Research Article

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An evaluation of tenuazonic acid, a potential biobased herbicide in cotton

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Abstract

BACKGROUND: Tenuazonic acid (TeA), a putative nonhost-selective mycotoxin isolated from *Alternaria alternata*, is the main causative agent of brown leaf spot disease of crofton weed (*Ageratina adenophora*) and some other crops. Previous studies revealed that it is a natural photosystem II inhibitor that binds the D1 protein to block electron transfer. Though the crude metabolite extract of *A. alternata* containing TeA has been bioassayed, the herbicidal activity of synthesized TeA has not been systematically evaluated yet.

RESULTS: TeA caused leaves of crofton weed to have brown spots that were more pronounced in older leaves than younger ones. It completely killed 92% of the four-leaf seedlings at 600 g ai/ha but only 81% or less of six-leaf seedlings or bigger. The bioassay of phytotoxicity of TeA to 67 plant species including 54 weeds and 13 crops, showed that TeA had a broad weed spectrum but low toxicity to Solanaceae and Malvaceae species. Further potted- plant experiments demonstrated that TeA had EC_{90} values that ranged from 119 to 795 μg/mL for 14 important weeds but was 2539 μg/mL for *Acalypha australis*. *Nicotiana tabacum* and *Gossypium hirsutum* had no injury symptoms at 1000 μg/mL. A field trial showed that TeA effectively controlled two important weeds, *Digitaria sanguinalis* and *Amaranthus retroflexus* without affecting cotton in the field.

CONCLUSION: TeA has potential as a biobased herbicide for controlling important dicotyledon and monocotyledon weeds in cotton and tobacco fields.

Keywords: herbicide; tenuazonic acid; PSII inhibitor; phytotoxicity; cotton

1 INTRODUCTION

Fungi of the genus *Alternaria* are commonly found in nature, including in many foods, food products, soil, household dust and decaying organic material and widely distributed pathogenic fungi in crops and plants. *Alternaria* spp. produce a number of secondary metabolites belonging to several classes of chemicals with different biological activities, such as cytotoxic, insecticidal, antiviral, antimicrobial, teratogenic, mutagenic, antiprotozoal, zootoxic, and phytotoxic.[1–3] Qiang *et al.*[4] first recognized *Alternaria alternata* as one of natural pathogens of crofton weed (*Ageratina adenophora*), a widely spread noxious weed in the world. *A. alternata* causes a brown leaf spot on this weed by producing the phytotoxin tenuazonic acid (TeA) (Fig. 1).[5] Qiang *et al.*[5] bioassayed the crude metabolite extract (AAC-toxin) containing 5% TeA produced by *A. alternata* for weed spectrum and crop safety and found that AAC-toxin has potential as a bio-based herbicide to control most grasses, broad leaf and sedge weeds in cotton fields.[6,7] Tenuazonic acid (TeA) was isolated from the crude toxin extract and determined it to be primarily responsible for the herbicidal effect.[8–10]

A previous study suggested the thylakoid membrane as the action site, because TeA inhibited photosynthesis by inhibiting photosystem II (PSII) but not PSI.[9] By blocking PSII, TeA affected chlorophyll fluorescence parameters such as the coefficient of photochemical quenching, the half-time value of the fluorescence rise, and the OJIP fluorescence rise kinetics.[11] Specially, TeA inhibited PS II electron transfer from Q_A to Q_B by competing for the Q_B-binding site in the D1 protein. The results of competitive replacement with [^{14}C] atrazine combined with the JIP test and D1 mutants suggested that TeA is a PS II inhibitor with a unique binding behavior within the Q_B niche.[12] Hence, TeA is a novel natural PS II (C2/7) inhibitor with a potential candidate for a broad spectrum herbicide.

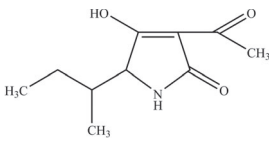

Figure 1. Structure of tenuazonic acid.

TeA, a tetramic acid derivative, was first isolated as an antitumor agent from the metabolites of *Alternaria tenuis* [13], and its structure was identified in 1959.[14] Although being obtained from a plant

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pathogen, few studies have investigated its phytotoxicity.[8] Zonno and Vurro[15] evaluated the inhibition of seed germination by TeA on the parasitic weed *Striga hermonthica*, and showed that TeA had an inhibitory effect of 52.9% at 10^{-4} M on its seed germination. AAC-toxin containing the main active ingredient of TeA had high herbicidal activity on the weeds such as crofton weed, *Digitaria sanguinalis*, *Echinochloa crus-galli*, *Amaranthus trtroflexus*, and *Eclipta prostrate* in the cotton field.[7,16] Cotton is an important economic crop which suffers from weed infestation in China. Because herbicide-resistant, transgenic cotton is not commercialized yet in China, limited commercialized herbicides can be chosen for weed control in cotton fields.

The relative phytotoxicity of TeA to weeds and crops has not been evaluated systematically. So, in this study, the phytotoxicity and specificity of TeA in laboratory, greenhouse, and field trials were conducted to assess the potential for using TeA as a selective, biobased herbicide in crops.

2 MATERIALS AND METHODS

2.1 Plant material

Seeds of *A. adenophora* were collected from different natural populations in Yunnan, Guangxi, and Guizhou provinces in China. The seeds of other weeds were collected in local fields, and the seeds of the crops (the cultivars of cotton, wheat, mung bean, tobacco and rape are Nannong 7, Lumai 21, Zhonglv 6, Yunyan 85, Youyan 7, respectively.) were bought from local markets. All the seeds were stored in the laboratory until used. The seeds of crofton weed and all other weeds were incubated for 5 days on moistened filter papers in petri dishes (9 cm in diameter) with a 12 h photoperiod at 25 °C. Then, the seedlings with roots and cotyledons were transplanted into 10-cm diameter plastic pots containing steam-pasteurized soil mix including one-third sand, one-third soil and one-third peat, and placed in a greenhouse with 28/20 °C day/night temperatures, natural light, and an average relative humidity of 35%. Young leaves of weeds and crops growing in fields were picked for the leaf puncture assay. The weeds and crops used in the spraying assay were direct-seeded into 6-cm diameter plastic pots. All pots were placed in a greenhouse and watered daily under the same conditions as above. Seedlings were thinned or transplanted to obtain 20 three-leaf stage seedlings per pot.

2.2 TeA production

TeA used in leaf puncture and seedling spraying assays was isolated and purified from the culture of *A. alternata* isolate NEW. The isolate was grown on a shaker (110 r.min^{-1}) for 6 days at 25 °C in 400 mL potato sucrose potassium dihydrogen phosphate medium per 1000-mL flask, and then the filtered culture was passed through a column of macroporous resin DA201 (Shanghai Yadong Heji Resin Inc., China), the column being eluted with alcohol. The alcohol-diluted extraction was concentrated by rotary evaporator under regular pressure at 80 °C. The condensate was extracted with same volume of ethyl acetate for three times. The extract was concentrated by rotary evaporator under regular pressure at 70 °C to obtain the crude toxin. The crude toxin was fractioned by column chromatography on Si gel, and then the fractions were prepared by silica thin layer chromatography until a brick red solid material, the purified toxin was obtained (> 98% purity). TeA (> 98% purity) used in the seedling spraying assay of crofton weed and in the field trial was synthesized by the method of Yang et al.[17] Using L-isoleucine as a starting material, TeA was synthesized following the five steps as esterification with alcohol, neutralization by sodium alcoholate, acylation with diketene, and cyclization and acidification in the presence of sodium alcoholate.[17]

2.3 Phytotoxicity of TeA to *A. adenophora*

2.3.1 *Leaf puncture assay*

In the leaf puncture assay, similar leaves of the same *A. adenophora* plant were picked, rinsed with tap water for 0.5 h, treated with 0.1% of mercury chloride for 3 to 5 min, rinsed with sterile water four times, blotted with sterile bibulous paper to remove the water on the surface of the leaves, placed in petri dishes (9 cm diameter) with the adaxial surface up on a wet filter paper, and lightly punctured with a sterile needle in the area 1 to 2 cm from the edge of the leaves. Then, 20 μL of 50 μg/mL TeA solution was dripped on the pinprick with a 200 μL pipettor, and the leaves were incubated for 48 h at 25 ± 1 °C, L: D = 12: 12 in an environmental chamber. The diameter of necrotic lesion was measured using a vernier caliper.[18,19]

2.3.2 *Seedling spraying assay*

In the seedling spraying assay, seedlings of crofton weed (population JH (Jinghong, Yunnan)) at four-leaf, six-leaf and eight-leaf stages were sprayed with TeA aqueous solutions. The concentrations of TeA were set as 300, 600, 900, 1200, and 1500 g/ha with 0.4% adjuvant JN (fatty alcohol polyoxyethylene ether (Jiangsu Zhongshan Chemical Inc., China) and laurocapram (Nanjing Longtan Fine Chemicals Inc., China), 1:3 [v/v]). The treatments and a water control were repeated four times. Five days later, phytotoxic effects were determined. The concentrations and determined effects were regressed, and EC_{50} and EC_{90} values were calculated. The effects were recorded with the following visual ratings: 0 = healthy (no apparent phytotoxicity), 1 = less than one-third of the leaves withered, 2 = one-third to one-half of the leaves withered, 3 = one-half to two-thirds of the leaves withered, 4 = more than two-thirds of the leaves withered, and 5 = completely withered individuals or 100% mortality. The effective results are presented as the percentage of damage rate, which is calculated as.

Percentage of damage rate = $\sum$ (injury rate × individuals)/(5 × total of individuals observed).

2.4 Phytotoxicity of TeA to plants

2.4.1 *Leaf puncture assay*

In the leaf puncture assay, the young leaves were picked from the healthy weed and crop plants selected in the farm fields, five concentrations of TeA solution with 0.4% adjuvant JN (fatty alcohol polyoxyethylene ether and laurocapram, 1:3 [v/v]) were prepared in the following concentrations: 25, 50, 100, 200, 400, and 800 μg/mL, and then treated as above. Phytotoxicity was classified into six grades according to the necrosis diameter: 0 = the diameter less than or equal to 0.2 mm, 1 = the diameter over 0.2 mm but less than or equal to 0.5 mm, 2 = the diameter over 0.5 mm but less than or equal to 0.8 mm, 3 = the diameter over 0.8 mm but less than or equal to 1.1 mm, 4 = the diameterover 1.1 mm but less than or equal to 1.4 mm, and 5 = the diameter over 1.4 mm. The percentage of damage rate was calculated as *Percentage of damage rate* = $\sum$ *(injury rate × individuals)/(5 × total of individuals observed).*

2.4.2 *Seedling spraying assay*

In the seedling spraying assay, five concentrations of TeA formulation with adjuvant were prepared in the following concentrations:

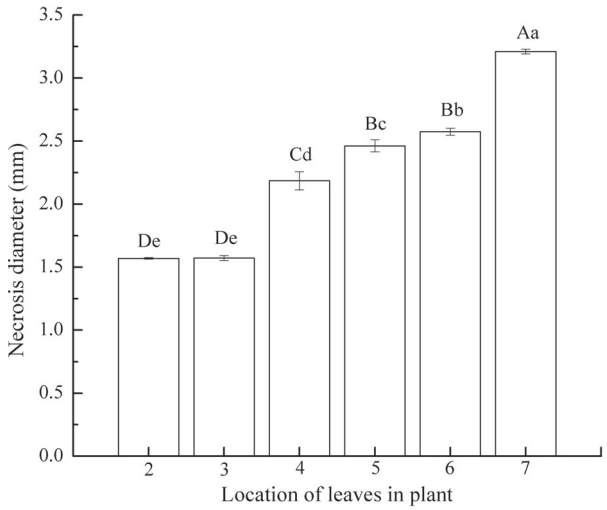

Figure 2. Phytotoxicity of TeA on *Eupatorium adenophorum* leaves of different maturity. Note: The different small letters in each column indicate significant difference at $P < 0.05$ level; the different capital letters in each column indicate significant difference at $P < 0.01$ level. The leaves are located from the top to the bottom of the plant with the numbers from 2 to 7.

62.5, 125, 250, 500, and 1000 µg/mL of TeA and 0.4% adjuvant (triton X-100 (Sinoparm Chemical Reagent Inc., China): sodium dodecyl benzene sulfonate (Sinoparm Chemical Reagent Inc., China): YZ905 (Beijing Ruidexing Chemical Inc., China): EF8108-II (Jingling Petrochemical Inc., China), 1: 1: 1: 1 [v/v]), with 5% alcohol. The seeds of 15 weeds and five crops were sown in 6 cm diameter plastic pots with soil, when the seedling grew up to three leaves, the solution was sprayed with a hand sprayer (100 mL/m^2) at 75 kPa pressure. Water control (CK0) and adjuvant control (CK1) were included, and all treatments were repeated three times. The effects were determined and analyzed as described above.

2.5 Herbicidal activity of TeA in a field trial

The field trial was carried out in a cotton field. The soil was sandy soil with pH of 6.7, organic content of 1.59%, and moisture content of 15% to 20%. Cotton seeds were grown in the laboratory to the two-leaf stage, and then seedlings were transplanted in the field. *Digitaria sanguinalis* and *Amaranthus retroflexus* were direct-seeded in the field, and when they grew at two- to three-leaf stage, the seedlings were thinned or transplanted to 300 plant/m^2. After being divided into some plots (20 m^2 per plot), TeA aqueous solution were sprayed (600 L/ha) with a separate sprayer for every treatment. The concentrations of TeA were set as 375, 750, 1125 and 1500 g/ha with 0.4% adjuvant JN (fatty alcohol polyoxyethylene ether and laurocapram, 1:3 [v/v]). Control effects were observed on day 7 and day 14 after spraying. Three samples (0.09 m^2 per sample) in each plot were randomly selected and the effects were calculated as follow: plant control effect (%) =100% × (weeds in control plot − weeds in treatment plot)/weeds in control plot, Fresh weight control effect (%) =100% × (weed fresh weight in control plot − weed fresh weight in treatment plot)/weed fresh weight in control plot. A herbicide positive control (bentazon) and a water control were also included, and the treatments were repeated four times.

2.6 Statistical analysis

Data were expressed as mean ± SE. Statistical analyses were performed using SPSS 11.0 for Windows. The difference in phytotoxicity of TeA to different weeds and crops were analyzed using one-way ANOVA followed by Dunnett's test for multiple comparisons.

3 RESULTS

3.1 Phytotoxicity of TeA to *A. adenophora*

3.1.1 Leaf puncture assay

Phytotoxicity of TeA to leaves of *A. adenophora* at different stages of maturity was determined by the leaf needle puncture assay. The assay indicated that the toxin is more effective on older leaves (Fig. 2). The diameter of necrosis increased with lower leaf position. There was no significant difference for phytotoxicity of the toxin between the second and the third pair of leaf ($P > 0.05$).

3.1.2 Seedling spraying assay

Seedlings of *A. adenophora* had varying necrosis 4 h after being sprayed with low concentrations of TeA solutions (Table 1 and 2). The necrotic area increased with increasing concentrations and decreasing leaf ages of plants. After 12 h, plants with 4 and 6 leaf-stages in the treatments of low and middle concentrations started to wilt, and some were dead in the high concentration treatment. The 8 leaf-stage plants appeared to wilt to some degree, but no plants died. Twenty-four h later, all 4 and 6 leaf-stage plants in the treatments of high concentration died, 85% to 90% died in the treatment of middle concentration, and 60% died in the treatment of low concentration. The weed control effect on 8 leaf plants was poor in low and middle concentration treatments, and was 95% in high concentration treatments.

Table 1. Phytotoxicity of TeA on *Ageratina adenophorum* at different leaf stages

	4 leaf-stage		6 leaf-stage		8 leaf-stage	
Treatment	Control effect	Significance of difference	Control effect	Significance of difference	Control effect	Significance of difference
1500 g ai./ha (25% TeA AS)	100	Aa	100	Aa	100	Aa
1200 g ai./ha (25% TeA AS)	100	Aa	98.9	Aa	96.1	Ab
900 g ai./ha (25% TeA AS)	95.0	Bb	91.1	Bb	79.5	Bc
600 g ai./ha (25% TeA AS)	92.2	Cc	81.1	Cc	76.4	Bc
300 g ai./ha (25% TeA AS)	78.4	Dd	63.9	Dd	59.5	Cd
water	0.0	Ee	0.0	Ee	0.0	De

Note: The different small letters in each column indicate significant difference at $P < 0.05$ level; the different capital letters in each column indicate significant difference at $P < 0.01$ level.

Table 2. Regression analysis of the dose–response of TeA on *Ageratina adenophorum* at different leaf-stages

Leaf-stage	Formula	R^2	EC_{50} (μg/mL)	EC_{90} (μg/mL)
Four leaf-stage	$y = 13.642 \ln(x) - 4.7211$	0.952	55.2	1036
Six leaf-stage	$y = 23.42 \ln(x) - 80.969$	0.989	268	1480
Eight leaf-stage	$y = 25.07 \ln(x) - 97.514$	0.950	359	1772

3.2 Phytotoxicity of TeA to plants

3.2.1 Leaf puncture assay

A leaf puncture assay of phytotoxicity of TeA to plant species showed that the main crop weeds demonstrated various sensitivities to TeA (Table 3). There were 42 susceptible plants belonging to 17 families, based on the value of EC_{90} no more than 500 ppm. These susceptible species included Amaranthaceae (e.g. *Alternanthera philoxeroides*, *Amaranthus retroflexus*, *Amaranthus tricolor* and *Celosia argentea*), Brassicaceae (e.g. *Arabidopsis thaliana*), Chenopodiaceae (e.g. *Chenopodium album*), Commelinaceae (e.g. *Commelina communis* and *Commelina bengalensis*), Compositae (e.g. *Ambrosia artemisiifolia*, *Conyza canadensis*, *Conyza sumatrensis* and *Erigeron annuus*), Convolvulaceae (e.g. *Pharbitis purpurea, Pharbitis nil*, and *Calystegia hederacea*), Cyperaceae (e.g. *Cyperus iria, Cyperus difformis*, and *Cyperus rotundus*), Euphorbiaceae (e.g. *Euphorbia humifusa*), Gramineae (e.g. *Digiteria sanguinalis*, *Leptochloa chinensis*, *Leptochloa panacea*, and *Microstegium vimineum*), Moraceae (e.g. *Humulus scandens*), Nyctaginaceae (e.*G. Mirabilis jalapa*), Onagraceae (e.g. *Ludwigia prostrata*), Oxalidaceae (e.g. *Oxalis corniculata*), Polygonaceae (e.g. *Polygonum jucundum*), Sterculiaceae (e.g. *Melochia corchorifol*), and Vitaceae (e.g. *Cayratia japonica*). Moderately susceptible plants with EC_{90} values between 500 and 2000 μg/mL included six species belonging to five families. The tolerant plants with EC_{90} values over 2000 μg/mL were 13 species in Malvaceae and Solanaceae.

3.2.2 Seedling spraying assay

3.2.2.1 Phytotoxicity on weeds. The results of seedling spraying assay showed that the efficacy of TeA to control the 15 weeds increased with the increasing toxin concentration (Table 4, Table 5). At 500 μg/mL, TeA provided 85% or higher control efficacy to Gramineae and Leguminosae and other four weed species. At the highest concentration of 1000 μg/mL, except for *Acalypha australis*, the efficacy on other 14 weeds were 92.7% or more. And the EC_{90} values for these 14 weeds were from 119 to 795 μg/mL.

3.2.2.2 Crop safety. The safety assay of TeA to five crops showed that *Gossypium hirsutum* and *Nicotiana tabacum* were highly tolerant to TeA and *Phaseolus radiatus* had low tolerance, but *Triticum aestivum* and *Brassica napus* were highly susceptible (Fig. 3, Table 6). At the highest concentration of 1000 μg/mL, the injury rates of *T. aestivum* and *B. napus* were over 90%, but those of *G. hirsutum* and *N. tabacum* were less than 3%.

3.3 Herbicidal activity of TeA in a field trial

3.3.1 Herbicidal activity on weeds

The control effects of aqueous TeA solution on the weeds were enhanced with increasing concentrations and time after spraying (Table 7). Seven days after spraying, the control effects of *D. sanguinalis* and *A. retroflexus* reached over 70% in the treatment of low concentration, and 14 days later, the effects were up to 90% in the treatment of high concentration. The fresh weight effects were also enhanced with increasing TeA concentrations. Even though there were no dead weeds in the treatment of low concentration, the growth of the weeds significantly was inhibited, and their size and weight were both lower than those of the control.

3.3.2 Injury on Gossypium hirsutum

As shown as Fig. 4, the injury rates of *Gossypium hirsutum* increased with higher TeA concentrations. Seven days after spraying, the injury rate reached 25% or so at the highest concentration, but fourteen days later, the injury rates gradually lowered, and the growth of *G. hirsutum* recovered gradually.

4 DISCUSSION

Fungal secondary metabolites are much more likely to have some type of phytotoxicity at lower concentrations than compounds derived from chemical synthesis programs. Furthermore, they may be more environmentally benign. Their phytotoxicity to a vast range of weeds can be the basis for herbicide development. Among *Alternaria* toxins, AAL-toxin isolated from *Alternaria alternata f.sp. lycopersici* was proposed as a herbicide, as a USA patent (USA Patent 5 256 628).[20] AAC-toxin containing 5% TeA produced by *A. alternata* had a wide herbicidal spectrum and showed a potential for developing as a bio-based herbicide to control most grass, broad leaf, and sedge weeds in cotton.[5–7] In this study, the results of the leaf puncture assay, seedling spraying assays, and a field trial showed that TeA not only could completely kill the seedlings of crofton weed but also had a broad herbicidal spectrum and was safe to tobacco and cotton. Although TeA has been detected in contaminated foodstuffs and fruits,[21,22] and has some cellular toxicity to 3 T3 mouse fibroblasts, Chinese hamster lung cells, and human hepatocytes[23] and animal toxicity to mouse,[24] it has little environmental and genetic toxicity.[23] It has a short half-life (about 3.22 d) in the field and a short residual period (about 20 d) in the soil.[25] These results suggested that TeA has the potential for the development of a bio-based herbicide.

Crofton weed seedlings at four-leaf stage were more sensitive to TeA than those at six- and eight-leaf stages. This is not surprising, as all herbicides are more phytotoxic to younger than older seedlings. For example, the toxicity of AAL-toxins is more effective on younger than older tomato plants.[26] However, old leaves of crofton weed were more sensitive to TeA than were younger leaves, which perhaps is due to structural differences in the leaf tissue and weaker defense capabilities of older leaves. So, TeA should be sprayed on the seedlings of croft weeds that are younger than the four-leaf stage to assure good weed control.

TeA also showed various phytotoxicity to plants in different families, which is similar to other phytotoxins such as AAL-toxin, tentoxin and destruxins produced by *A. alternate*.[27–29] The target of TeA could be different for these plants with different responses. Previous studies indicated that TeA inhibited photosynthesis of crofton weed,[9] by blocking electron transfer from Q_A to Q_B by binding the Q_B-binding site in the D1 protein in PSII of *Chlamydomonas reinhardtii*.[12] The inhibition of PSII leads to increases in destructive

Table 3. Phytotoxicity of TeA to weeds and crops by leaf punctured assay

Family	Plant species	Fitting equation(concentration and injury rate)	R^2	EC_{50} (μg/mL)	EC_{90} (μg/mL)
Amaranthaceae	*Alternanthera philoxeroides*	$y = 102.39 - 102.32/(1 + (x/207.32)^{2.36})$	0.988	203	480
	Amaranthus retroflexus	$y = 105.78 - 105.54/(1 + (x/102.63)^{3})$	0.986	98.8	183
	Amaranthus tricolor	$y = 106.74 - 106.36/(1 + (x/127.43)^{1.90})$	0.994	119	308
	Celosia argentea	$y = 110.14 - 110.04/(1 + (x/92.14)^{1.31})$	0.973	79.9	289
Brassicaceae	*Arabidopsis thaliana*	$y = 105.14 - 102.15/(1 + (x/63.16)^{2.17})$	0.988	58.7	141
Chenopodiaceae	*Chenopodium album*	$y = 103.54 - 102.66/(1 + (x/77.82)^{2.12})$	0.983	74.7	189
	Chenopodium serotinum	$y = 104.36 - 104.38/(1 + (x/156.88)^{1.55})$	0.976	149	513
Commelinaceae	*Commelina bengalensis*	$y = 103.09 - 102.36/(1 + (x/87.57)^{2.07})$	0.994	74.7	189
	Commelina communis	$y = 105.25 - 102.66/(1 + (x/91.06)^{2.05})$	0.987	84.5	214
Compositae	*Ageratina adenophora*	$y = 96.49 - 96.36/(1 + (x/70.60)^{3})$	0.991	72.3	170
	Ageratum conyzoides	$y = 102.31 - 102.42/(1 + (x/67.38)^{3})$	0.996	66.4	131
	Ambrosia artemisiifolia	$y = 100.62 - 100.62/(1 + (x/49.88)^{1.69})$	0.990	74.7	177
	Conyza canadensis	$y = 95.04 - 95.36/(1 + (x/159.30)^{3})$	0.989	141	417
	Conyza sumatrensis	$y = 105.16 - 105.28/(1 + (x/115.84)^{3})$	0.991	112	210
	Eclipta prostrata	$y = 97.17 - 97.28/(1 + (x/97.34)^{3})$	0.987	116	230
	Erigeron annuus	$y = 110.88 - 110.81/(1 + (x/74.10)^{3})$	0.989	72.9	121
	Solidago canadensis	$y = 94.82 - 94.96/(1 + (x/135.70)^{3})$	0.986	211	540
	Sonchus asper	$y = 96.31 - 96.42/(1 + (x/51.48)^{3})$	0.995	51.5	125
	Sonchus oleraceus	$y = 100.80 - 100.79/(1 + (x/89.36)^{2.47})$	0.987	88.8	211
	Youngia japonica	$y = 100.36 - 100.35/(1 + (x/102.70)^{3.30})$	0.987	102	197
Convolvulaceae	*Calystegia hederacea*	$y = 111.46 - 111.567/(1 + (x/92.29)^{1.54})$	0.986	80.8	234
	Ipomoea triloba	$y = 108.82 - 109.07/(1 + (x/95.44)^{1.29})$	0.986	113	322
	Pharbitis nil	$y = 117.80 - 117.92/ (1 + (x/96.60)^{1.28})$	0.988	76.3	242
	Pharbitis purpurea	$y = 107.10 - 106.44/(1 + (x/78.39)^{2})$	0.984	72.9	179
Cyperaceae	*Cyperus difformis*	$y = 102.87 - 102.43/(1 + (x/67.42)^{2.18})$	0.994	65.4	164
	Cyperus iria	$y = 103.21 - 103.28/(1 + (x/72.32)^{1.62})$	0.993	109	237
	Cyperus rotundus	$y = 104.76 - 104.39/(1 + (x/102.68)^{1.59})$	0.986	106	319
Euphorbiaceae	*Acalypha australis*	$y = 101.54 - 100.98/(1 + (x/532.11)^{2.01})$	0.989	512	1474
	Euphorbia humifusa	$y = 103.44 - 101.28/(1 + (x/118.05)^{1.08})$	0.989	113	267
Gramineae	*Digiteria sanguinalis*	$y = 92.4 - 92.4/(1 + (x/87.19)^{3})$	0.997	45.2	143
	Echinochloa crus-galli	$y = 115.94 - 115.95/(1 + (x/179.78)^{1.52})$	0.994	150	408
	Eleusine indica	$y = 103 - 100/(1 + (x/379.93)^{3})$	0.984	365	716
	Leptochloa chinensis	$y = 105.16 - 105.36/(1 + (x/88.01)^{1.6})$	0.992	83.0	268
	Leptochloa panicea	$y = 110.01 - 109.84/(1 + (x/74.84)^{3})$	0.996	78.5	226
	Microstegium vimineum	$y = 105.42 - 105.35/(1 + (x/175.63)^{3})$	0.998	170	319
	Oryza sativa	$y = 105.42 - 105.35/(1 + (x/175.63)^{3})$	0.991	361	673
	Setaria viridis	$y = 115.67 - 115.19/(1 + (x/118.62)^{1.07})$	0.985	91.1	382
	Triticum aestivum	$y = 112 - 112/(1 + (x/134.32)^{2.16})$	0.991	84.4	258
	Zea mays	$y = 109 - 109/(1 + (x/82.07)^{2.15})$	0.997	76.0	169
Malvaceae	*Abutilon theophrasti*	$y = 140\,279.87 - 140\,279.87/(1 + (x/5.37 \times 10^{6})^{0.94})$	0.984	1156	2156
	Althaea rosea	$y = 127\,273.79 - 127\,273.23/(1 + (x/5.37 \times 10^{6})^{0.94})$	0.982	1282	2392
	Gossypium hirsutum	$y = 392\,969.43 - 392\,969.22/(1 + (x/5.37 \times 10^{6})^{0.94})$	0.975	1604	2992
	Hibiscus rosa-sinensis	$y = 96\,616.60 - 96\,616.84/(1 + (x/5.37 \times 10^{6})^{0.94})$	0.982	1720	3207
	Hibiscus syriacus	$y = 148\,548.03 - 148\,548.53/(1 + (x/5.37 \times 10^{6})^{0.94})$	0.982	1088	2029
	Sida acuta	$y = 149\,569.93 - 149\,569.93/(1 + (x/5.37 \times 10^{6})^{0.94})$	0.983	1730	3226
Moraceae	*Humulus scandens*	$y = 102.01 - 101.84/(1 + (x/70.45)^{2.29})$	0.987	69.1	170
Nyctaginaceae	*Mirabilis jalapa*	$y = 104.56 - 102.60/(1 + (x/111.07)^{2.06})$	0.985	104	266
Onagraceae	*Ludwigia prostrata*	$y = 108.05 - 107.54/(1 + (x/113.79)^{1.52})$	0.977	103	326
Oxalidaceae	*Oxalis corniculata*	$y = 102.45 - 102.63/(1 + (x/66.78)^{3})$	0.987	65.8	129
Plantaginaceae	*Plantago asiatica*	$y = 103.31 - 103.24/(1 + (x/176.61)^{1.57})$	0.985	169	596
Polygonaceae	*Polygonum jucundum*	$y = 102.44 - 102.60/(1 + (x/110.72)^{1.81})$	0.997	108	331
Portulacaceae	*Portulaca oleracea*	$y = 103.8 - 103.73/(1 + (x/61.72)^{2.18})$	0.993	59.6	145
Solanaceae	*Capsicum annuum*	$y = 110\,551.69 - 110\,551.69/(1 + (x/5.37 \times 10^{6})^{0.94})$	0.971	1490	2779
	Lycium chinense	$y = 126\,344.79 - 126\,344.79/(1 + (x/5.37 \times 10^{6})^{0.94})$	0.977	1292	2410
	Nicotiana tabacum	$y = 134\,705.84 - 134\,705.84/(1 + (x/5.37 \times 10^{6})^{0.94})$	0.976	1207	2251
	Physalis alkekengi	$y = 149\,569.93 - 149\,569.93/(1 + (x/5.37 \times 10^{6})^{0.94})$	0.981	1367	2550
	Solanum lyratum	$y = 119\,841.75 - 119\,841.75/(1 + (x/5.37 \times 10^{6})^{0.94})$	0.971	1151	2147
	Solanum melongena	$y = 111\,480.69 - 111\,480.69/(1 + (x/5.37 \times 10^{6})^{0.94})$	0.976	1477	2754
	Solanum nigrum	$y = 140\,279.87 - 140\,279.87/(1 + (x/5.37 \times 10^{6})^{0.94})$	0.981	1156	2156
Sterculiaceae	*Melochia corchorifolia*	$y = 102.20 - 101.96/(1 + (x/63.00)^{2.20})$	0.996	61.6	156
Vitaceae	*Cayratia japonica*	$y = 103.70 - 100.84/(1 + (x/102.58)^{2.39})$	0.987	97.1	222

Table 4. Phytotoxicity of TeA to some weeds by seedling spraying assay

Plant species	Efficacy (%)						
			Concentration (μg/mL)				
	Water control (CK0)	Adjuvant control (CK1)	62.5	125	250	500	1000
Acalypha australis	0 ± 0	0 ± 0	3.4 ± 2.2	7.5 ± 1.3	20.7 ± 6.6	37.3 ± 4.8	70.7 ± 2.7
Alopecurus japonicus	0 ± 0	0 ± 0	4.9 ± 0.6	24.5 ± 5.4	55.3 ± 6.6	86.2 ± 3.2	93.3 ± 3.8
Ambrosia artemisiifolia	0 ± 0	0 ± 0	19.1 ± 0.9	37.6 ± 2.6	75.4 ± 2.2	88.2 ± 1.3	93.4 ± 0.9
Commelina communis	0 ± 0	0 ± 0	8.5 ± 1.5	30.4 ± 2.8	57.7 ± 4.2	78.1 ± 2.7	92.7 ± 2.5
Cyperus iria	0 ± 0	0 ± 0	15.2 ± 1.3	31.4 ± 1.2	58.0 ± 3.0	82.9 ± 3.2	100 ± 0
Cyperus difformis	0 ± 0	0 ± 0	22.8 ± 2.1	56.4 ± 5.5	73.6 ± 8.0	98.2 ± 1.8	100 ± 0
Digitaria sanguinalis	0 ± 0	0 ± 0	56.4 ± 8.8	90.4 ± 2.1	99.8 ± 0.2	100 ± 0	100 ± 0
Echinochloa crus-galli	0 ± 0	0 ± 0	6.0 ± 0.6	30.8 ± 7.5	65.0 ± 0.8	92.8 ± 3.8	97.7 ± 0.4
Eclipta prostrata	0 ± 0	0 ± 0	4.5 ± 1.1	22.7 ± 0.7	42.5 ± 2.4	73.3 ± 4.7	99.2 ± 0.5
Erigeron annuus	0 ± 0	0 ± 0	4.9 ± 0.4	18.8 ± 5.7	47.7 ± 2.3	76.9 ± 5.9	94.2 ± 2.9
Geranium carolinianum	0 ± 0	0 ± 0	9.4 ± 2.1	34.4 ± 11.4	70.4 ± 6.4	87.4 ± 4.5	100 ± 0
Leptochloa chinensis	0 ± 0	0 ± 0	19.0 ± 1.9	66.0 ± 7.7	77.0 ± 7.5	100 ± 0	100 ± 0
Sonchus asper	0 ± 0	0 ± 0	4.8 ± 0.9	30.4 ± 1.1	55.4 ± 2.6	85.8 ± 2.6	100 ± 0
Trifolium repens	0 ± 0	0 ± 0	12.9 ± 1.1	69.1 ± 1.1	88.0 ± 0.7	100 ± 0	100 ± 0
Vicia sativa	0 ± 0	0 ± 0	6.8 ± 0.8	23.3 ± 1.7	63.2 ± 1.8	85.8 ± 3.1	100 ± 0

Table 5. Regression analysis of the concentration-response of TeA on weeds

Plant species	Formula	R^2	EC_{50} (μg/mL)	EC_{90} (μg/mL)
Acalypha australis	$y = 0.03 + 0.68/(1 + (2.7867/x)^{9.8849})$	0.988	612	2539
Alopecurus japonicus	$y = 0.05 + 0.88/(1 + (2.3342/x)^{11.2067})$	0.958	216	684
Ambrosia artemisiifolia	$y = 0.19 + 0.74/(1 + (2.1761/x)^{9.1295})$	0.949	147	607
Commelina communis	$y = 0.08 + 0.85/(1 + (2.3145/x)^{8.872})$	0.976	206	795
Cyperus iria	$y = 0.15 + 0.85/(1 + (2.2765/x)^{9.1832})$	0.988	189	609
Cyperus difformis	$y = 0.23 + 0.77/(1 + (2.0564/x)^{9.0711})$	0.958	114	345
Digitaria sanguinalis	$y = 0.56 + 0.34/(1 + (1.7618/x)^{13.2662})$	0.924	57.5	119
Echinochloa crusgalli	$y = 0.06 + 0.92/(1 + (2.2546/x)^{12.0721})$	0.951	180	474
Eclipta prostrata	$y = 0.04 + 0.95/(1 + (2.4265/x)^{10.7006})$	0.976	267	748
Erigeron annuus	$y = 0.05 + 0.89/(1 + (2.41/x)^{10.9666})$	0.982	257	795
Geranium carolinianum	$y = 0.09 + 0.91/(1 + (2.2212/x)^{10.9955})$	0.960	166	508
Leptochloa chinensis	$y = 0.19 + 0.81/(1 + (2.0192/x)^{10.61})$	0.935	105	292
Sonchus asper	$y = 0.05 + 0.95/(1 + (2.3072/x)^{10.8345})$	0.976	203	544
Trifolium repens	$y = 0.13 + 0.87/(1 + (2.0072/x)^{15.5677})$	0.904	102	257
Vicia sativa	$y = 0.07 + 0.93/(1 + (2.3003/x)^{12.299})$	0.962	200	521

reactive oxygen species,[30] resulting in cell membrane destruction and cell death. This can result in the brown leaf spots associated with TeA. So, we suggest TeA application should be done on sunny days to enhance its efficacy.

Chemical synthesis is an effective method for producing larger amounts of toxin and to alter their structures to enhance the desired biological activity while lowering mammalian toxicity. *Alternaria* toxins such as AAL-toxin, AS-I toxin, tentoxin etc., were all synthesized for those purposes.[31–33] The synthesis of tenuazonic acid and its analogues has been reported recently in our laboratory.[34,35] Using L-isoleucine as a starting material, TeA was synthesized following only five steps with the advantages of low cost, simple and safe production, and high yields.[17] Synthetic TeA caused as rapid injury symptoms to crofton weed (after 4 h) and other weeds as did natural TeA. However, synthetic TeA demonstrated lower activity than natural TeA based on pure TeA of crude extract.[7] This may be explained by two reasons. First, the crude extract may contain other herbicidally active substances. In addition, the TeA structure has two asymmetric carbon atoms, and synthesized TeA may be a mixture of four isomers, each with different biological activities.

TeA had little toxicity to Solanaceae and Malvaceae plants, including cotton and tobacco, in the leaf puncture and seedling spraying assays. These two crops were highly tolerant to TeA, but it was toxic to wheat, rice, corn, and rape. Hasan also reported that TeA is toxic to barley, wheat, and sorghum.[36] In our cotton field trial, TeA caused minor injury to cotton at the highest concentration of 1500 g/ha, however, the plants gradually recovered. At lower concentrations, TeA was safe to cotton, but still killed several important weeds. Its safety on other crops should be investigated.

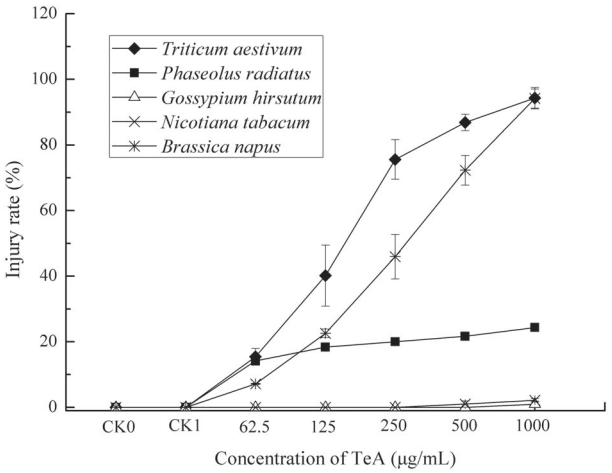

Figure 3. Phytotoxicity of TeA to five economic crops by the seedling spraying assay. Note: The error bars were ± 1 standard error.

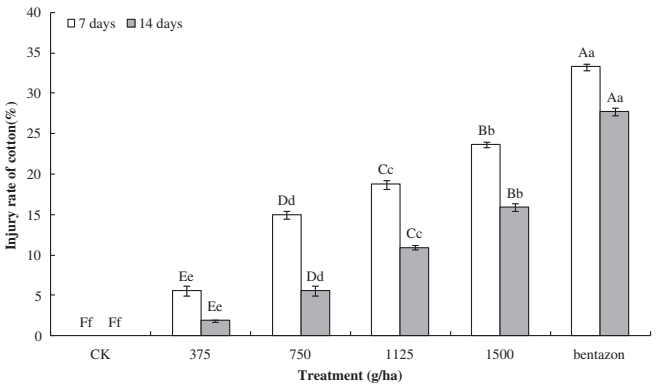

Figure 4. The injury rate of cotton after 7 days and 14 days
Note: The different small letters in each column indicate significant difference at $P < 0.05$ level; the different capital letters in each column indicate significant difference at $P < 0.01$ level.

5 CONCLUSION

TeA was phytotoxic to crofton weed and the main weeds in cotton fields, but was not phytotoxic or had little phytotoxicity to *Nicotiana tabacum* and *Gossypium hirsutum*. Especially in cotton field trials, it showed good control effects on the two main weeds *D. sanguinalis* and *A. retroflexus*, but was relatively safe to cotton. In addition, the half-life of TeA was only about 3.22 d in the field, its residual period was about 20 d in the soil, and it had low environmental and genetic toxicity. Hence, TeA has the potential for development as a bio-based herbicide in cotton fields.

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The opinions expressed and arguments employed in this paper are the sole responsibility of the authors and do not necessarily reflect those of the OECD or of the governments of its Member countries.

Table 6. Regression analysis of the concentration-response of TeA on crops

Population	Formula	R^2	EC_{50} (μg/mL)	EC_{90} (μg/mL)
Brassica napus	$y = 19.706x - 28.609$	0.971	264	898
Gossypium hirsutum	$y = 0.085x^2 - 0.4592x + 0.4762$	0.786	17 069	95 143
Nicotiana tabacum	$y = 0.1776x^2 - 0.8443x + 0.7937$	0.966	15 517	89 612
Phaseolus radiatus	$y = -1.2075x^2 + 12.618x - 9.4268$	0.939	2279	5687
Triticum aestivum	$y = 20.601x - 20.034$	0.960	157	576

Table 7. Control effect of TeA on *Digitaria sanguinalis* and *Amaranthus retroflexus* in field trial

	Digitaria sanguinalis			*Amaranthus retroflexus*		
Treatment	Plant control effect (7d)	Plant control effect (14d)	Fresh weight control effect (14d)	Plant control effect (7d)	Plant control effect (14d)	Fresh weight control effect (14d)
1500 g ai./ha (25% TeA AS)	92.8 ± 1.8Aa	87.7 ± 3.8Aa	90.0 ± 11.9Ab	86.4 ± 7.0Aa	83.9 ± 4.7Bb	85.5 ± 10.4Aa
1125 g ai./ha (25% TeA AS)	83.8 ± 0.8Bb	80.5 ± 4.9ABa	85.7 ± 9.1Ab	77.3 ± 2.6ABb	64 ± 18.1ABab	73.4 ± 10.2Aab
750 g ai./ha (25% TeA AS)	72.6 ± 4.1Cc	70.7 ± 9.6Bbc	76.4 ± 7.5Aab	68.9 ± 4.9BCb	60.1 ± 8.6Aba	67.7 ± 8.6Aab
375 g ai./ha (25% TeA AS)	56.4 ± 3.5Dd	50.2 ± 4.7Bc	64.9 ± 2.2Aa	61.1 ± 4.3Cc	54.0 ± 7.2Aa	59.5 ± 13.8Ab
300 g ai./ha (48% Bentazon)	63.9 ± 3.0Dd	53.6 ± 3.9Bc	52.6 ± 9.2Ab	76.3 ± 8.4ABab	69.7 ± 5.6Aa	67.9 ± 6.6Ab
water	0.0 ± 3.7Ee	0.0 ± 18.6Cd	0.0 ± 34.2Bc	0.0 ± 17.1Dd	0.0 ± 18.8Cc	0.0 ± 32.7Bc

Note: The different small letters in each column indicate significant difference at $P < 0.05$ level; the different capital letters in each column indicate significant difference at $P < 0.01$ level.

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