fresh samples of two weeds for further work.

Extracts preparations

Before the extract preparation, we wash the samples, shade them dry for 24 to 72 hours, chopped and macerated. When samples were completely dry, we took the sample and organic solvent at a particular ratio (1:10) in soxhlet apparatus for extract preparations.

Culturing of pathogens

Fungal phytopathogens were isolated from the infected Bengal gram (*Cicer arietinum*) crop as *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium rolfsi*. These fungal phytopathogens were isolated on a PDA medium by pure culture technique methods. We took the infected part of Bengal gram as root, stem and leaf and put it on a basic fungal culture medium *i.e.* potato dextrose agar and after that, all the Petri plates were incubated at culturing temperature *i.e.* 28°C for 24 hours. After that growth appeared in all the plates. Based on basic morphology we isolate the fungus and inoculate the fungal culture into a fresh Petri plate of PDA for further identification.

Antioxidant activity of weed extracts by different methods

DPPH methods

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical is an antioxidant in an electron-transfer-based process that produces a violet way out in ethanol. This free radical, firm at room temperature, is compact in the existence of an antioxidant molecule, giving rise to a colorless ethanol solution. Uses of the DPPH assay provide an effortless way to estimate antioxidants by spectrophotometer, so it can be constructive to assess various goods at a time. The antioxidant activity percentage (AA %) of each substance was assessed by DPPH free radical assay. DPPH radical measurement scavenging activity was performed as described by methodology Brand-Williams. The samples were reacted with the stable DPPH radical in an ethanol solution. The reaction mixture consisted of adding 0.5 ml of sample, 3 ml of absolute ethanol, 0.3 ml of DPPH radical solution and 0.5 mm of ethanol. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The color turn (from deep violet to light yellow) was recorded at 517 nm Absorbance after 100 min of reaction using a UV-VIS spectrophotometer (Brand-Williams *et al*., 1995).

FRAP methods

The ferric-reducing ability of studied plant materials was assessed following the method described by Benzie and Strain (1996). The final results were articulated and have ferric reducing ability equivalent to that of 1 mm $FeSO_4$,

particularly expressed as mol Fe (II) equivalent/g sample in dry weight.

ABTS methods

Free radical scavenging activity of plant samples was determined by ABTS radical cation decolorization assay (Pellegrini *et al*., 1999). The ABTS·+ cation radical was produced by the reaction between 7 mm ABTS in water and 2.45 mm potassium persulfate (1:1), stored in the dark at room temperature for 12-16 h before use. The ABTS·+ solution was then diluted with methanol to obtain an absorbance of 0.700 at 734 nm. After the addition of 5 µl of plant extract to 3.995 ml of diluted ABTS·+ solution, the absorbance was measured at 30 min after the initial mixing. An appropriate solvent blank was run in each assay.

***In vitro* testing of isolated fungal phytopathogens on legume crop Bengal gram (*Cicer arietinum*)**

Seeds of Bengal gram were obtained from the local market of Meerut. They were sown in pots of equal size. Each pot had 100 gm of soil. Each treatment was replicated three times with 10 seeds per pot. Pots were arranged in a completely randomized design (CRD) and were maintained at field capacity in a uniform open environment. One disc of all the fungal phytopathogens (*Fusarium oxysporum, Rhizoctonia solani* and *Sclerotium rolfsi*) cultures was added to each pot and then irrigation was done with tap water. Five healthy seedlings were left in each pot. Thereafter, pots were irrigated with different concentrations (5%, 10% and 15%) of all solvents of both *Dactyloctenium aegyptium* and *Chenopodium album* root extracts (original stock solution) by diluting it with the appropriate amount of distilled water. Control pots were irrigated with tap water. At the time of maturity of the Bengal gram crop, data were collected based on different growth parameters, such as germination % disease symptoms in leaves, stems and roots.

***In vivo* testing of pathogens on culture media**

This testing is done using food poisoning techniques. It is a very effortless and normal method to resolve the inhibition zone. The weed extracts were used beside the fungal phytopathogens *Fusarium oxysporum, Rhizoctonia solani* and *Sclerotium rolfsi*. One gm crude samples of both weeds diluted with 10 ml of each solvent *i.e.* Ethyl acetate, butyl alcohol, benzene, methanol and sterile water and from this suspension, 1250 µl, 2500 µl and 3750 µl is added to the PDA melted medium to obtain the desired concentration to form a volume of 25 ml of suspension was poured into each sterile Petri plate but in controlled Petri plate free from extracts concentration and allowed to be stabilized. Three duplicates (plates) were utilized for each treatment. For test fungal cultures, each plate was infected with a 6 mm diameter disc and incubated at 28°C until the control plates reached maximal growth.

Statistical analysis

At maturity, data on different extracts at different concentrations were recorded and statistically analyzed using a weighted mean.

The weighted mean was applied in the present study. The following mean was used for the study:

Weighted mean

$$\frac{\Sigma WiXi}{\Sigma Wi}$$

Where:
Xi = Worth of a thing.
Wi = Item's weight.

RESULTS AND DISCUSSION

Dactyloctenium aegyptium and *Chenopodium album* is a multi-property weedy plant. The root of these weeds shows effectiveness due to the presence of an active compound, which is responsible for the positive result. Antioxidant tests were measured by respective methods, like FRAP, DPPH and ABTS patterns. Comparative analysis of *D. aegyptium* and *C. album* root by FRAP, DPPH and ABTS was shown in Table 1.

Antifungal activeness of both (*Dactyloctenium aegyptium* and *Chenopodium album*) weed extracts was measured by radial methods (food poisoning) using the root part in different organic solvents, in particular ratio (1:10) at different concentrations (5%, 10% and 15%). The observation revealed that if we increase the extract concentration it reduces the fungal growth. The different extracts in the various solvents show that all the extracts inhibit the growth of the soil-borne fungal phytopathogens. *Dactyloctenium aegyptium* methanol extract at a maximum concentration of 15% showed fungal growth, 1.68E+00 of *Sclerotium rolfsi* compared to control 5.25E+00 Along with that ethyl acetate extract in concentration 15% showed the fungal growth 1.08E-01 of *Sclerotium rolfsi* as compare to control 5.16E+00 Butyl alcohol extract of *Dactyloctenium aegyptium* at 15% concentration showed up the growth 1.68E+00 of *Fusarium oxysporum* as compare to control 5.68E+00. Benzene extract of *Dactyloctenium* at 15% concentration showed growth of 1.14E+00 of *Sclerotium rolfsi* as compared to control 5.54E+00. Water extract of *Dactyloctenium* showed growth of 1.4E+00 of *Fusarium oxysporum* as compared to control i.e 5.68E+00 shown in Table 2.

Table 1: Antioxidant activity of *Dactyloctenium aegyptium* and *Chenopodium album root* by FRAP, ABTS and DPPH methods.

Samples	FRAP (%)	DPPH (%)	ABTS (%)
Dactyloctenium aegyptium (R)	64.15	37.45	7.69
Chenopodium album (R)	0.4	44.51	9.15

R = Root.

Showed in another weed, *Chenopodium album* methanol extract at a maximum concentration of 15% showed fungal growth of 0.80E+00 of *Sclerotium rolfsi* as compared to control 5.41E+00 Along with that ethyl acetate extract increased concentration of 15% showed up the fungal growth 1.25E+00 growth of *Fusarium oxysporum*

Table 2: Antifungal activity of *Dactyloctenium aegyptium* root extracts on fungal phytopathogens.

Extracts	Concentration	Fungal phytopathogens (mean in scientific)		
		Fusarium oxysporum	*Rhizoctonia solani*	*Sclerotium rolfsi*
Methanol	5%	2.88E+00	3.39E+00	3.13E+00
	10%	1.98E+00	2.68E+00	2.14E+00
	15%	9.80E-01	1.48E+00	1.68E+00
	C%	9.80E-01	5.44E+00	5.25E+00
Ethyl acetate	5%	3.88E+00	2.98E+00	2.12E+00
	10%	2.78E+00	1.66E+00	1.59E+00
	15%	1.68E+00	5.90E-01	1.08E-01
	C%	4.89E+00	4.36E+00	5.15E+00
Butyl alcohol	5%	2.98E+00	3.36E+00	1.43E+00
	10%	2.01E+00	2.28E+00	2.48E+00
	15%	1.68E+00	1.98E+00	3.37E+00
	C%	5.97E+00	5.68E+00	5.68E+00
Benzene	5%	2.14E+00	3.48E+00	3.14E+00
	10%	1.38E+00	3.10E+00	2.14E+00
	15%	1.11E+00	2.44E+00	1.14E+00
	C%	4.99E+00	4.98E+00	5.54E+00
Water	5%	1.78E+00	5.15E+00	2.19E+00
	10%	1.39E+00	4.48E+00	1.58E+00
	15%	1.40E-01	4.13E+00	5.80E-01
	C%	5.68E+00	5.87E+00	5.98E+00

Table 3: Antifungal activity of *Chenopodium album* root extracts on fungal phytopathogens.

Extracts	Concentration	Fungal phytopathogens (mean in scientific)		
		Fusarium oxysporum	*Rhizoctonia solani*	*Sclerotium rolfsi*
Methanol	5%	2.18E+00	3.45E+00	2.25E+00
	10%	1.70E+00	2.98E+00	1.55E+00
	15%	1.00E+00	1.75E+00	0.80E+01
	C%	4.96E+00	4.89E+00	5.41E+00
Ethyl acetate	5%	3.96E+00	2.65E+00	2.29E+00
	10%	2.25E+00	2.10E+00	2.88E+00
	15%	1.25E+00	1.40E+00	1.40E+00
	C%	4.25E+00	5.78E+00	6.21E+00
Butyl alcohol	5%	2.45E+00	3.45E+00	3.25E+00
	10%	1.80E+00	2.95E+00	2.01E+00
	15%	1.20E+00	1.85E+00	1.75E+00
	C%	5.32E+00	5.80E+00	5.41E+00
Benzene	5%	2.78E+00	3.85E+00	2.44E+00
	10%	1.98E+00	2.78E+00	1.49E+00
	15%	1.48E+00	1.89E+00	5.50E-01
	C%	5.99E+00	4.10E+00	5.48E+00
Water	5%	3.89E+00	2.98E+00	3.74E+00
	10%	2.30E+00	2.55E+00	2.10E+00
	15%	1.85E+00	2.40E+00	1.14E+00
	C%	5.62E+00	5.79E+00	5.88E+00

as compare to control 4.25E+00 Butyl alcohol extract of *Chenopodium album* at 15% concentration showed up the growth 1.75E+00 of *Fusarium oxysporum* as compare to control 5.68E+00. Benzene extract of *C. album* at 15% concentration showed growth 1.48E+00 of *Fusarium oxysporum* as compared to control 5.99E+00. Water extract of *C. album* showed the growth 1.14E+00 of *Fusarium oxysporum* as compared to control *i.e* 5.88E+00 shown in (Table 3).

At all over analysis we compare the each weeds extracts against all selected fungal phytopathogens, all details mentions in Table 4 is given in average/mean of 3 replicate at 15 ml extract concentration.

Different concentration extracts of *C. album* and *D. aegyptium* induce germination, physiological characteristics and reduce infectious symptoms on fungal phytopathogens (*Fusarium oxysporum, Rhizoctonia solani and Sclerotium rolfsi*) on infected Bengal gram crop part (leaf, stem and

Table 4: Comparative analysis of both weeds root extracts against fungal phytopathogens.

Variety of extract	Inhibition (%) of fungal phytopathogens		
	F. oxysporum	*R. solani*	*S. rolfsi*
W1M	84	86	90
W1EA	60	72	75
W1BA	68	60	49
W1BE	70	49	75
W1WA	53	29	50
W2M	79	75	89
W2EA	65	62	58
W2BA	69	50	68
W2BE	65	61	58
W2W	67	53	70

W1= *Dactyloctenium* root , W2= *Chenopodium* root, M= Methanol, EA= Ethyl acetate, BA= Butyl alcohol, BE= Benzene, W= Water.
*Data is given in average/ mean of 3 replicate at 15 ml extract concentration.

Table 5: Physiological changes in infected Bengal gram crop, treated with *Dactyloctenium aegyptium* root extracts (mg/ml).

Extract concentration		Germination %	Infected Bengal gram crop		
			Fusarium oxysporum (leaf)	*Rhizoctonia solani* (root)	*Sclerotium rolfsi*- (stem)
Methanol	5%	30	+	=	-
	10%	30	++	==	-
	15%	70	++	==	-
	C	20	+	=	-
Ethyl acetate	5%	40	+	=	-
	10%	30	+	=	-
	15%	40	++	==	-
	C%	20	+	=	-
Butyl alcohol 5%	30	++	==	-	
	10%	30	++	==	-
	15%	60	++	==	-
	C%	30	++	=	-
Benzene	5%	20	+	==	-
	10%	20	++	==	-
	15%	60	++	==	-
	C%	20	+	=	-
Water	5%	30	++	=	-
	10%	50	++	=	-
	15%	60	++	==	-
	C %	20	+	=	-

+ (yellow leaf) ++ (green leaves) - (short stem) - - (long stem) = (short root) = = (long root) C (control).

root), especially at higher concentrations in pot/field conditions However, lower to higher concentration (5%, 10% and 15%) had shown these effects on all these parameters (Table 5, 6, 7 and 8).

The present study shows both inhibitory (at higher concentrations) and stimulatory (at lower concentrations) effects of all extracts of *Dactyloctenium* and *Chenopodium*. The antifungal activity of *D. aegyptium* and *C. album* could be related to the presence of phenolics and alkaloid compounds in their leaves and roots (Malik *et al*., 1994). All extracts of both weeds reduce the growth of all selected fungal phytopathogen, but two fungi, *Fusarium oxysporum*

Table 6: Physiological changes in infected Bengal gram crop, treated with *Chenopodium album* root extracts (mg/ml).

Extract concentration		Germination %	Infected Bengal gram crop		
			Fusarium oxysporum (leaf)	*Rhizoctonia solani* (root)	*Sclerotium rolfsi* (stem)
Methanol	5%	0	NA	NA	NA
	10%	60	+	=	-
	15%	90	+	= =	-
	C	0	NA	NA	NA
Ethyl acetate	5%	20	+	=	-
	10%	60	++	==	-
	15%	80	++	==	-
	C%	20	+	=	-
Butyl alcohol 5%	60	+	=	-	
	10%	50	++	=	-
	15%	70	++	==	-
	C%	60	+	=	-
Benzene	5%	40	+	=	-
	10%	40	++	==	-
	15%	70	++	==	-
	C%	30	+	=	-
Water	5%	20	+	=	-
	10%	50	+	=	-
	15%	80	++	==	-
	C %	30	+	=	-

+ (yellow leaf) ++ (green leaves) - (short stem) - - (long stem) = (short root) = = (long root) NA (not appear) C (control).

Table 7: Disease symptoms show in Bengal gram crop after treatment with *Dactyloctenium* root extracts.

Extract concentration		Infected Bengal gram crop		
		Fusarium oxysporum (leaf)	*Rhizoctonia solani* (root)	*Sclerotium rolfsi* (stem)
Methanol	T	+++	+++	+++
	C	-	-	-
Ethyl acetate	T	+	+++	+++
	C	-	-	-
Butyl alcohol	T	++	++	-
	C	-	-	-
Benzene	T	+++	+	++
	C	-	-	-
Water	T	+	-	+
	C	-	-	-

(+) Less affected; (++): Much affected; (+++): Highly affected; (-): Unaffected T (test); C (control).

Table 8: Disease symptoms show in Bengal gram crop after treatment with *Chenopodium* root extracts.

Extract concentration		Infected Bengal gram crop		
		Fusarium oxysporum (leaf)	*Rhizoctonia solani* (root)	*Sclerotium rolfsi* (stem)
Methanol	T	+++	+++	+++
	C	-	-	-
Ethyl acetate	T	++	++	+++
	C	-	-	-
Butyl alcohol	T	+++	+	+
	C	-	-	-
Benzene	T	++	++	+++
	C	-	-	-
Water	T	+	+	++
	C	-	-	-

(+) Less affected; (++) Much affected; (+++): Highly affected; (-): Unaffected T (test); C (Control).

and *Sclerotium rolfsi* are highly infected as compared to control.

CONCLUSION

At increased concentrations (10% and 15%), improvement of leaf color (becomes yellow due to blight disease by *F. oxysporum*) root length (root rot by *R. solani*) and stem length (becomes brown or color rot by *S. rolfsi*) because the % rate of active fungal spore is induced. Similarly, higher concentrations of extracts containing phenolics and alkaloids might have higher absorption of minerals and water and their translocation from roots to other plant parts. We conclude that the methanol root extract of both weeds shows good results in laboratory and field conditions.

Conflict of Interest

All authors declared that there is no conflict of interest.

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