Allelopathic interference of *Ageratum conyzoides* L. against some crop plants

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Summary *Ageratum conyzoides* L., commonly known as billygoat weed, is an annual aggressive weed native to tropical America and has invaded and is now naturalised in several parts of India. It severely infests cultivated land and interferes with the growth and development of crops. A study was conducted to explore the nature and mechanism of allelopathic interference of the weed against three crop plants, viz. chickpea (*Cicer arietinum* L.), mustard (*Brassica campestris* L.) and rice (*Oryza sativa* L.). Test crops were grown under green house conditions in soil collected from an area heavily infested with *A. conyzoides*. A significant reduction in seedling growth and dry weight were observed compared with the control (grown in uninfested soil). Further, growth studies were carried out in soils amended with weed residues. The growth and dry weight accumulation of crops was severely reduced in the amended soils, thereby indicating that residues of *A. conyzoides* release some inhibitory substances in the soil. Quantitative analysis showed these to be phenolics. An appreciable amount of phenolics was also detected in the soils amended with residues. Likewise, phenolics were also present in the soil collected from the area infested with *A. conyzoides*. The study indicated that *A. conyzoides* might exert allelopathic effects towards these crops by releasing water-soluble phenolics in the soil.
Keywords Billygoat weed, allelopathy, phenolics, rice, chickpea, mustard.

INTRODUCTION

Ageratum conyzoides L. (family Asteraceae), commonly known as billygoat weed, is an annual, aromatic invasive weed native to tropical America. It is found extensively in various tropical parts of the world including South-East Asia. In India also the weed can be seen growing luxuriantly in various places especially in the hilly areas. Though a weed of cultivated lands, it can grow well in a variety of habitats such as grasslands, wastelands and other vacant areas. In India the weed is known to interfere with the growth and establishment of both summer and winter season crops causing huge economic losses to the farmers. However, no exact figures are available regarding the percent loss, yet infestation of *A. conyzoides* has even forced some farmers to abandon their fields (Negi 1988). Likewise, in the rangelands the interference of the weed adversely affects the availability of native grasses or, in other words, causes fodder scarcity. All these observations suggest that there must be some interference mechanism adopted by the weed that leads to its dominance over other vegetation. Allelopathy is a well-known interference mechanism in which living or dead plant parts, including litter, release chemicals exerting an effect (mostly negative) on the associated plants (Wardle *et al*. 1998). Most of the weeds that are particularly invasive in nature have adopted this as part of a strategy to successfully establish in a given area. Qasem and Foy (2001) have reported this phenomenon in as many as 240 weed species. Under natural conditions however, a number of other interactions may also be operating simultaneously, so it is really difficult to demonstrate the phenomenon as a sole reason for weed dominance. Nevertheless, there are protocols available that can demonstrate the phenomenon of allelopathy as an ecological mechanism for the successful invasion of a given ecosystem.

In the case of *Ageratum conyzoides*, however, the cause of interference is unknown, as only a few studies are available that indicate a chemical mediated effect of this weed. These reports are preliminary in nature and indicate that root and shoot extracts of *A. conyzoides* are phytotoxic in nature (Jha and Dhakal 1990, Prasad and Srivastava 1991). However, there are reports indicating that a volatile essential oil of the weed is allelopathic and this is attributed to the presence of precocenes and their derivatives and several mono- and sesquiterpenes (Kong *et al*. 1999, 2002). However, there is a lack of information regarding the role of leachable water-soluble allelochemicals in imparting allelopathic activity to this weed. In order to fill this information gap, we thus hypothesised that such allelochemicals are involved in the suppressive effects of the weed. The objectives of the present study therefore, were to demonstrate the phytotoxic effect of residues of *A. conyzoides* towards some crop plants and to determine the nature of growth inhibiting substances.

MATERIALS AND METHODS

Collection of plant and soil material Soil and leaf residues of billygoat weed (*Ageratum conyzoides* L.) were collected from an area heavily infested with the weed selected on the campus of Panjab University, Chandigarh, India. Collection of the soil was made from the upper 0–10 cm profile. Likewise, soil was also collected from a nearby area free of *A. conyzoides* (but with other native plants) so as to serve as a control. The soils were brought to the laboratory, air-dried, sieved through a 2 mm sieve and stored in polythene bags for two weeks until used. For bioassay studies, certified seeds of chickpea (*Cicer arietinum* L.), mustard (*Brassica campestris* L.) and rice (*Oryza sativa* L.) were also procured from the Plant Breeding Department of the Punjab Agricultural University, Ludhiana, India.

Growth of test crops in *A. conyzoides* soil Seven hundred grams of soil collected either from an *A. conyzoides* infested area or uninfested area was placed in 12.5 cm diameter plastic pots. Five seeds each of chickpea, mustard and rice were placed 1 cm below the soil surface in each pot and allowed to germinate. After emergence, pots were thinned to two seedlings each and allowed to grow for one month. Five replicates were maintained for each crop in a completely randomised block design. After a month the seedling length (from tip of root to tip of shoot) and dry weight of the germinated seeds were determined.

Preparation of residue amended soils Ten and 20 g of leaf residues were mixed into 1 kg of the control soil. It was determined on the basis of the amount of leaf residues added to the soil under field conditions using clipped quadrats (nearly 1.5 to 2 kg residues m^{-2} of soil). Amended soils were sprayed with 750 mL of distilled water and left at 25°C for 16 h. Some 250 g of respective amended soil was taken for growth experiments.

Growth studies in amended soils Fifteen seeds of each of the test crops were sown in Petri dishes containing each type of amended soil. Five replicates were maintained for each treatment in a randomised manner. All the Petri dishes were maintained in a seed germinator at 25 ± 1°C, 75 ± 3% relative humidity and 16/8 light/dark photoperiod. Each Petri dish was sprayed daily with 25 mL of distilled water. After eight days, the seedling length and dry weight of 10 seedlings from each treatment were measured.

Preparation of residue extracts In order to find out whether leaf residues of *A. conyzoides* contain any water-soluble phytotoxins, 2 g of leaf residues of *A. conyzoides* were dipped in 100 mL of distilled water for 16 h at 24°C and filtered through a muslin cloth followed by Whatman No. 1 filter paper. These were further diluted so as to get 1% solutions. These extracts were kept in refrigerator at 4°C until further use.

Bioassay study with residue extracts In a laboratory bioassay, effects of different concentrations of leaf residue extracts were studied on the seedling growth and biomass of chickpea, mustard and rice. For this, 20 seeds of each of these crops were placed in a 15 cm Petri dish lined with a Whatman No. 1 filter paper moistened with 8 mL of either 1% or 2% of leaf residue extracts. Treatment in a similar manner but with distilled water served as a control. For each treatment five replicates were maintained in a completely randomised design. After seven days, the seedling length and dry weight of the seedlings were determined. The whole experiment was then repeated.

Total phenolics content Total water-soluble phenolics in the aqueous residue extracts and residue amended soils were estimated as per the method of Swain and Hillis (1959) using Folin-ciocalteu reagent. The amount was determined spectrophotometrically at 700 nm against a standard of ferulic acid. For each estimation five replicates were maintained.

Statistical analysis Data from the growth experiments with *A. conyzoides* infested soils, bioassay with residue extracts and growth studies in amended soils were analysed by two sample *t*-tests, at $P < 0.05$ and $P < 0.01$.

RESULTS AND DISCUSSION

The results indicate that growth of all three test crops, viz. chickpea, mustard and rice, was severely affected when grown in soil collected from *A. conyzoides* infested areas compared with the control (Table 1).

Both seedling length and dry weight were significantly reduced when compared with the control. Among the three test crops, the maximum effect was seen on mustard followed by chickpea and rice (Table 1). In mustard, seedling length and dry weight were reduced by nearly 35 and 40%, respectively, compared with nearly 25 and 10% in rice (Table 1). Such growth inhibition is similar to field observations where, in general, the growth of crops is poor in fields earlier infested with *A. conyzoides*. This indicates the presence of some growth-inhibiting substances in the *A. conyzoides* infested soil. Probably, these phytotoxins are released by the weed since there was no such effect in the soil collected from an area devoid of *A. conyzoides.*

In order to further ensure the phytotoxicity of *A. conyzoides,* its residues, which accumulate in large amounts in fields, were collected and mixed into the

Table 1. Seedling length (SL, cm) and dry weight (DW, mg plant^{-1}) of test crops after one-month when grown in soil infested with *A. conyzoides*.

Test crop	Parameter studied	*Ageratum* infested area soil	Control area soil
Chickpea	SL	31.8*	40.5
	DW	128.9*	180.3
Mustard	SL	27.0*	42.1
	DW	73.1*	121.8
Rice	SL	18.7*	24.8
	DW	8.9*	9.9

* represents significant difference from control at P <0.01, applying *t*-test.

soil. It was observed that the growth of all three test crops was significantly reduced in the soils amended with residues of *A. conyzoides* (Table 2). Here also, the maximum effect was observed on mustard and minimum on chickpea. With the amendment of 20 g of residues per kg of soil, seedling length was reduced by nearly 57, 47 and 40% in mustard, rice and chickpea, respectively (Table 2).

The inhibition of growth in the soils amended with *A. conyzoides* residues indicates the presence of some phytotoxic principles. To confirm this, a laboratory bioassay was planned with aqueous extracts of *A. conyzoides* residues. It was observed that seedling growth of test crops was significantly reduced in response to the residue extracts (Table 3). The maximum effect was seen on chickpea. At 2% concentration seedling length and weight were reduced by nearly 72 and 65%, respectively (Table 3). It indicated the presence of water-soluble phytotoxins in the residues.

These bioassay studies suggest the presence of some water-soluble phytotoxins in the residues of *A. conyzoides*, which upon release accumulate in bioactive concentrations in the soil and bring about the suppression of other plants. Under natural conditions the release of these chemicals into the environment occurs through different mechanisms such as leaching from living and dead parts, volatilisation, microbial decay and root exudation (Rice 1984). However, leaching is the most prevalent method for the escape of allelochemicals from plants into the environment (Patrick 1971, Rice 1984). Phenolics are the most ubiquitous phytotoxins that are implicated in allelopathy (Mizutani 1999). In the present study significantly higher amounts of phenolics were found in the *A. conyzoides* infested soils compared with the control soil (Table 4). Even the leaf residue extracts

Table 2. Seedling length and dry weight of one-week-old seedlings of three test crops in the soil amended with *A. conyzoides* residues.

Crop	Amount of residue (g kg^{-1} soil)	Seedling length (cm)	Seedling weight (mg)
Chickpea	0	22.2	46.4
	10	18.9*	26.5**
	20	13.2**	19.4**
Mustard	0	18.1	11.7
	10	11.2*	7.8*
	20	8.0**	6.4*
Rice	0	16.5	8.9
	10	11.1*	5.1*
	20	8.8**	4.3*

Within a crop and parameter, *, ** represent significant differences from the control at P <0.05 and 0.01, respectively, applying *t*-test.

Table 3. Effect of *A. conyzoides* residues extracts on seedling length and weight of test crops (% of control).

Crop	Conc. (%)	Seedling length	Seedling weight
Chickpea	1	44.8**	50.7**
	2	27.9**	35.2**
Mustard	1	61.5*	68.0*
	2	40.7**	48.2**
Rice	1	53.2**	74.3*
	2	34.5**	51.7**

*, ** represent significant differences from the control at P <0.05 and 0.01, respectively, applying *t*-test.

Table 4. Phenolic content in the *A. conyzoides* residue extracts, residue amended soil and soil from an infested area.

Treatment	Amount of phenolics
	(µg g^{-1})
Control soil	13.87 ± 1.28
A. conyzoides infested soil	30.07 ± 2.56*
Residue amended soil (20g)	38.33 ± 0.48*
	(µg mL^{-1})
Residue extracts (2%)	44.72 ± 1.48

* represents significant difference from control at P <0.05 applying *t*-test.

and soils amended with *A. conyzoides* residues were rich in phenolics (Table 4).

The lesser amount of phenolics in the *A. conyzoides* infested soils compared with residue extracts could be due to their transformation upon entering the complex and dynamic soil environment. Phenolics upon entering the soil are known to undergo a series of transformations (Blum *et al*. 1999, Singh *et al*. 2001) and only a small fraction is available in soil that brings about the growth inhibitory effect (Elliot and Cheng 1987). However, the inhibitory effect depends upon the bioactive concentration of the accumulated phenolics in the soils, which itself is dependent upon continuous flux from the donor plant. Although the water-soluble phenolics involved in the present study were not identified, a number of allelochemicals have been identified from the volatile oils of *A. conyzoides* and these have been implicated in allelopathy (Kong *et al*. 1999, 2002).

Besides phenolics, other factors such as physico-chemical properties of soil, pH of the extracts, availability of nutrients and interactions of allelochemicals with nutrients may also affect the growth and development of crops (Blum *et al*. 1999). However, these possibilities were not explored in this study. Thus, based on the present study, it is indicated that phenolics may be responsible for growth retardatory effects of *A. conyzoides* on crops. However, whether they bring about growth inhibition directly or indirectly needs to be explored.

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