PRINT: ISSN 0973-5070 ONLINE: 2456-6772
Ethno Med, 10(2): 106-110 (2016)
DOI: 10.31901/24566322.2016/10.02.01

Inhibitory Potential of *Smilax medica* on Growth of Maize (*Zea Mays*) Grown in District Bannu

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KEYWORDS Allelopathic. Antioxidant. Herbicides. Phytotoxicity. Weeds

ABSTRACT *Smilax medica* is traditionally used as an anti-microbial, antifungal, antioxidant and anti-inflammatory agent. Current study is designed to evaluate the allelopathic properties of the crude methanolic extract of *Smilax medica* against the root and seedling growth. Roots of *Smilax medica* were dried grounded to powdered form and were then saturated with methanol. Phytotoxicity activity of *Smilax medica* plant was done against *Zea Mays* seeds. Result obtained from the study showed maximum root and stalk inhibition and minimum stalk growth. Due to the presence of bioactive element, that is allelochemicals in the roots of *Smilax medica*, difference was seen in the fresh and dry weight of *Zea Mays*. Plant product based natural herbicides could serve as an alternative to synthetic herbicides. Use of *Smilax medica* may be promoted as a natural herbicides.

INTRODUCTION

Plants with medicinal qualities are used all over the globe for the treatment of numerous diseases because they have minimum or usually no side effects in competition to synthetic chemicals (Khan et al. 2009). One of the chief biotic menaces are weeds which interfere with cotton field through rivalry and allelopathy which eventually cause substantial reduction in crop production. Utilizing allelopathic properties of plants provide a great chance to control weeds effectively (Muhammad et al. 2016). Allelopathy is the release of compounds which have both promoting and inhibitory effects. These compounds are actually secondary metabolites which are produced by the plants during some specific reactions (Bhowmik and Inderjit 2003). Whenever phytotoxic are leached in the adjacent rhizosphere, then they strongly inhibit with the propagation and growth of neighboring unwanted species of plants (Muhammad and Majeed 2014). Allelochemicals are normally present in both the reproductive and vegetative regions of the plants but they are also present in the roots of the plants (Muhammad et al. 2016). The allelopathic influence which is caused by the chemicals released by the plants, may either carry stimulatory or inhibitory potential depending on the concentration of the chemicals (Mahmood et al. 2013). These secreted compounds obstruct cell division, seed germination, leaf expansion, inhibit stomatal movement (Muhammad et al. 2016). Damages by weed to crops are estimated about 13 percent every year. To overcome this problems, a significant investigation resulted in the production of effective herbicides (Bridges 1994). Plant having medicinal values play an important role to control the harmful effects caused by the pathogens (Sahreen et al. 2010; Khan et al. 2009; Khan et al. 2010a, b). Various treatments to infectious diseases all over the globe and especially in Pakistan are done through herbal drugs (Khan et al. 2009). Allelopathic effects are also reported by (Khan et al. 2010c). Extensive use of synthetic herbicides leads in the production of resistant weed species (Heap 2014) as well as pollution of the environment (Akhtar and Roger 2009). Resolving the issue, plant's extracts play their role against these weeds. In this context, an emerging interest of researchers is observed throughout the world (Fujii et al. 1991; Piyatida and Kato-Noguchi 2010). The medicinal plant's extract are now thoroughly used to see their effectiveness against these weeds and other harmful pathogens (Mominul Islam and HisashiKato-Noguchi 2014).

Genus *Smilax* contain about 350 species, which are widely distributed in tropical region of East Asia, South and North America (Marc et al. 2005). Some species of genus *Smilax* were found to possess anti-inflammatory (Jiang and Zu 2003), NO-modulating (Chung et al. 2003) and anti-leprosic activity (Paris et al. 1952), but till now no active biological essential activity have been reported on neither *Smilax medica* nor on its constituents (Marc et al. 2005). *Smilax medi-*

ca belongs to family *Smilacacea*, traditionally used as anti-microbial, anti-fungal, antioxidant, and anti-inflammatory agent. It also plays some other vital role as it detoxify organs, cleanse blood, aids absorption, kills bacteria, rise urination and most obviously helps in the fortification of liver. People of central and South America are using the roots of *Smilax medica* for centuries in order to cure sexual incapabilities, skin diseases and as a tonic for different types of flaws. In Pakistan, it has been used by the people for headaches and joint pain and against common cold. Roots of *Smilax medica* have a long previous history of use for syphilis and other sexually-transmitted diseases throughout the globe. Due to its repute as a blood purifier, it was registered as an official herb in the *U.S. Pharmacopoeia* as a syphilis treatment from 1820 to 1910 (Leslie Tayler 2012).

The current study was carried out in order to see the inhibitory effects of plant on maize seeds grown in district Bannu.

MATERIAL AND METHODS

Plant Collection

The whole plant of *Smilax medica* was collected from Ghoriwala area, district Bannu and was properly identified by a botanist at Department of Botany, UST Bannu. The plant specimen was deposited in the Herbarium, department of Botany, UST Bannu for future reference as voucher no WK-107. The plant sample was cleaned and shed dried, crushed mechanically up to mesh size 0.1mm.

Plant Extraction

200gm powder of *Smilax medica* was extracted in 1000 ml methanol with constant shaking at shaking machine for 5 hours and then was placed at room temperature for 7 days. The plant material was filtered by using Whatman filter paper No.1 and the filtrate was concentrated with the help of the Hedolph Rotavap. The rotavap product was lyophilized immediately. The lyophilized powder was stored at 4°C for further use.

Phytotoxic Assay

The plant growth of *Zea mays* was tested against the phytotoxic activity of *S. medica* methanolic extract. This standard protocol of McLaughlin and Rogers (1998) was used to assess the activity. Stock solution was prepared and different concentrations of the stock solution of *S. medica* extract were prepared i.e. 3 mg/ml, 1.5 mg/ml, 0.75 mg/ml and 0.37 mg/ml. 500µl from all concentrations was taken and was put on the pre-labelled petri plates. Distilled H_2O was used as a control. Filter paper were placed in the plates before pouring the samples. 5 ml distilled H_2O was spread on each plate. Methanol was evaporated before applying water. Five seeds of *Zea mays* from already washed by 1% $HgCl_2$ and soaked in distilled H_2O. Seeds were kept in each petri dish. All the petri dishes were incubated in the growth chamber and after five days, the first reading, that is, length of root/ radical was taken and distilled water to each plate was added to maintain the moist condition. After ten days, the last reading of the seed's growth was taken and the percent inhibition of growth was calculated. Fresh and dry weight was also recorded.

RESULTS

During the study allelopathic properties of the crude methanolic extract of *Smilax Medica* roots were identified. Phytotoxicity activity of *Smilax Medica* plant was carried out against *Zea Mays* seeds. Result obtained from the research disclosed that crude methanolic extract of *Smilax Medica* roots results in maximum root and stalk inhibition and minimum stalk growth on 3 mg/ml with respect to control. The effect of *Smilax Medica* roots on stalk and roots growth of *Zea Mays* is as follows; 3 mg/ml > 1.5 mg/ml > 0.75 mg/ml > 0.37 mg/ml. At the same time due to the presence of bioactive element, that is, allelochemicals in the roots of *Smilax Medica*, difference was seen in the fresh and dry weight of *Zea Mays.*

Phytotoxicity Assessment

Effect of Smilax medica on Stem and Root Growth (After 5 Days Readings)

Allelopathic effect of *Smilax medica* was assessed against maize (*Zea mays*) growth under appropriate conditions in the growth chamber. Methanolic extract of *Smilax medica* (MESM) showed minor stem growth and maximum stalk inhibition on 3 mg/ml as compared to

control. The root growth at 3mg/ml, 1.5mg/ml, .75mg/ml, and .37mg/ml was 2.9cm, 3.6cm, 4.2cm, 5.8cm respectively. Whereas at control the root growth of maize is 5.9 cm. Hence we can see a significant change in the root growth as compared to the change in the concentration of the S*milax medica* roots extract (Table 1).

Table 1: Effect of *S. medica* on maize (*Zea mays*) root and stem growth after 5 days (cm)

Samples	*Root growth*	*Stem growth*
Control	5.9	6.6
3 mg/ml	2.9	3.5
1.5 mg/ml	3.6	4.1
0.75 mg/ml	4.2	4.7
0.37 mg/ml	5.8	6.4

Similarly methanolic extract of *Smilax medica* showed minimum root growth and maximum root inhibition at concentration of 3mg/ml. The stem growth at 3mg/ml, 1.5mg/ml, .75mg/ml, and .37mg/ml is 3.5cm, 4.1cm, 4.7cm, 6.4cm respectively. Whereas at control the stem growth of maize seed is 6.6 cm. We can clearly see a handsome change in the stem growth as compared to the change in the concentration of the *Smilax medica* roots extract after 5 days of examination.

The effect of *Smilax medica* on stalk and root growth after 5 days is as under; 3 mg/ml > 1.5 mg/ml > 0.75 mg/ml > 0.37 mg/ml.

Effect of Smilax Medica Plant on Stalk and Root Growth after 10 Days

Minimum stalk growth and maximum stalk inhibition was seen after 10 days of experiment on methanolic extract of *Smilax medica* on 3mg/ml as compared to control. At the same time minimum root growth and maximum root inhibition was seen on methanolic extract of *Smilax medica* on 3mg/ml. The root growth after 10 days of assessment at 3mg/ml, 1.5mg/ml, .75mg/ml, and .37mg/ml is 3.5cm, 4.8cm, 6.4cm, 7.7cm respectively. Whereas at control the root growth of maize is 6.4cm (Table 2). Hence we can see a significant retardation in the root growth as compared to the change in the concentration of the *smilax medica* roots extract.

Similarly methanolic extract of *Smilax medica* showed minimum stem growth and maximum stem inhibition at concentration of 3mg/ml after 10 days of examination. The stem growth at 3mg/ml, 1.5mg/ml, .75mg/ml, and .37mg/ml is 4.2cm, 5.3cm, 7.1cm, 8.3cm respectively. Whereas at control the stem growth of maize seed is 7.2 cm. We can clearly see a handsome change in the stem growth as compared to the change in the concentration of the *smilax medica* roots extract after 5 days of examination.

Table 2: Effect of *S. medica* on maize (*Zea mays*) root and stem growth after 10 days (cm)

Samples	*Root growth*	*Stem growth*
Control	6.4	7.2
3 mg/ml	3.5	4.2
1.5 mg/ml	4.8	5.3
0.75 mg/ml	6.4	7.1
0.37 mg/ml	7.7	8.3

The effect of *Smilax medica* on stalk and root growth after 10 days is as under; 3 mg/ml > 1.5 mg/ml > 0.75 mg/ml > 0.37 mg/ml.

Effect of Smilax Medica Plant on Fresh and Dry Weight

Difference in the reduction of fresh and dry weight was observed in methanolic extract of *Smilax medica* after 10 days, which showed the presence of bio-active allelochemicals in the methanolic extract of *Smilax medica.* At the end of experiment, when seedlings were dried under suitable conditions, it was seen that the mass of methanolic extract of *Smilax medica* was efficiently less than as compared to that of control. Fresh weight at 3mg/ml, 1.5mg/ml, .75mg/ml and .37mg/ml was 1.50gms, 2.75gms, 3.48gms, 3.93gms respectively, whereas dry weight of the maize seeds at 3mg/ml, 1.5mg/ml, .75mg/ml and .37mg/ml was .95gms, 1.77gms, 2.39gms, 2.83gms (Table 3) respectively. The fresh and dry weight of the seeds in control was 4.28gms and 2.94gms respectively.

Table 3: Dry and fresh weight after 10 days (gms)

Samples	*Fresh weight*	*Dry weight*
Control	4.28	2.94
3 mg/ml	1.50	0.95
1.5 mg/ml	2.75	1.77
0.75 mg/ml	3.48	2.39
0.37 mg/ml	3.93	2.83

DISCUSSION

Globally, medicinal plants are used since ancient time for the treatment of numerous diseas-

es by having minimum or no side effects compared to synthetic chemicals (Khan et al. 2009). In the present study crude methonlic exttact of *Smilax medica* was evaluated as a potent phytoxic potential. Previously the metanolic extract of the roots of the plants was tested for antifungal potential only (Sautour et al. 2006). The rhizome of some of the *Smilax* species have been used since long time as both food and folk medicine around the globe (Chaun et al. 2015). Large number of bioactive chemical constituents are found within plants naturally in most proficient ways with specific selectivity. Germination of plants is suppressed when exposed to allelopathic compounds (Asgharipour 2011). Phytotoxicity of medicinal plants is very important aspect because growth inhibition of weeds and other unwanted plants (Wasi et al. 2015). In the present study the retardation of germination and subsequent growth of maize seeds showed that allelochemicals possessed by *Smilax medica* roots perhaps directly affect the maize seeds. Not every but some of the medicinal plants might possess phytotoxic activities which inhibit the growth of unwanted plant and weeds. Current study showed that methanolic extract of *Smilax medica* have significant herbicidal potency, which might be the presence of allelochemicals. The result from the phytotoxic bioassay on methanolic extract of *Smilax medica* showed that they inhibited the growth of both stalk and roots of *Zea Mays* comparatively lower to other medicinal plants. The allelopathic effect of sorghum and sunflower with inhibition of propagation and growth of cotton, wheat, rice and mungbean in the laboratory, pot and field experiments and weeds have been documented (Muhammad et al. 2016). Present study showed that crude methonolic extract could be used as an alternative medicine by having novel bioactive metabolites. The allelopathic chemicals hinder cell division, seed germination, leaf expansion, stomatal movement, respiration (Muhammad et al. 2016), which is observed in the present study by the reducing the leaf and shoot size. Also, in the current study, seed germination was retarded in case of both shoots and roots after 5 days of assessment. The root elongation was limited to 2.9cm at 3mg/ml and stem elongation was 3.5cm at 3mg/ml. When this data was taken into account with the control where the roots elongation was 5.9cm and stem elongation was 6.6cm, indicating the inhibitory role of methanolic extract of *Smilax medica*. Similar results of seed growth retardation was observed after 10 days of assessment, as reported by (Kordali et al. 2008) stating that Turkish *Origamum acutidens* totally retarded the growth of roots and shoots. Similarly, Javid in 2009 reported that the water extracts of *Datura alba* and *Withania somnifera* containing potent bioactive metabolites which significantly inhibited the growth of shoots and roots of *Rumex dentatus* which is one of the highly feasible weeds in wheat. Further studies should be carried out to evaluate their herbicidal effects in vivo and the mechanisms of action by which effects are mediated.

CONCLUSION

Control of weeds is one of the greatest challenging tasks in crop production, which can affect the overall yield of the crop. Access use of synthetic herbicides can cause severe environmental pollution besides being developed herbicide resistant weed biotypes.

RECOMMENDATIONS

Plant product based natural herbicides could serve as an alternatives to the synthetic herbicides that are biodegradable and environment friendly, which could be easily obtained from *Smilax medica*.

ACKNOWLEDGEMENTS

Praying and dedicating my research article to All Mighty Allah, the God of knowledge and wisdom. The authors are grateful to Adeel Khan and Wasi Ullah Khan for their insightful suggestions during manuscript write up. The research work would have been a dream, had it not been enlightened, by my well-wishers and the above respectable.

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Paper received for publication November 2015
Paper accepted for publication April 2016