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RESEARCH ARTICLE

Phytotoxic activity of Tamarixdioica extract against four plant species

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Abstract

TamarixdioicaGl Webster is used ethno-pharmacologically for the treatment of many diseases in Pakistan. The need to reduce harmful effects from the unsystematic use of herbicides has facilitated the development of weed management systems, which are based on ecological manipulations rather than agrochemicals. On this aspect, utilizing allelopathic plants to suppress or stop the weed growth may be the most cost-effective and environment-friendly method of weed control. Efforts were made to investigate the phytotoxic efficacy of T. dioicaagainst the growth of four plants Brachiariaramosa, Vignaradiata, ZeamaizandEchinochloa crus-galli under field and an in-vitro study. Three concentrations of crude methnolic extract; 1000µg ml^{-1}, 600µg ml^{-1} and 200µg ml^{-1} were used under both the field and in-vitro studies. The T. dioicca extract showed significant inhibition both in hypocotyls/shoot and radicals/roots growth. T. dioica showed maximum inhibition in the growth of B. ramosa (43.34%±0.02) while, minimum in V. radiata (34.93%±0.02). The extract caused maximum reduction in the fresh and dry weights of E. crus-galli (1.86±0.09, 0.70±0.006), while minimum reduction was shown by V. radiata (8.36±0.09, 2.07±0.00).Overall, our results suggest that T. dioicamight be used affectively, as bioherbicide for weed control. Consequently, it will reduce dependency on synthetic agrochemicals

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Introduction

Phytotherapy means ‘’ treatment by plants” Some time it is also known as ‘’alternative medicines (Adanet al., 2010). The higher plants have a great potential against the growth and development of certain microbes, due to the presence of some bioactive compounds, like phenolic compounds, flavonoids and essentials oils etc. They are easily available, safer and affordable. Based on on-going progress in controlling the disease causing microorganisms and parasites and lake of secure (antimicrobial) medicines for universal chronic diseases, it is imperative to search for new antibiotics from alternate resources (Kaydanet al., 2012). Pharmacological evaluation is the initial step in the standardization of crude drugs. Pharmacological studies bring forth valuable information regarding the anatomy and morphology of plant and also the corporal characteristics of the crude drugs (Shinwari, 2010). Usually, curative plants fabricate a diversity of compounds of recognized restorative characters (Bruneton, 1995). In the recent years, unsystematic and excessive use of modern agrochemicals has made our soil unwell, through undesired ecological contamination, increased resistance in parasites and addition of poisonous residues in the foodstuff (Khan et al., 2010). Prolonged use of synthetic herbicide might perceptibly cause an incredible ecological weakening, ensuing chemical contamination in the soil and dropping the soil productivity (Jamil, 2005). According to the International Allelopathy Society (IAS) Phytotoxicity / allelopathy, “Any procedure concerning bioactive compounds formed by plants, microbes, and fungus that

manipulate the development and growth of natural and agricultural systems (including negative and positive effects)" are termed as phytotoxin or allelochemicals (Whittaker and Feney 1997).

T. dioicabelongs to Family, Tamaricaceae and is commonly known as Ghaz orkhagal (Pashto) and Tamarisk (Eng). The tree is native to Pakistan, Afghanistan, Iran, India, Bangladesh, Bhutan, Kashmir, Nepal, and Myanmar. T. dioica is abundantly found in Khyber Pakhtunkhwa and Sindh provinces of Pakistan (Khan et al 2012). It is an evergreen tree, often expressive with green loose twig-lets and thin leaves. The leaves are minute, down warding the shoot, tips oppressed greenish with whitish edge (Khan et al., 2008). The T.dioica flowers are hermaphrodite, violet or pinkish and closely attached, spikes, 2-5.1 cm long. Stamens are six and have a capsular fruit. They are evergreen growing up to–18 meter in height and forming dense shade (Chouore, 2005). Usually they are growing in saline areas because they are highly salt tolerant and also called as salt cedar. It is well adapted to alkaline environment (Wu et al., 2000).

The chief phytochemicals of T. dioica flowers and leaves are steroids, phlo-batannins, tannins, phenols, flavonoids, terpenoids and saponin (Qasimet al 2013). Tameradone and Tamadonalkaloides had obtained from the aerial parts of T. dioica. (Shafiqueet al., 2010). Different bioactive compounds namely, Hexacosyl-p-coumarate, GardeninsNevadensin and Apigenin were isolated from T.dioica leaves and barks (Romeo &Weidenhamer, 2010). Different researchers have reported that T. dioica can be used as antibacterial, antifungal and antiprotozoans.It is also used as diuretic, carminative and for the treatment of hepatic and splenic disorders (Baum 1978).

It is traditionally used from ancient time as antimicrobial agent (Indergit and Dashun, 1998). Literature survey shows that, no work has been done on phytotoxic screening of T. dioica. The present study was designed to carry out the phytotoxic activity of T.dioica flowers and leaves for the first time.

Materials and Methods

Plant Collection:

The Tamarixdioica (leaves and flowers) was collected from District LakkiMarwat (KPK) Pakistan in July, 2012. Identification of the plant was confirmed through a well known taxonomist Professor Abdur-Rehman, Chairman Botany Department, Govt Post Grageuate College Bannu) and was further authenticated by Pro. Dr. M. Rashid Awan (Incharge, Herbaruim of Hazara University, Mansehra). The Tamarixdioica(voucher no 3684) was deposited at the Herbarium of Hazara University. The plant was shed dried for two weeks and grinded to powder form.

Preparation of Plants Extracts:

200 gm of each plant powder was dissolved in 1± 0.02 liter of (80%) methanol, to get crude methanolic extracts of T. dioica. The extract was kept at room temperature for 48 hours, filtered and was kept open to evaporate the methanol. The extract was stored in refrigerator at (4 ^{0}C) for further investigation.

Phytotoxic Assay (Field Study):

Solution preparation

The aqueous stock solutions of 100mg /10ml (W/V) of methanolic crude extract of T.dioca was prepared by using the formula M1V1=M2V2.

Assay Procedure

An experimental plot of 10 m^2 was prepared for testing the seed growth of four experimental plants (Vignaradiata, Echinochloa crus-galli, Zea mays andBrachiariaramosa) against the extracts of Tamarixdioica. Four healthy seeds of each of the above mentioned plantswere sown in separate rows.After 5 days of germination, shoot length of all the seedlings developed from the grown seeds were carefully measured in cm with the help of a ruler and the average was calculated. Following the measurement was recorded, 100mg/ml, sample solution of the extracts from Tamarixdioicawas sprayed on the specified seedling rows whereas the control for each experimental plant was sprayed by tap water. Protector; a synthetic herbicide manufactured by Synjanta, Pakistan was sprayed on each 7^{th} row and accurate record was maintained. In order to avoid desiccation, all the plants were watered each 2^{nd} day. After five days of treatment, the shoot lengths were again measured and the average was recorded. The percentage was noted with respect to control. The fresh and dry weight was calculated by digital electronic balance and then average measurement was recorded. The difference in the soot length was noted between the treatments and their respective controls.

In-Vitro Phytotoxic Assay

Solution Preparation:
The stock solutions (10 ml) of the concentrations 10mg/10ml (W/V) of methanolic crude extract from Tamarixdioica was prepared by using the formula M1V1=M2V2. The stock solutions were diluted accordingly, up to 1000µg /ml, 600µg/ml and 200µg/ml.

Assay Procedure:
Assay Procedure:
For this assay modified protocol of Mc Laughlin (1998) was used. Solutions of different concentrations of the extract from Tamarixdioicaas mentioned under the section of solution preparation were applied to the seeds of the four tested plants. The seeds of Zeamaiz, Vignaradiata, Echinochloa crus-galliand Brachiariaremosa were washed with distill water (DW). All the seeds were then washed with 1% mercuric chloride for surface sterilization. The standard filter papers (Watsman No1) were set in autoclaved petriplates. Following sterilization, 5 ml solution of each concentration was loaded to the labeled petriplates, except their respective controls. The methanol was evaporated in order to avoid its toxicity on germination. The experiment was conducted in three replications by placing 4 seeds of each of Zeamaiz, Vignaradiata, Echinocoloa crus –galli, Brachiariaramosa in already marked separate petriplates at uniform spacing between the seeds. For the purpose of soaking the seeds to germinate, 5 ml of distilled water was added to each petriplate. The plants were placed in growth room. After 3 days and 5 days interval, length of shoot/ hypocotyls and radical /root were carefully measured with a scale in centimeters and the readings were recorded. The average increase in shoots and roots lengths were calculated. The percentage inhibition of root and shoot were compared with respect to those of the controls. Using electronic digital balance, the fresh and dry weight was recorded in grams.

Statistical Analysis

Usingslide writer and SPSS 13.0 computer software, the recorded data for In vitro and field study were separately analyzed.

Results and discussion:

Phytotoxic efficacy under field condition:
The present phytotoxic study revealed diverse results regarding the growth inhibition of the four tested plants Viz. Vignaradiata, Echinochloa crus-galli, Zea mays andBrachiariaramosa against different doses of the extracts from Tamarixdioica. 100mg/ml aqueous concentrations of sample solutions were used under field study. The extract from the selected plant (Tamarixdioica) depicted significant inhibitory effects against the tested plants (Table 1, 2). Significant reduction in the fresh and dry weights at($P<0.01$) of the four tested plants was observed against the extract. T. dioicacaused maximum reduction in fresh and dry weights of B. ramosa (4.±0.06, 0.2±0.003), while minimum reduction was shown by Vignaradiata (8.0±0.06, 0.7±0.008). Over all, Maximum inhibition (43.34%) was shown by B.ramosaagainst T. dioica under the field study as compared to the respective control (0.00).Based on the toxic effects of Tamarixdioicaextract under field conditions, the tested plants for growth inhibition were ranked as: B. ramosaa> E. crus-galli> Z. mays >V. radiate, respectively. The present results are in agreement with those of Javaidet al., (2009).

In Vitro Phytotoxic Efficacy:

The present phytotoxic studies also revealed a wide range of results regarding growth inhibition of the tested plants against the extract of T. dioicawhichwas applied to the seeds of V. radiata, E. crus-galli, Z. mays and B. Ramose sown in separate patridishaes. 1000µg/ml, 600µg/ml and 200µg/ml Methanolic concentrations of sample solution were used. The maximum inhibition was recorded at 1000µg/ml.

The results of our present screening assays revealed that the extract from T. dioicaas a biohebicide, effectively inhibited the growth of the tested plants. In the cases of T. dioica, significant inhibition was noted both in shoot and roots growth as compared to those of the control. The fresh and dry weights weresignificantly reduced (P

<0.05) after the 5th day of treatment. From Table 3, it is evident that T. dioicashowed maximum inhibition at shoot levels against B. raosa(50.97%), followed by Z. mays (45.83%), E. crus-galli (41.78%) and V. radiata(37.52%), while at root levels T. dioicadepicted maximum inhibition against E. crus-galli (54.99%), followed by B. ramosa (50.97%), Z. mays (44%) and V. radiata(43.54%), respectively. After the 5th day of treatment, fresh weights of all the treated groups was calculated and observed that T. dioica, significantly reduced the fresh weights 2.2 to 1.86 g) as well as dry weights (0.60 to 0.70) of Z. mays, V. radiata, E. crus-galliand B. ramosaat (P<0.05) at all the three level of concentration. Thus the reduction in fresh and dry weight of Z. mays indicated that T. dioica contains bioactive allelochemicals (Phenols and essential oils).

Using the sandwich method while examining the phytotoxic effect of the extracts from 14 plant species of plain areas on the growth of lettuce seeds, Fujii and Aziz, (2005) have also forwarded similar results which justify and support the findings of the present study.Over all, T. dioica caused significant reduction in the fresh and dry weights of E. crus-galli at all the three levels of concentration.The presently available literature relevant to phytotoxic investigation suggests thatTamarixdioicahas significant phytotoxic effects on plants species tested in the present study. At field level, the T. dioicashowed remarkable inhibitory effects against E. crus-galli, B. ramosa, V. radiata and Z. mays. The T. dioica showed maximum growth inhibition against B. ramosa (43.34 %), while minimum inhibition was recorded for V. radiata(34.93). T. dioica showed maximum inhibition against E. crus-galli (50.97%) and minimum against V. radiata(43.52%) at shoot levels, while at root levels maximum inhibition was revealed against E. crus-galli (54.49%) where as it was minimum against Z. mays (24.54). Our results in accordance with those forwarded previously, by Jamil, (2008). As a summary of our results, the plant under investigation (T. dioica) indicates the presence of phytotoxic chemicals which have significant phytotoxic efficacy against the control of weeds. The plant is therefore, recommended to be used effectively as biological control measure for weeds.

Table 1.Phytotoxic Efficacy of Tamarixdioicaagainst Vignaradiata, Z. mays, B.ramosaandE.cruss-galli(Field study).

Sample plants	S.G. (cm) before spray	S.G. (cm) after spray	(%) Growth	(%) Inhibition	Total F.W. (gram)	Total D.W (gram)
V.radiata	2.07±0.003	4.75±0.04	65.07±0.2	34.93±0.02	8±0.06	0.5±0.002
Control	2.50± 0.002	7.30±0.05	100±0.3	0.00±0.01	12±0.06	3.6±0.003
Z. mays	1.85±0.012	2.65±0.09	59.48±0.002	36.52±0.06	6±0.03	0.40±0.003
Control	1.65±0.009	3.66±0.02	100 .0±0.03	0.00±0.00	10.3±0.04	2.60±0.008
B.ramosa	2.22±0.003	3.50±0.04	57.66±0.02	43.34±0.002	04±0.06	0.2.4±0.003
Control	2.67±0.002	6.07±0.04	100.0±0.04	0.00±0.007	18.5±0.07	3.6±0.003
E. cruss-galli	1.95±0.012	2.25±0.09	61.48±0.002	39.52±0.06	5±0.03	0.3.10±0.003
Control	1.65±0.009	3.66±0.02	100 .0±0.03	0.00±0.00	10.3±0.04	2.60±0.008

Mean ± SD
S.G. = Shoot growth; F.W. = Fresh weight; D.W. =Dry weight.

Table 2. Phytotoxic Efficacy of Tamarixdioicaagainst Vignaradiata, Z. mays, B.ramosaandE.cruss-galli(In vitro study).

Sample Plants	Conc. µg /ml	S.G. (cm) after 3rd day	S.G (cm) after 5th day	(%) Inhibition
V. radiata	1000	1.95±0.004	2.95±0.03	37.52±0.06
	600	2.45±0.003	3.2±0.09	33.67±0.05
	200	2.66±0.02	3.6±0.09	25.21±0.04
Control	DW	3.15±0.06	4.75±0.05	0.0±0.00
Z. mays	1000	1.09±0.06	1.09±0.06	2.21± 0.05
	600	1.22±0.07	1.22±0.07	2.63±0.07
	200	1.65±0.05	1.65±0.05	2.84±0.03
Control	DW	2.49±0.09	2.49±0.09	4.91±0.08
E. cuss-galli	1000	0.57±0.04	0.57±0.04	3.1±0.06
	600	0.8±0.007	0.8±0.07	3.23±0.09
	200	0.43±0.08	0.43±0.08	3.53±0.05
Control	DW	1.68±0.00	1.68±0.00	4.2±0.02
B. ramose	1000	0.75±0.04	2.47±0.008	45.83±0.06
	600	0.85±0.07	2.85±0.06	37.50±0.09
	200	0.91±0.06	3.09±0.05	32.24±0.04
Control	DW	2.25±0.08	4.56±0.04	0.00±0.008

Mean ± SD: S.G. = Shoot growth

Table3**. Phytotoxic Efficacy of Tamarixdioica against Vignaradiata, Z. mays, B.ramosaandE.crussgalli (In vitro study)**

Sample Plants	Conc. µg /ml	R.G. (cm) after 3rd day	R.G. (cm) after 5th day	(%) Inhibition	Total F.W. (gram)	Total D.W. (gram)
V. radiata	1000	1.15±0.006	2.62±0.0.07	41.78±0.08	1.86 ± 0.09	0.70 ± 0.09
	600	1.48±0.003	2.8±0.09	37.78±0.09	1.99 ± 0.08	0.50 ± 0.06
	200	1.75±0.007	2.95±0.50	34.44±0.00	2.2± 0.003	0.6 0± 0.12
Control	DW	2.28±0.00	4.05±008	0.00±0.00	4.04 ± 0.05	1.62 ±0.09
Z. mays	1000	1.15±0.006	2.62±0.0.07	41.78±0.08	1.86 ± 0.09	0.70 ± 0.09
	600	1.48±0.003	2.8±0.09	37.78±0.09	1.99 ± 0.08	0.50 ± 0.06
	200	1.75±0.007	2.95±0.50	34.44±0.00	2.2± 0.003	0.6 0± 0.12
Control	DW	2.28±0.00	4.05±008	0.00±0.00	4.04 ± 0.05	1.62 ±0.09
E. cuss-galli	1000	0.11±0.005	1.76±0.06	50.97±0.04	2.06 ± 0.03	0.7 0± 0.02
	600	0.15±0.003	2.28±0.04	36.49±0.09	2.09 ± 0.03	0.93 ± 0.02
	200	0.38±0.005	2.93±0.02	18.38±0.08	2.95± 0.08	1.04 ± 0.05
Control	DW	1.40±0.003	3.59±0.05	0.00±0.05	4.95 ± 00.4	1.92 ±0.02
B. ramosa	1000	2.12±0.06	3.02±0.02	44.28±0.03	2. 00± 0.08	0.70 ± 0.05
	600	2.42±0.05	3.9±0.05	28.04±0.04	2.29 ± 0.05	0.80 ± 0.06
	200	2.57±0.03	4.12±0.06	23.99±0.04	2.60± 0.02	1.04 ± 0.09
Control	DW	3.11±0.05	5.42±0.02	0.00±0.04	6.04 ± 0.04	2.92 ±0.08

Mean ± SD: R.G. = Root growth; F.W. = Fresh weight; D.W. =Dry weight.

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