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Table: -, Figs : 2

SHORT COMMUNICATION

Response of purple nutsedge (*Cyperus rotundus* L.) to crop extracts prepared in various solvents

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ABSTRACT

Plant allelochemicals have different solubilities in organic solvents. Laboratory studies were conducted to know the better solvent for extraction of sorghum (*Sorghum bicolor* L. Moench), *Brassica* (*Brassica napus*) and sunflower (*Helianthus annuus*) allelochemicals. Extraction efficiency of five solvents (water, methanol, ethanol, xylene and n-hexane) was compared with distilled water (control) and determined their effects on purple nutsedge growth. Extracts from three crops studied were used to irrigate as herbicides in pots under room conditions. Extracts of sorghum, *Brassica* and sunflower inhibited the shoot length and fresh and dry weight of purple nutsedge. The inhibition order of solvents was: water (highest inhibition) ≥ methanol ≥ ethanol >xylene > n-hexane and the order of inhibition of crop extracts was sorghum > *Brassica* > sunflower. Extracts with 50% concentration cause more suppressive effects than 5% concentration, because higher concentrations are supposed to have more amounts of phytotoxins in the extract. These findings showed that water is best solvent to extract naturally occurring allelochemicals in crop plants.

Keywords: Allelochemicals, *Brassica*, crop extracts, purplenutsedge, sorghum, sunflower.

INTRODUCTION

Laboratory bioassays are an integral part of allelopathic research to generate data to support the field studies (15,16). Chemical characteristics of solvent and diverse structure and composition of the natural products ensures that product recovery in extracts will show different behavior in different solvent system (36). Water, methanol, ethanol, xylene and n-hexane are the solvents to extract the natural products of different molecular weights (27,28,29). Large molecular weight compounds are soluble in water, while lower molecular weight compounds are soluble in methanol (28). Selection of a solvent is very important for the best extraction of allelochemicals from plant material (31). Solvents with high polarity extract greater quantity of total allelochemicals than solvents of less polarity or non-polar ones (24). Many allelochemicals are water soluble owing to its high polarity,

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strong dipolar moment and very weak ionization (13). Water is inexpensive and eco-friendly solvent, ideal for allelochemicals extraction (19).

Purple nutsedge (*Cyperus rotundus* L.) is worst weed, widely distributed through out the tropics and subtropics in 52 different crops and in 92 countries (4,37). It is very common throughout South East Asia (30). In Pakistan, it is most common summer weed in Indus valley in major field crops (cotton, sugarcane and maize) and causes 23-89% yield reductions in various crops (5,14). Its control is difficult by manual, mechanical and chemical methods due to various modes of propagation (seeds, rhizomes, tubers) and varying degree of dormancy (4,9). Moreover, chemical weed control is generally unsafe and enhances human and environmental health risks (10). Hence, there is need to investigate the environmentally safe and sustainable weed management strategies to reduce the cost of production.

Crop allelopathy is an environment friendly, economical and sustainable approach to overcome the problems raised by synthetic chemicals (9,38). Crop plants like sorghum (1,6), sunflower (2) and *Brassica* (12,42) exert allelopathic effects on the associated weeds. The allelopathic effects of sorghum spp. (*Sorghum bicolor*, *Sorghum vulgare*, *Sorghum sudanese* and *Sorghum halepense*) and bioactive allelochemicals have been identified in sorghum forage (11, 18, 34). Sunflower extracts, root exudates and leaf leachates have many allelochemicals viz., chlorogenic acid and isochlorogenic acids (43), sesquiterpenes lactones, helivypolide D and helivypolide E, and the bisnorsesquiterpene annuionone D (25), compounds with a heliespirone skeleton: heliespirones C, D, and E (26), heliannuols A-E (phenolic sesquiterpenes; 41), hydroquinone, gentistic acid, beta-resorcyclic acid, vanillic acid, caffeic acid, ferulic acid and quercetin (35). Sunflower is allelopathic to many weeds (23, 32) similar to herbicide treatments (21). Aqueous extracts from leaf and stem of 13 sunflower varieties showed mixed responses to seed germination of weeds, however, leachates of dry leaves and stem inhibited the growth of weeds (22). Turk *et al.* (42) investigated the allelopathic effects of various black mustard (*Brassica nigra* L.) plant parts on alfalfa (*Medicago sativa* L.) germination and seedling growth. He found that aqueous extracts of all plant parts significantly inhibited the alfalfa seed germination and seedling growth than distilled water control. Iqbal and Cheema (17) reported effective control of purple nutsedge in the field with crop water extracts from sorghum, sunflower and *Brassica*.

This research was aimed (i) to find the suitable solvent because organic compounds in the plant materials are multicomponent mixtures, their separation and purification need suitable solvent for extraction and (ii) the allelopathic control of purple nutsedge with different crops extracts.

MATERIALS AND METHODS

These studies were conducted in 2005. The treatments consisted of two factors: (i) Plant materials for extraction : 3 (Sorghum cv. 'JS-263', *Brassica* cv. 'Hyola 401' and sunflower cv. 'Hysun-33') and (ii). Solvents: 5 (water, methanol, ethanol, n-hexane and xylene). The solvents were used to extract the phytotoxins from the plant material collected from our Research Farm. It was air dried at about 35±3°C, ground in a mill (CYCLOTEC 1093 sample mill, Hoganas- Sweden) and stored in airtight jars. Ten g of each plant material were used to obtain extracts using different solvents [methanol, ethanol, n-hexane, xylene (Merck Germany) and distilled water] in Soxhlet extractor. The process was repeated four times. Extracts were then evaporated to dryness under reduced pressure at 50°C by using a rotary evaporator (EYELA, Rotary Vacuum Evaporator. N.N. Series equipped with an Aspirator and a Digital Water Bath SB-651, Rikakikai Co. Ltd. Tokyo, Japan). Initially the dried extract was dissolved in 30 ml distilled water. Two concentrations [Ratio 1:1 (50% concentration) and 1:20 (5% concentration)] of these extracts were prepared in distilled water. The sprouted tubers of purple nutsedge (*Cyperus rotundus*) were collected from our fields in the morning. Their shoots were cut and one tuber of purple nutsedge was transplanted in each pot (9 cm x 9 cm) with 75 g sandy clay loam soil (sun dried and sieved). Crop extracts were applied at 12 ml per pot as per treatments just after tubers transplanting. Control treatment received only 12 ml distilled water per pot. Pots were placed under room conditions (temperature 30±2ºC). Purple nutsedge shoot lengths (cm) and dry weight (g) were recorded 40 days after transplanting. Inhibition percentage over control (Distilled water application) was calculated from these data. The experiment was laid out in completely randomized design (CRD) with four replicates. Water was applied when required to avoid water stress. Data were analyzed for the statistical significance by using Statistical software package" MSTAT C" (3). Least significance difference (LSD) test was applied at 5% level of significance to compare treatment means. Figures were drawn in MS Excel (Microsoft Office manufactured by Microsoft Corporation, New York, USA).

RESULTS

Shoot length

The 5 and 50% concentrations of sorghum aqueous extracts caused maximum inhibition in shoot length followed by methanol extracts at 50% concentration (Fig. 1). However, the extracts in n-hexane were least inhibitory to purple nutsedge shoot length (Fig. 1). The general order of inhibition was: extract in water > methanol > ethanol > xylene > n-hexane. Higher concentrations of extracts (50%) caused more inhibition in purple nutsedge shoot length than the lower concentration (5%; Fig.1).

The water extracts, methanol and ethanol extracts of *Brassica* at 50% concentration were more inhibitory (Fig. 1). The *Brassica* extracts in xylene and n-hexane of 50% concentration also suppressed the purple nutsedge growth but was less suppressive than water and methanol extracts. N-hexane (5% conc.) extract was least effective, but was similar to ethanol and xylene extract. The general order of suppression of purple nutsedge shoot length was: extract in water > methanol ≥ ethanol > xylene ≥ n-hexane. Purple nutsedge shoot length was drastically suppressed at higher concentration (50%) than at lower concentration (5%; Fig.1).

The sunflower extracts in water and methanol at 50% concentration proved most inhibitory to shoot length (Fig. 1). N-hexane was least harmful, however, it was identical to ethanol and xylene extract at lower concentration (5%). The inhibition of purple nutsedge shoot length with sunflower extracts in different solvents followed the order: in water ≥ methanol > ethanol > xylene > n-hexane.

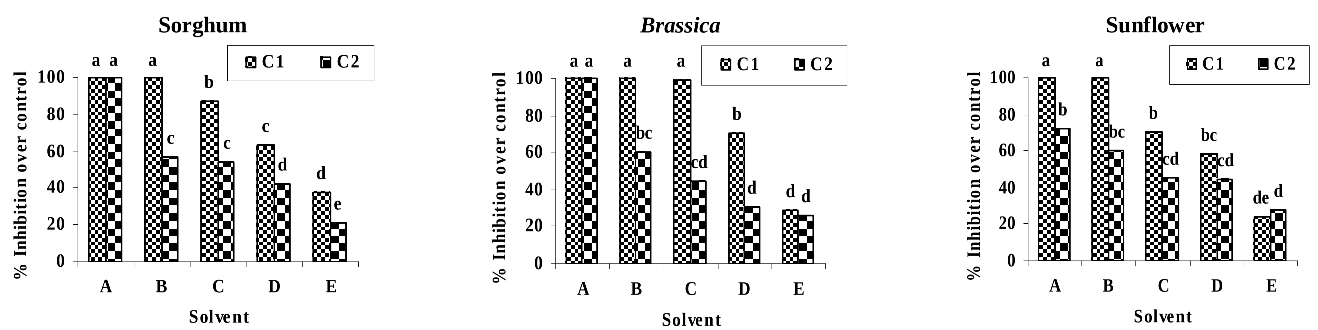

Figure 1. Effect of crop extracts of sorghum, Brassica and sunflower on shoot length of purple nutsedge. Inhibition percentage was calculated over control (Distilled water); (A) water extract (B) methanol extract (C) ethanol extract (D) xylene extract (E) n-hexane extract; C1= 50% concentration, C2= 5% concentration.

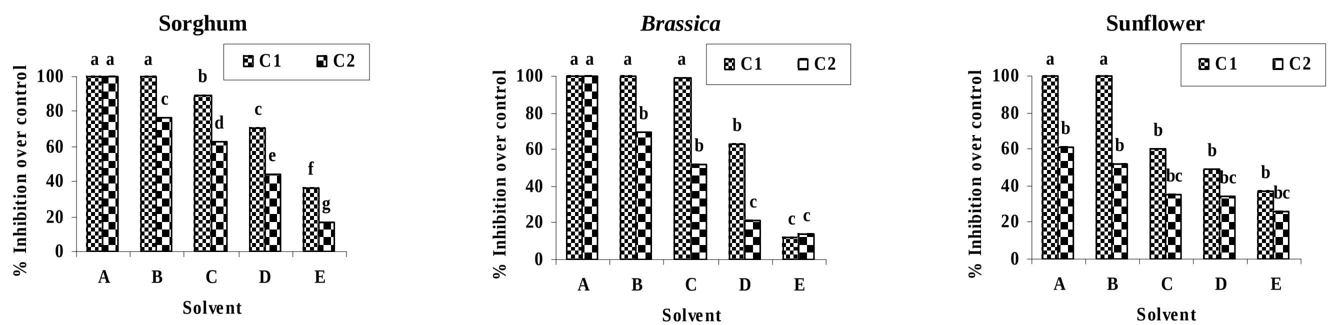

Figure 2. Effect of crop extracts of sorghum, sunflower and Brassica on dry matter of purple nutsedge. Inhibition percentage was calculated over control (Distilled water); (A) water extract (B) methanol extract (C) ethanol extract (D) xylene extract (E) n-hexane extract; C1= 50% concentration, C2= 5% concentration.

Dry weight

The sorghum water extracts at 5% and 50% concentration caused maximum inhibition in dry weight and was similar to methanol extracts at 50% concentration (Fig. 2). Sorghum extract in n-hexane was least effective. The sorghum extract inhibition followed the order: in water > methanol > ethanol > xylene > n-hexane. Higher concentrations (50%) of sorghum extract caused more suppression in dry weight of purple nutsedge than lower concentration (5%; Fig. 2).

Maximum reduction in dry weight was observed in *Brassica* extracts in water at 5 and 50% concentrations and methanol and ethanol extracts at 50% concentration. N-hexane at both concentrations (5 and 50%) and xylene extract at 5% concentrations did not affect the dry weight. The higher concentrations of extracts were more effective in suppressing purple nutsedge than lower concentrations.

Sunflower extracts (50%) in water and methanol proved most suppressive of dry weight of purple nutsedge (Fig. 2). However, there was no significant difference among all the solvent extracts at lower concentrations. Purple nutsedge dry weight was drastically decreased at higher concentration (50%) in water and methanol than at lower concentration (5%; Fig. 2). All other solvent extracts, showed similar reduction at both concentrations (5 and 50%).

DISCUSSION

Extracts of sorghum, *Brassica* and sunflower in different solvents inhibited the shoot length and dry weight of purple nutsedge. Water extracts proved most inhibitory to purple nutsedge grown in both concentrations and inhibition was similar to methanol at 50% concentration. The inhibition order of growth with different solvents was: water ≥ methanol ≥ ethanol >xylene > n-hexane and for crops as sorghum (highest inhibition)> *Brassica* > sunflower. The differences in inhibition of purple nutsedge with different solvent extracts may be attributed to the extraction capability of respective solvent. The solvents with high polarity (e.g. water) dissolved more quantity of allelochemicals than solvents with less polarity or non polar (28,39). Lin and Giusti (24) extracted isoflavones from pulverized Manokin soybeans using six solvent systems, which are the combination of three polarity levels (83% acetonitrile, 80% methanol, and 58% acetonitrile) and two acidity levels (Nonacidified and acidified). The solvent with higher polarity extracted higher amount of total isoflavones. For individual isoflavones, 58% acetonitrile (Highest polarity) extracted either the highest amount or similar to other solvents, while 83% acetonitrile (lowest polarity) extracted either the lowest amount or no more than other solvents. Mallik (25) reported that high molecular weight phytotoxins are soluble in water, while, low molecular weight phytotoxins are soluble in alcohol.

Many researchers have proved that water is the best solvent to extract allelochemicals from different plant materials. Solomon and Bhandarai (40) found the water a better solvent to extract allelochemicals followed by methanol and chloroform. Chun *et al*. (8) observed that phenolic compounds from four weed species were greater in the aqueous extract than in the methanol extract. Chon *et al*. (7) stated that the aqueous extracts from *Lactuca sativa*, *Xanthium occidentale* and *Cersium japonicum* were completely inhibitory to lucerne root growth, but hypocotyls growth of lucerne was less sensitive. Although allelopathic effects of methanol extracts were much less than coumarin or alachlor. The hazardous organic solvent used in extraction processes can be replaced with water (20). Naczk *et al*. (33) found methanol the most effective alkanol used to extract total flatulence-causing sugars, while isopropanol exhibited the poorest extraction efficiency. They added that the removal of flatulence-causing sugars depend on their molecular weight, interaction with the seed components and polarity of the solvent system used.

However some prominent allelochemicals that are not soluble in water e.g. sorgoleone in sorghum cannot be extracted by water. Pinelo *et al*. (36) subjected byproducts of grape to an extractions process and found the decreasing capacity order to extract soluble materials as ethanol> methanol > water, methanol was the most selective for extracting phenolic compounds. Moller and Nyberg (31) suggested the selection of extraction solvent mixture for achieving the true analytical value. Iqbal and Cheema (17) suggested the use of water extracts in combination with lower doses of herbicides for effective control of weeds. To have larger amount of extracts, it is impossible to take extracts in different expensive and hazardous organic solvents.The water is an inexpensive and environment friendly and ideal solvent for extraction (19) of many allelochemicals, these can be used directly with herbicides to control weeds.

Extracts with 50% concentration showed higher suppressive effects than 5% concentration, because higher concentrations are supposed to have higher amounts of phytotoxins in the extracts. These results agree with Mallik *et al.* (29) who stated that germination of radish seeds was reduced by 40 and 95% at 2 and 4 mg g^{-1} sand amended with unextracted lambsquarter shoots, respectively. He further stated that shoot dry weight and plant height were also significantly reduced at 4 mg g^{-1} but not at 2 mg g^{-1} concentration.

CONCLUSIONS

These studies showed that water is the best solvent to extract naturally occurring allelochemicals present in allelopathic plants biomass. Higher concentrations of extracts were more inhibitory to purple nutsedge due to higher amount of phytotoxins than the lower ones.

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