

Full Length Research Paper

Potential allelopathic effects of *Xanthium italicum* Moretti on wheat

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The allelopathic potential of invasive *Xanthium italicum* Moretti on seedling growth of wheat (*Triticum aestivum* Linn) was investigated. Aqueous (0.05 g/ml) and ethanol (1 mg/ml) extracts reduced root elongation of wheat seedlings by 71 and 87%, respectively; chloroform fraction of the ethanol extract exhibited the strongest phytotoxic effect as compared to other fractions, inhibiting root growth of wheat seedlings by 95% at 1 mg/ml. Incorporation of *X. italicum* plant residues (20, 50 and 100 g/kg soil) into soils affected not only photosynthetic pigment content, but also photosynthetic parameters of wheat seedlings. Chlorophyll a, b and carotenoid contents of wheat seedlings decreased significantly when plant residues were applied at 100 g/kg soil. Net photosynthetic rate (P_n), transpiration rate (T_r) and stomatal conductivity (g_s) decreased greatly when seedlings were treated with plant residues at 20, 50 and 100 g/kg soil, whereas CO₂ concentration (C_i) increased significantly at 50 and 100 g plant residue/kg soil. Shoot length, fresh and dry weight were also significantly affected starting from 20 g plant residue/kg soil treatment in a dose-dependent manner. Furthermore, our results indicated that soil conductivity, soil pH and soil nutrients were unlikely responsible for the aforementioned changes. Our results suggest that allelopathy might contribute to the invasion success of *X. italicum*, and its invasion could possibly pose potential threat to wheat production in China.

Key words: Allelopathy, phytotoxin, wheat, *Xanthium italicum*.

INTRODUCTION

Invasive exotic plant species can negatively affect native plant community by either displacing resident species or inhibiting the establishment of new individuals (Yurkonis et al., 2005). Besides natural habitats, exotic plants could also invade agricultural fields where they compete with crops for light, nutrients, water and space; moreover, they may alter soil nutrient dynamics as well as soil microbial community structure and function which presumably create unfavorable growth conditions for crops and consequently result in lower yields (Ehrenfeld, 2003;

Kourtev et al., 2002). Despite the fact that biological invasions occur worldwide at an alarming rate, the mechanisms underlying the successful invasion of exotic species remain unclear. There have been a number of hypotheses attempting to elucidate the possible mechanisms involved in the process of exotic invasions; among them, the "novel weapon hypothesis" posits that allelopathy might contribute to the invasion success of exotics through production of novel allelochemicals which possess potent phytotoxic effects on native species due to the lack of coevolution (Callaway and Aschehoug, 2000; Callaway and Ridenour, 2004; Thorpe et al., 2009). However, this theory has been controversial ever since it was proposed (Blair et al., 2006; Duke et al., 2009), despite that many exotic plants have been speculated or

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even confirmed to be allelopathic (Gibson et al., 2011; Shao et al., 2005; Wang et al., 2012). The exotic plant *Xanthium italicum* Moretti (family Asteraceae, Compositae) was first spotted in China in the late 20th century. Traditionally, *Xanthium* species, including *X. italicum*, have been used as folk medicines worldwide for treatment of sinusitis, fever, leucoderma, herpes, and cancer (Saxena and Mondal, 1994; Hartwell, 1968; Tran et al., 2003).

This plant has spread to six provinces in the past two decades (Li et al., 2010; Liu et al., 2002; Wang and Wan, 2010). Interestingly, all six provinces are located either on the border with foreign countries or on the coast, indicating that this plant was possibly transported to China via international trade. These six provinces are not only geographically far away but also characterized by distinctive climates, suggesting that *X. italicum* has the ability to adapt to various environmental conditions. *X. italicum* was reported to be able to tolerate salinity and drought stress; it is also known as a strong competitor against annual plants (Liu et al., 2002; Takakura and Fujii, 2010). In China, *X. italicum* is often seen invading corn, wheat, cotton and soybean. It is estimated that 8% *X. italicum* coverage could result in a 60% reduction in yield (Liu et al., 2002). Our previous study revealed that allelopathy might contribute, at least in part, to the invasion success of *X. italicum* (Shao et al., 2012).

This plant contains several kinds of phytotoxins including xanthinosin, xanthatin and xanthinin (Vasas and Hohmann, 2011), which were all reported to possess plant inhibitory activity (Geissman et al., 1954; Khan, 1963; Shao et al., 2012). These putative allelochemicals are present mainly in leaves and fruits of *X. italicum*, which are possibly released to the environment via leaching or litter decomposition, thus affect neighboring plants' growth. However, it is insufficient to demonstrate that a certain plant is allelopathic simply by isolating and identifying phytotoxins from it; these phytotoxins need to be demonstrated that they can be released to the environment, either in the air or in the soil, and persist at effective concentration for a certain period of time so that they could function as active allelochemicals (Inderjit, 2000).

Our previous study confirmed that seedling growth of radish and wheat were significantly inhibited when grown in soils collected from a field heavily infested by *X. italicum* (data not shown), implying the possible involvement of phytotoxic compounds in the soils.

In the current study, we not only investigated the allelopathic potential of *X. italicum* plant extracts; we also grew wheat seedlings in soils mixed with mature *X. italicum* plant residues to mimic the litter decomposition process and verify whether allelochemicals can enter the soil and retain their phytotoxicity long enough to affect seedlings growth of wheat, which could help us understand whether allelopathy contributes to the invasiveness of *X. italicum*, and predict the possible consequences of

this invasion for agriculture in China.

MATERIALS AND METHODS

Plant

Aboveground parts of *X. italicum* plants were collected from a dense monoculture growing along the roadside in Urumqi, Xinjiang province in September, 2011 (N43°54'40.2", E87°17'7.6"). Most plants were harvested at their fruiting stage. Plant materials were air dried at room temperature in the shade for 14 days before use.

Phytotoxic effects of aqueous and ethanol extracts of *X. italicum* plant

Air dried plants of *X. italicum* were first ground into powder. Five grams of the powder were macerated in 100 ml distilled water (pH = 6.8) for 24 h and filtered to afford 0.05 g/ml extract. The pH of the aqueous extract was adjusted to 6.8 with 0.1 M HCl and 0.1 M NaOH. Three ml of distilled water (control) or the aqueous extracts were then added to each Petri dish (9 cm diameter) lined with Whatman No. 3 filter paper, followed by addition of 10 wheat seeds on each filter paper.

Seeds were surface sterilized in 0.5% HgCl₂ before use. Petri dishes were sealed with Parafilm to prevent water loss and stored in the dark at 25°C for 4 days before root and shoot lengths were measured. Phytotoxic bioassays with ethanol extract were conducted in a similar manner with a few changes. Five grams of *X. italicum* plant powder were soaked in 100 ml 95% ethanol for 24 h and filtered to give 0.05 g/ml extract. Three milliliter of ethanol (control) or ethanol extract were added to Petri dishes and allowed for complete evaporation of the solvent. After that, 3 ml distilled water and 10 wheat seeds were added to each Petri dish which was subsequently allowed to incubate at 25°C for 4 days. All phytotoxic assays were conducted in triplicate, and in total 30 seedlings were measured (N = 30).

Phytotoxic effects of organic fractions of ethanol extract of *X. italicum* plant

Two hundred grams of dried plant powder of *X. italicum* were first extracted in ethanol for a week and were then subjected to solvent-solvent partition using the following organic solvents sequentially: petroleum ether, chloroform, ethyl acetate and *n*-butanol, to afford corresponding fractions. The fractions along with the remaining water phase were filtered and evaporated to dryness *in vacuo*, weighed, and redissolved in 95% ethanol (for organic fractions) or distilled water (for the water phase) to give 1 mg/ml solutions, which were then tested for their phytotoxic effects using the same method for plant extracts.

Phytotoxic effects of *X. italicum* plant residues

Air dried aboveground *X. italicum* plant was ground into powder and incorporated into soils at rates of 20, 50, and 100 g/kg (residue/soil), respectively. Fifteen wheat seeds were sown in each 15 cm diameter plastic pot filled with 1 kg soil (control) or plant residue/soil mixture (five replicates were made for the control and treatments). Seedlings were allowed to grow in a greenhouse with 16 h photoperiod at an irradiance of 180 μmol m⁻² s⁻¹ photosynthetically active radiation (PAR; pot height) at 20/16°C day/night for 45 days

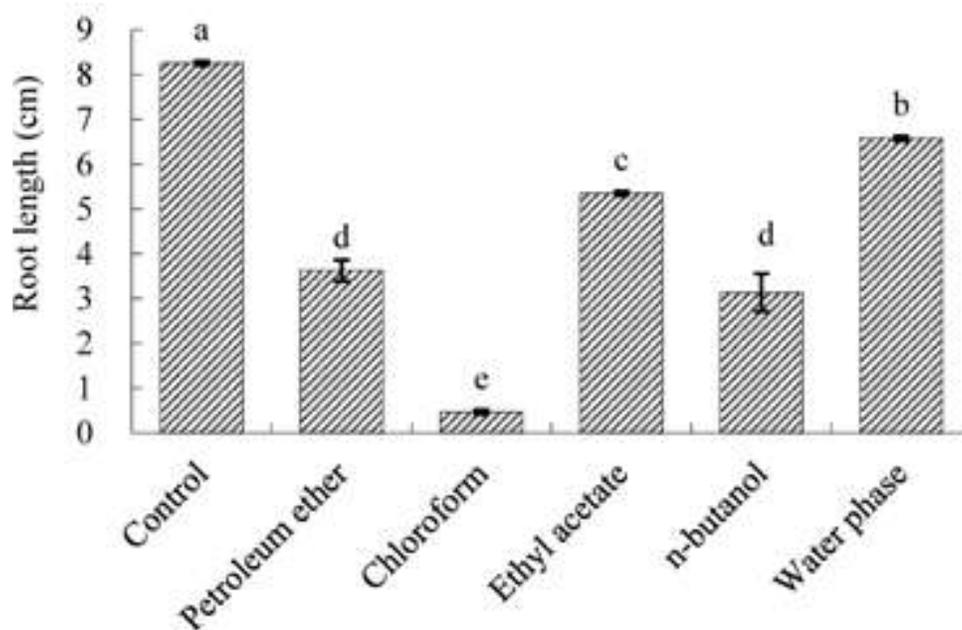


Figure 1. Phytotoxic effects of different organic fractions of the ethanol extract of *X. italicum* at 1 mg/ml on root growth of wheat seedlings. Values with the same letter are not different at $P = 0.05$ level according to Duncan's test.

after germination. The pots were watered every other day without fertilizer addition. The following measurements were then made: 1) shoot length, fresh and dry weight; 2) total chlorophyll a, b and carotenoid content; 3) net photosynthetic rate (P_n), intercellular CO_2 concentration (C_i), transpiration rate (T_r) and stomatal conductivity (g_s). Chlorophyll a, b and carotenoid content were calculated according to Wellburn (1994). Photosynthetic parameters were measured using a LI-6400 Photosynthetic Measurement Systems (LI-COR, Lincoln, NE, USA).

Soil properties analysis

To evaluate possible negative influences brought by plant residues other than allelochemicals, the pH, electrical conductivity (EC) as well as soil nutrients were also analyzed before wheat seeds were sown. Soil pH was determined on a 1:5 soil:deionised water suspension using a glass electrode; EC was measured with digital meters (EC 215, Hanna Instruments) also from 1:5 slurries of deionised water and soil. Measurements of soil nutrient were as follows: total organic: Walkley–Black dichromate wet digestion method; total nitrogen (N): Kelvin's method; total: HCl-HF digestion molybdenum antimony impedance colorimetry; total potassium (K): atomic absorption spectrophotometry; available N: micro-diffusion technique after alkaline hydrolysis; available phosphorus (P): colorimetric method; available K: ammonium acetate extract method.

Statistical analysis

The significance of effects of extracts and residues of *X. italicum* on wheat seedling growth was first examined by ANOVA ($P < 0.05$) and then analyzed using Duncan's test at $P < 0.05$ level.

RESULTS AND DISCUSSION

Phytotoxic effects of aqueous and organic extracts of *X. italicum* on seedling growth of wheat

Aqueous and ethanol extracts of *X. italicum* plants greatly inhibited root elongation of wheat seedlings by 71 and 87%, respectively (data not shown). Among the five organic fractions obtained from solvent partition of the ethanol extract, chloroform fraction exhibited the most potent inhibitory activity, reducing root length of wheat seedlings to 5% of control at 1 mg/ml (Figure 1). Petroleum ether and *n*-butanol fractions showed moderate phytotoxic effect, which resulted in a 57 and 63% reduction on root growth, respectively, whereas ethyl acetate fraction and the remaining water phase only exhibited weak inhibitory effect, which inhibited root growth by 36% and 21%, respectively. Our results suggested that major phytotoxins were present in the chloroform fraction of the ethanol extract of *X. italicum*. These organic fractions exhibited similar effects on shoot growth of wheat seedlings, but to a much lesser extent (Figure 2).

Phytotoxic effect of *X. italicum* plant residues on seedling growth of wheat

An apparent decreasing trend on shoot length, fresh and dry weight was observed in a dose-dependent manner

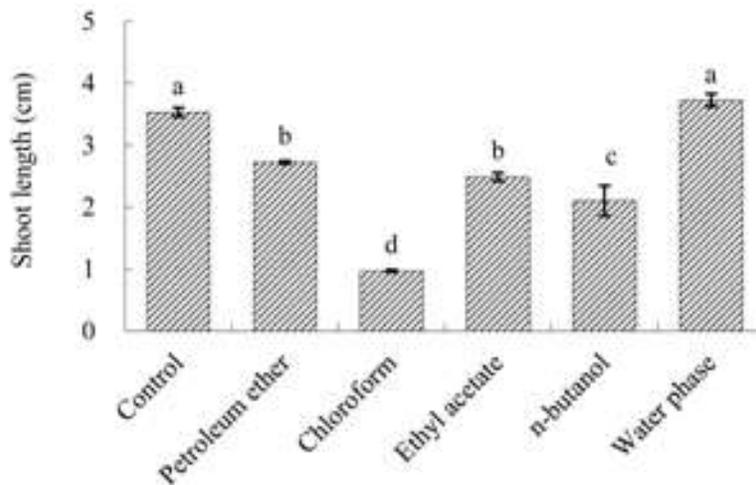


Figure 2. Phytotoxic effects of different organic fractions of the ethanol extract of *X. italicum* at 1 mg/ml on shoot growth of wheat seedlings. Values with the same letter are not different at $P = 0.05$ level according to Duncan's test

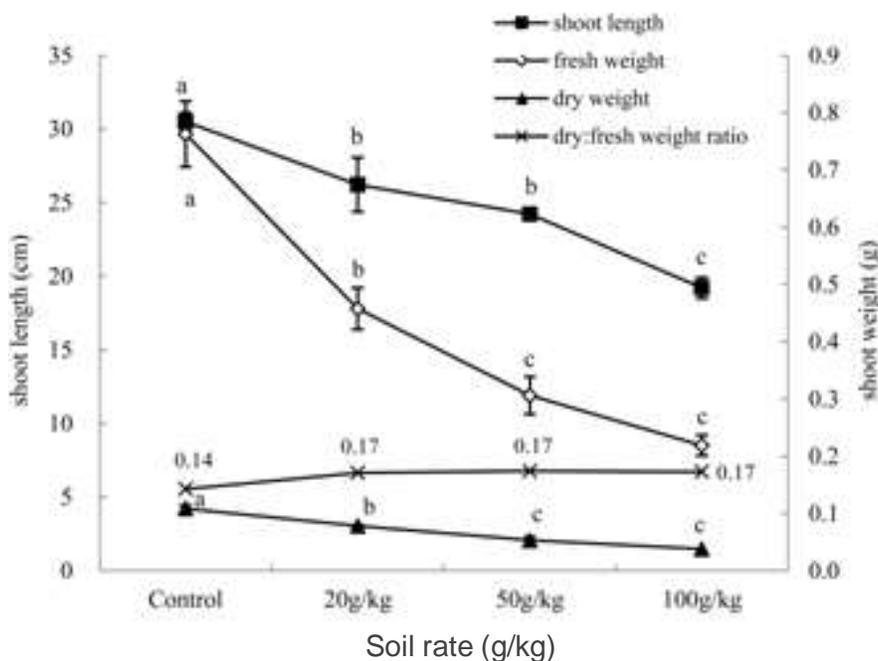


Figure 3. Shoot length, fresh and dry weight of wheat seedlings grown in *X. italicum* plant residue/soil mixture. Means within a line followed by the same letter are not different at $P = 0.05$ level according to Duncan's test.

when wheat seedlings were grown in soils incorporated with *X. italicum* plant residues. Starting from 20 g plant residue/kg soil treatment, shoot length, fresh and dry weight were all significantly affected. At 100 g plant residue/kg soil, shoot growth was reduced to 63% of control, while fresh and dry weight became 29 and 35% of control, respectively (Figure 3).

Phytotoxic effect of *X. italicum* plant residues on photosynthetic pigment content and photosynthetic activity

Photosynthetic pigment content as well as the photosynthetic parameters of wheat seedlings were mostly negatively affected by *X. italicum* plant residues except for

Table 1. Phytotoxic effect of *X. italicum* plant residues on photosynthetic pigment content and photosynthetic parameters of wheat seedlings.

Residue amount (g/kg soil)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Carotenoid (mg/g)	P_n ($\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	g_s ($\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	C_i ($\mu\text{mol CO}_2\text{mol}^{-1}$)	T_r ($\text{mmolH}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)
0(Control)	2.03 ± 0.08 ^a	0.61 ± 0.02 ^a	0.40 ± 0.01 ^a	6.42 ± 1.01 ^a	0.05 ± 0.01 ^a	414.12 ± 42.69 ^b	1.18 ± 0.18 ^a
20	1.97 ± 0.01 ^a	0.61 ± 0.01 ^a	0.40 ± 0.00 ^a	3.00 ± 0.25 ^b	0.01 ± 0.00 ^b	363.56 ± 14.67 ^b	0.37 ± 0.04 ^b
50	2.02 ± 0.02 ^a	0.63 ± 0.02 ^a	0.39 ± 0.01 ^a	2.28 ± 0.95 ^b	0.02 ± 0.00 ^b	546.11 ± 30.75 ^a	0.49 ± 0.12 ^b
100	1.56 ± 0.06 ^b	0.50 ± 0.02 ^b	0.31 ± 0.01 ^b	2.23 ± 0.86 ^b	0.02 ± 0.01 ^b	589.13 ± 36.29 ^a	0.54 ± 0.14 ^b

Each value is the mean ± standard error (SE). Means within a column followed by the same letter are not different at $P = 0.05$ level according to Duncan's test.

Table 2. Effect of *X. italicum* plant residues on soil electrical conductivity, pH and soil nutrients.

Residue amount (g/kg soil)	Total organic (g/kg)	Total N (g/kg)	Total P (g/kg)	Total K (g/kg)	Available N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)	EC (ms/cm)	pH
0 (Control)	144.03 ± 3.44 ^c	6.41 ± 0.45 ^c	0.92 ± 0.01 ^b	21.21 ± 0.23 ^a	449.17 ± 108.15 ^a	65.86 ± 1.31 ^d	14.14 ± 0.42 ^b	1.93 ± 0.09 ^b	7.46 ± 0.06 ^a
20	190.82 ± 4.29 ^b	6.92 ± 0.56 ^{ab}	0.93 ± 0.02 ^b	21.37 ± 0.42 ^a	325.50 ± 71.47 ^a	369.78 ± 7.36 ^c	15.24 ± 0.20 ^b	2.33 ± 0.12 ^{ab}	7.38 ± 0.01 ^{ab}
50	183.87 ± 5.98 ^b	7.41 ± 0.77 ^{ab}	0.97 ± 0.02 ^b	21.41 ± 0.62 ^a	344.17 ± 61.87 ^a	571.85 ± 14.34 ^b	20.44 ± 0.27 ^a	2.20 ± 0.21 ^{ab}	7.37 ± 0.03 ^{ab}
100	220.85 ± 7.44 ^a	8.52 ± 0.37 ^a	1.06 ± 0.04 ^a	21.24 ± 0.49 ^a	369.25 ± 64.75 ^a	1281.20 ± 29.97 ^a	21.39 ± 0.73 ^a	2.93 ± 0.42 ^a	7.33 ± 0.02 ^b

Each value is the mean ± standard error (SE). Means within a column followed by the same letter are not different at $p = 0.05$ level according to Duncan's test.

intercellular CO_2 concentration (C_i) (Table 1). Chlorophyll a, b and carotenoid contents were not significantly affected until plant residue concentration reached 100g/kg soil. At this rate, chlorophyll a, b, and carotenoid were reduced to 77, 81 and 76 of control. Meanwhile, net photosynthetic rate (P_n), stomatal conductivity (g_s) and transpiration rate (T_r) of wheat seedlings decreased significantly when grown in 20, 50 and 100 g plant residue/kg soil mixture, whereas intercellular CO_2 concentration (C_i) increased significantly when 50 and 100 g plant residue/kg soil mixture was applied. At 100 g plant residue/kg soil, P_n , g_s and T_r decreased by 71, 77 and 74%, respectively, whereas C_i increased by 28%.

Soil properties analysis

Plant residue application did not significantly

change the EC and pH of the soils until the rate increased to 100 g/kg soil (Table 2). Soil nutrient analysis revealed that total organic, total and available N, P, K increased when plant residues were applied to the soil. Total organic was 32, 28 and 53% higher than the control, and total N was 8, 16 and 33% higher than the control, when plant residues were added to the soil at 20, 50 and 100 g/kg soil; total P was not significantly affected until 100 g plant residue/kg soil was used, which caused a 15% increase as compared to the control. Plant residue application did not cause significant change in Total K and available N, but increased available K by 8, 45 and 53% at 20, 50 and 100 g plant residue/kg soil, respectively. Available P was greatly affected; 20 g plant residue/kg soil resulted in a 4.6 time increase, and 100 g plant residue/kg soil raised its amount to nearly 20 times of the control.

Our results confirmed the presence of phytotoxins in *X. italicum* plants, which can be extracted with water and organic solvents, and possibly pass into and persist in soil to influence wheat seedlings' growth. Application of plant residues resulted in significant change in seedling growth, photosynthetic pigment content and parameters, indicating that this application had adverse effect on wheat seedlings. Previously, plants with allelopathic activity have been reported to suppress seedling growth of receiver plants, including their growth rate, seedling height, fresh and dry weight (Iqbal et al., 2007; Jose and Gillespie, 1998; Yarnia et al., 2009); this phenomenon was speculated to be caused by reduction in total chlorophylls of plants treated with phytotoxic compounds, which consequently result in decreased photosynthetic activity (Einhellig and Rasmussen, 1979). Moreover, net photosynthetic

rate, respiration rate and transpiration rate were found to decrease when treated with allelochemicals, while intercellular CO₂ usually increased significantly, which are consistent with our results.

On the other hand, application of plant residues also changed the properties of soil, including its nutrient status, EC and pH. Soil nutrient analysis revealed a significant increase in total organic, total nitrogen, total and available phosphorus and available potassium, which presumably should enhance seedling growth of wheat seedlings. It is noteworthy to mention that seedling growth, photosynthetic pigment content and photosynthetic parameters were mostly affected starting from 20 g plant residue/kg soil treatment; at this rate, soil EC and pH was not significantly different from the control, and soil NPK were not greatly different as compared to the control, except for available P, which became 4.6 times higher than the control.

Furthermore, release of phytotoxins into soil may result in complicated ecological consequences other than their toxicity alone, which may subsequently alter soil properties and affect growth of vegetation in the vicinity. Previous studies found that phytotoxins in soil had important influences on soil nutrient dynamics, such as organic matter dynamics and nutrient cycling (Kuiters, 1990; White, 1994); in addition, phytotoxins may alter the composition and function of soil microbial community, which presumably could create unfavorable growth conditions for neighboring plants (Ehrenfeld, 2003; Kourtev et al., 2002). Further study is needed to investigate the possible mechanism underlying the phytotoxic activity of allelochemicals from *X. italicum* on other plants' growth.

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REFERENCES

- Blair AC, Nissen SJ, Brunk GR, Hufbauer RA (2006). A lack of evidence for an ecological role of the putative allelochemical (+/-)-catechin in spotted knapweed invasion success. *J. Chem. Ecol.* 32:2327-2331.
- Callaway RM, Aschehoug ET (2000). Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science* 290:521-523.
- Callaway RM, Ridenour WM (2004). Novel weapons: invasive success and the evolution of increased competitive ability. *Front. Ecol. Environ.* 2:436-433.
- Duke SO, Blair AC, Dayan FE, Johnson RD, Meepagala KM, Cook D, Bajsa J (2009). Is (-)-catechin a novel weapon of spotted knapweed (*Centaurea stoebe*)? *J. Chem. Ecol.* 35:141-153.
- Ehrenfeld JG (2003). Effects of Exotic Plant Invasions on Soil Nutrient Cycling Processes. *Ecosystems* 6:503-523.
- Einhellig FA, Rasmussen JA (1979). Effects of three phenolic acids on chlorophyll content and growth of soybean and grain sorghum seedlings. *J. Chem. Ecol.* 5:815-824.
- Geissman TA, Deuel P, Bonde EK, Addicott, FA (1954). Xanthinin: a plant growth-regulating compound from *Xanthium pennsylvanicum*. *J. Amer. Chem. Soc.* 76:685-687.
- Gibson DM, Krasnoff SB, Biazzo J, Milbrath L (2011). Phytotoxicity of antofine from invasive swallow-worts. *J. Chem. Ecol.* 37:871-879.
- Hartwell JL (1968) Plants used against cancer. *J. Nat. Prod.* 31: 71-170.
- Inderjit WLA (2000). Are laboratory bioassays for allelopathy suitable for prediction of field responses? *J. Chem. Ecol.* 26:2111-2118.
- Iqbal J, Cheema ZA, An M (2007). Intercropping of field crops in cotton for the management of purple nutsedge (*Cyperus rotundus* L.). *Plant soil* 300:163-171.
- Jose, S, Gillespie AR (1998). Allelopathy in black walnut (*Juglans nigra* L.) alley cropping. II. Effects of juglone on hydroponically grown corn (*Zea mays* L.) and soybean (*Glycine max* L. Merr.) growth and physiology. *Plant soil* 203:199-205.
- Khan AA (1963). Isolation and characterization of inhibitors from *Xanthium* and their relation to photomorphogenesis. Ph.D. thesis, University of Chicago, IL, USA.
- Kourtev PS, Ehrenfeld JG, Häggblom M (2002). Exotic plant species alter the microbial community structure and function in the soil. *Ecology* 83:3152-3166.
- Liu QR, Yu M, Zhou YL (2002). A preliminary study on the invasive plants in Beijing. *J. Beijing Norm. Univ. (Natural Science)* 38:399-404.
- Saxena VK, Mondal SK (1994) A xanthanolide from *Xanthium strumarium*. *Phytochemistry* 35: 1080-1082.
- Shao H, Huang XL, Wei XY, Zhang C (2012). Phytotoxic effects and a phytotoxin from the invasive plant *Xanthium italicum* Moretti. *Molecules* 17:4037-4046.
- Shao H, Peng SL, Wei XY, Zhang, DQ, Zhang C (2005). Potential allelochemicals from an invasive weed *Mikania micrantha* HBK. *J. Chem. Ecol.* 31:1657-1668.
- Takakura K, Fujii S (2010). Reproductive interference and salinity tolerance differentiate habitat use between two alien cockleburbs: *Xanthium occidentale* and *X italicum* (Compositae) *Plant Ecol.* 206:309-319.
- Thorpe AS, Thelen GC, Diaconu A, Callaway RM (2009). Root exudate is allelopathic in invaded community but not in native community: Field evidence for the novel weapons hypothesis. *J. Ecol.* 97:641-645.
- Tran QL, Tezuka Y, Ueda J, Nguyen NT, Maruyama Y, Begum K, Kim HS, Wataya Y, Tran QK, Kadota S (2003). *In vitro* antimalarial activity of antimalarial medicinal plants used in Vietnamese traditional medicine. *J. Ethnopharmacol.* 86: 249-252.
- Vasas A, Hohmann J (2011). *Xanthane sesquiterpenoids*: Structure, synthesis and biological activity. *Nat. prod. Rep.* 28:824-842.
- Wang R, Wan FH (2010). Prediction of the potential survival area of *Xanthium italicum* in China. *Acta Prataculturae Sinica* 199:222-230.
- Wang RL, Rehman SU, Liang XT, Song YY, Su YJ, Baerson S, Zeng RS (2012). Effects of simulated acid rain on the allelopathic potential of invasive weed *Wedelia trilobata*. *Allelopathy J.* 30:23-32.
- Wellburn AR (1994). The special determination of chlorophylls a and b as well as total carotenoids using various solvents with spectrophotometers of different resolution. *J. Plant Physiol.* 144:307-313.
- White CS (1994). Monoterpenes: their effect on ecosystem nutrient cycling. *J. Chem. Ecol.* 20:1381-1406.
- Yarnia M, Benam MBK, Tabrizi EFM (2009). Allelopathic effects of sorghum extracts on *Amaranthus retroflexus* seed germination and growth. *J. Food Agric. Environ.* 7:770-774.
- Yurkonis KA, Meiners SJ, Wachholder BE (2005). Invasion impacts diversity through altered community dynamics. *J. Ecol.* 93:1053-1061.