

Combined herbicidal effect of two natural products (sorgoleone and hairy root extract of tartary buckwheat) on crops and weeds

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Abstract

Sorgoleone, a root exudate of sorghum [*Sorghum bicolor* (L.) Moench] and hairy root extract of tartary buckwheat (*Fagopyrum tataricum* Gaertn.) were mixed and applied to different broadleaf and grass weed species and crop species in the greenhouse arranging a randomized complete block design with four replications to evaluate their combined herbicidal activity and crop selectivity. A mixture of these two natural products (sorgoleone and root extract of tartary buckwheat) showed significantly greater inhibitory effects than either one alone. Broadleaf weed species were more susceptible to the application of the mixture than grass weed species. *Galium spurium*, *Rumex japonicus*, *Aeschynomene indica*, and *Amaranthus retroflexus* were the most susceptible among the broadleaf species and *Setaria viridis* was the most susceptible among the grass species. Broadleaf weed species demonstrated more growth suppression than grass weed species. For example, growth of *G. spurium* was reduced by 80.9% at 150 $\mu\text{g ml}^{-1}$ of sorgoleone alone and by 33.7% at 7.5 mg ml^{-1} of hairy root extract alone; however their mixture reduced *G. spurium* growth by 100%. Moreover, the mixture reduced studied crop growth slightly more than sorgoleone or hairy root extract used individually. The ability of weed growth suppression using sorgoleone and hairy root extract of tartary buckwheat together offers interesting possibilities for effective weed management.

Keywords: Allelochemicals; Crop selectivity; Hairy root extract; Sorgoleone; Weed management.

Abbreviations: ANOVA- Analysis of variance, HPLC-High Performance Liquid Chromatography, IBA- indole-3-butyric acid, LSD-Least Significance difference, MS, Murishage and Skoog, SAS- Statistical Analysis Systems, SH- Schenk and Hildebrandt's, USDA-ARS- United States Department of Agriculture-Agricultural Research Service.

Introduction

Allelochemicals, which are naturally derived plant products, may provide new, effective, and reduced-risk alternative methods for suppressing weeds in crop fields with lower impact on the environment. Natural products relatively have short half-life and therefore considered safe at environmental toxicology standpoint (Duke et al., 2002). Natural products such as allelochemicals offer an attractive alternative to synthetic herbicides because they are generally more environmental friendly (Dayan et al., 2009 a). In addition, many allelochemicals and other natural phytotoxins may inhibit molecular target sites distinct from those targeted by commercially available herbicides (Duke et al., 2002; Dayan et al., 2012). Many plant species possess the capability to produce and release allelochemicals to suppress the growth of other plants (Weston, 1996). Sorgoleone, the oily exudate secreted by the root hair of sorghum (*Sorghum bicolor*), contains the lipid benzoquinone sorgoleone (2-hydroxy-5-methoxy-3-[(8'Z, 11'Z)-8', 11',14'-pentadecatriene]-p-benzoquinone), which is a potent allelochemical (Netzly and Butler, 1986; Czarnota et al., 2001; Inderjit and Duke, 2003; Dayan et al., 2010). Plants in the genus *Fagopyrum* produce a wide array of biologically active constituents. Common buckwheat (*Fagopyrum esculentum* Moench) and tartary buckwheat (*Fagopyrum tataricum* Gaertn.) are important members of this genus. Tartary buckwheat has

various pharmacological and biological properties, including anticancer (Guo et al., 2007), antidiabetic (Yao et al., 2008) and herbicidal activities (Fujii et al., 2005). The importance of mixtures of allelochemicals is recognized both in herbicide research and in research exploring plant chemical interference (allelopathy) *in situ*. Previously, mixtures are often used to enhance efficiency and efficacy, and reduce selectivity (Green et al., 1995). Latter, it has been stated (Einhellig, 1995) that nearly all allelopathic activities are due to mixtures of two or more compounds. Therefore, in this study, herbicidal potential of a combination of natural products-sorgoleone from sorghum root and hairy root of tartary buckwheat to different weeds and crops were studied, in order to determine their efficacy as a potential bioherbicide.

Results and discussions

Combined effect of sorgoleone and hairy root extract on weeds

Combination of both natural products (sorgoleone and hairy root extract) inhibited weed growth more than either product used singly, with the broadleaf weed species being more susceptible than grass weed species (Fig. 1 and Fig. 2). The broadleaf weeds *G. spurium*, *R. japonicus*, and *A. indica*

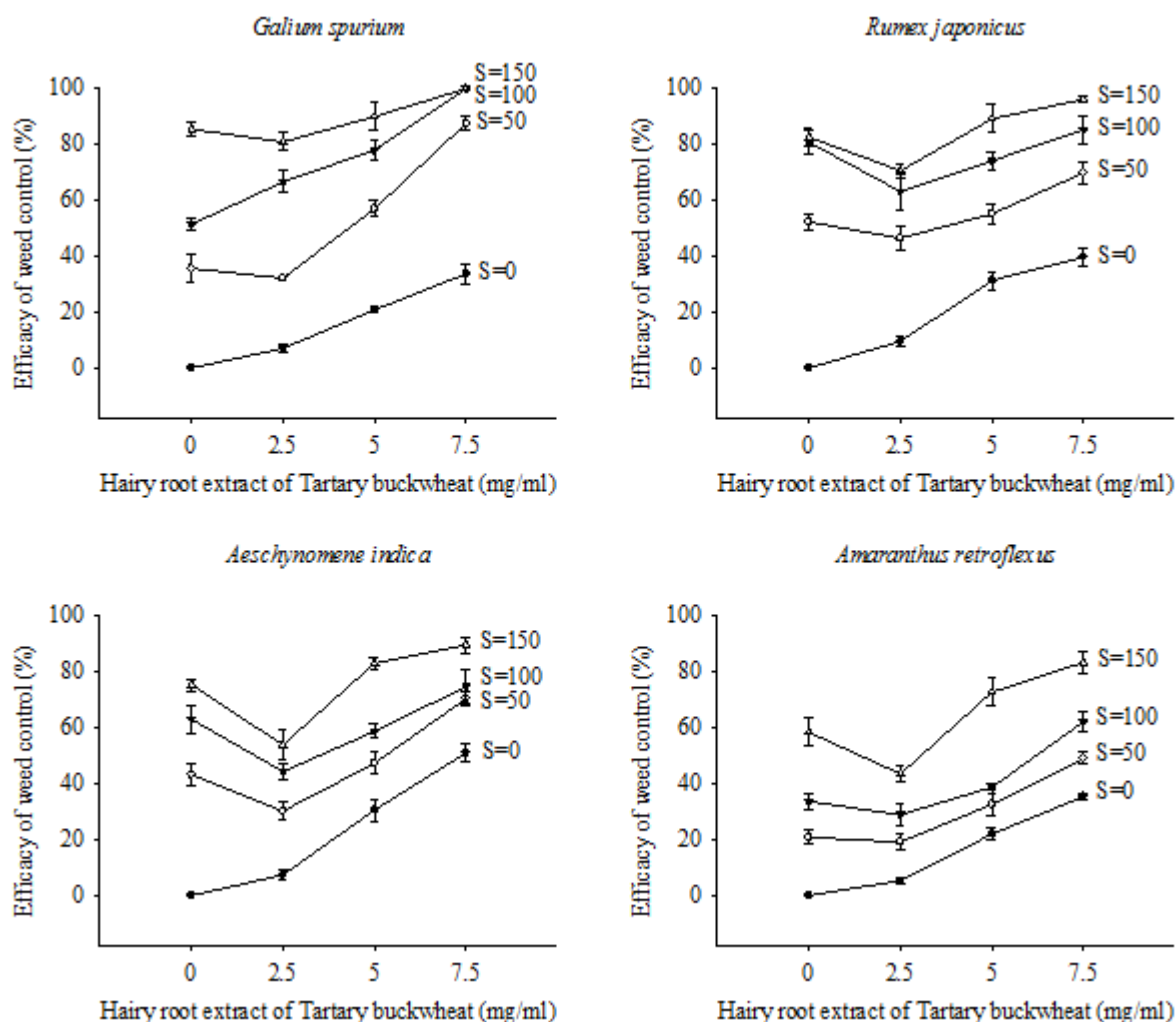


Fig 1. Combined effect of foliar application of sorgoleone and hairy root extract of *Fagopyrum tataricum* on control of broadleaf weeds. S=0, Sorgoleone (0 µg/ml); S=50, Sorgoleone (50 µg/ml); S=100, Sorgoleone (100 µg/ml); S=150, Sorgoleone (150 µg/ml).

were the most susceptible species to the combined application of the allelochemicals. Application of sorgoleone at 150 µg/ml inhibited the growth of *G. spurium*, *R. japonicus*, and *A. indica* by 80.9%, 82.6%, and 75.4%, respectively (Fig. 1). The growth inhibition with the application of hairy root extract at 7.5 mg/ml was 33.7%, 39.7%, and 51.1% for *G. spurium*, *R. japonicus*, and *A. indica*, respectively (Fig. 1). However, when 150 µg/ml of sorgoleone combined with 7.5 mg/ml of hairy root extract was applied; growth inhibition was 100%, 96.2%, and 89.7% for *G. spurium*, *R. japonicus*, and *A. indica*, respectively (Fig. 1). The trend was slightly lower for the other broadleaf weed species. *S. viridis* was the most susceptible grass weed species; application of 7.5 mg/ml of hairy root extract inhibited the growth of *S. viridis* by 53.6%; 150 µg/ml of sorgoleone inhibited growth by 52.3%; and 150 µg/ml of sorgoleone combined with 7.5 mg/ml of hairy root extract applied together inhibited growth by 78.2% (Fig. 2).

Combined effect of sorgoleone and hairy root extract on crop

The variation of crop growth inhibition, as measured by shoot biomass was not significantly different in most cases. However, crop growth inhibition was slightly greater with

combined application of the natural products than with their individual use (Fig. 3 and Fig. 4). Among the crop species, lettuce, soybean, and cucumber were most susceptible. On the other hand corn, wheat and rice were most tolerant. The growth inhibition using 150 µg/ml of sorgoleone combined with 7.5 mg/ml of hairy root extract for lettuce, soybean, and cucumber was 33%, 19.6%, and 18.8%, respectively (Fig. 4). The crop species of corn, wheat and rice displayed growth inhibition between 10-12% with 150 µg/ml sorgoleone combined with 7.5 mg/ml hairy root extract (Fig. 3). This study investigated the herbicidal potential of a combination of the natural product sorgoleone obtained from the sorghum root and hairy root of tartary buckwheat on different weeds to determine their potential synergism and efficacy as a potential bioherbicide. Modern agriculture now faces the challenge of reducing environmental damage and health hazards from chemical inputs while maintaining a high level of production. Hence, alternative weed control methods that have no adverse effect on the environment are required for crop fields. Allelochemicals may play a key role in suppressing weeds in crop fields without impairing the environment and results in significant yield increases. The importance of allelopathy in agricultural practices has been recognized with the main objective of using this phenomenon in biological weed control (Rice, 1984). One approach to utilize this phenomenon is to screen accessions

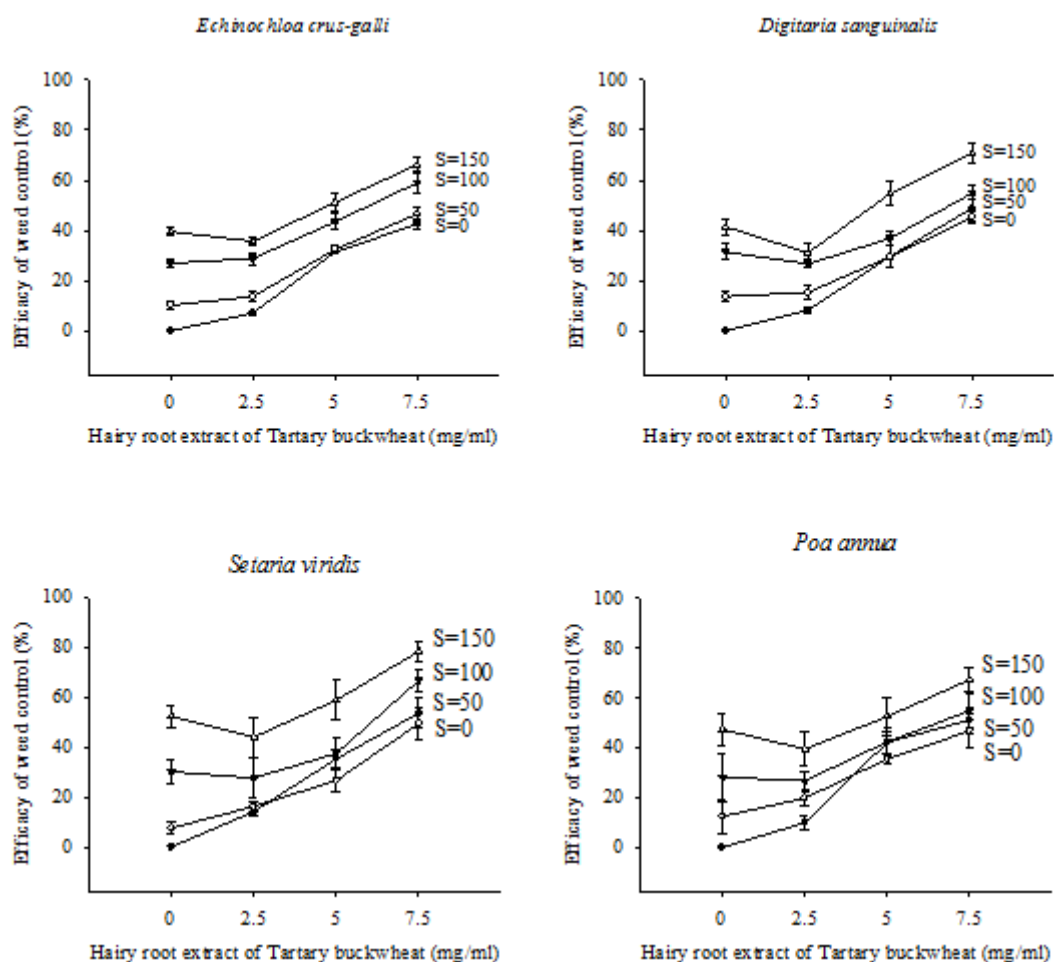


Fig 2. Combined effect of foliar application of sorgoleone and hairy root extract of *Fagopyrum tataricum* on control of grass weeds. S=0, Sorgoleone (0 µg/ml); S=50, Sorgoleone (50 µg/ml); S=100, Sorgoleone (100 µg/ml); S=150, Sorgoleone (150 µg/ml).

of allelopathic crops for their ability to reduce weeds (Olofsdotter et al., 2002; Gealy et al., 2003). The efficacy of natural products is not as strong as that of synthetic herbicides and scientists are currently attempting to use synergistic combinations of various allelochemicals to increase the efficacy of these natural products. In this study, a combination of sorgoleone and hairy root extract were chosen for their synergistic potential. Most weed species showed synergistic effects with the combination of sorgoleone and hairy root extract of tartary buckwheat and synergistic effects were greater at higher mixture ratios. The weed species *G. spurius*, *R. japonicus*, *A. retroflexus*, and *A. indica* showed the most synergistic effects in this study. Previous reports have given the evidence to use mixtures of chemical with profound results. Berenbaum and Neal (1985) found a significant interaction between the two compounds, resulting in a 5-fold increase in toxicity for xanthotoxin in the presence of myristicin. Myristicin alone did not result in mortality at any dose level tested.

Materials and Methods

Plant materials

Sorghum seeds (cultivar Chalsusu) were used in this study. Seeds were treated with the fungicide benomyl for 4 h and then rinsed several times in distilled water. Seeds were

grown in Petri dishes (100 × 40 mm) on the surface of sterile Whatman No.1 filter paper (diameter, 90 mm) for collection of root exudates. Cultures were then placed in a growth chamber at 30°C under standard cool-white fluorescent tubes with a flux rate of 550 µmol s⁻¹ m⁻² and a 16-h photoperiod for 10 days. Roots were harvested from 10-day-old seedlings. Root samples were stored in sealed clear polyethylene plastic bags at -80°C until use.

Sorghum root extraction

The lipophilic benzoquinone sorgoleone is produced exclusively by sorghum root hairs (Dayan et al. 2007; Baerson et al., 2008; Cook et al., 2010). Therefore, a crude extract of sorgoleone was obtained from sorghum root according to the procedures described by (Netzly and Butler, 1986; Czarnota et al., 2003), except that methanol (Uddin et al., 2010) was used as a solvent instead of methylene chloride. Seedling roots were excised and immersed in methanol (1:20 w/v) for 30 sec for extraction. The methanol extract was filtered and evaporated under vacuum to dryness. The crude extract was stored at -80°C until further use.

Sorgoleone analysis by HPLC

The dried extract was dissolved in methanol (1 mg mL⁻¹) and

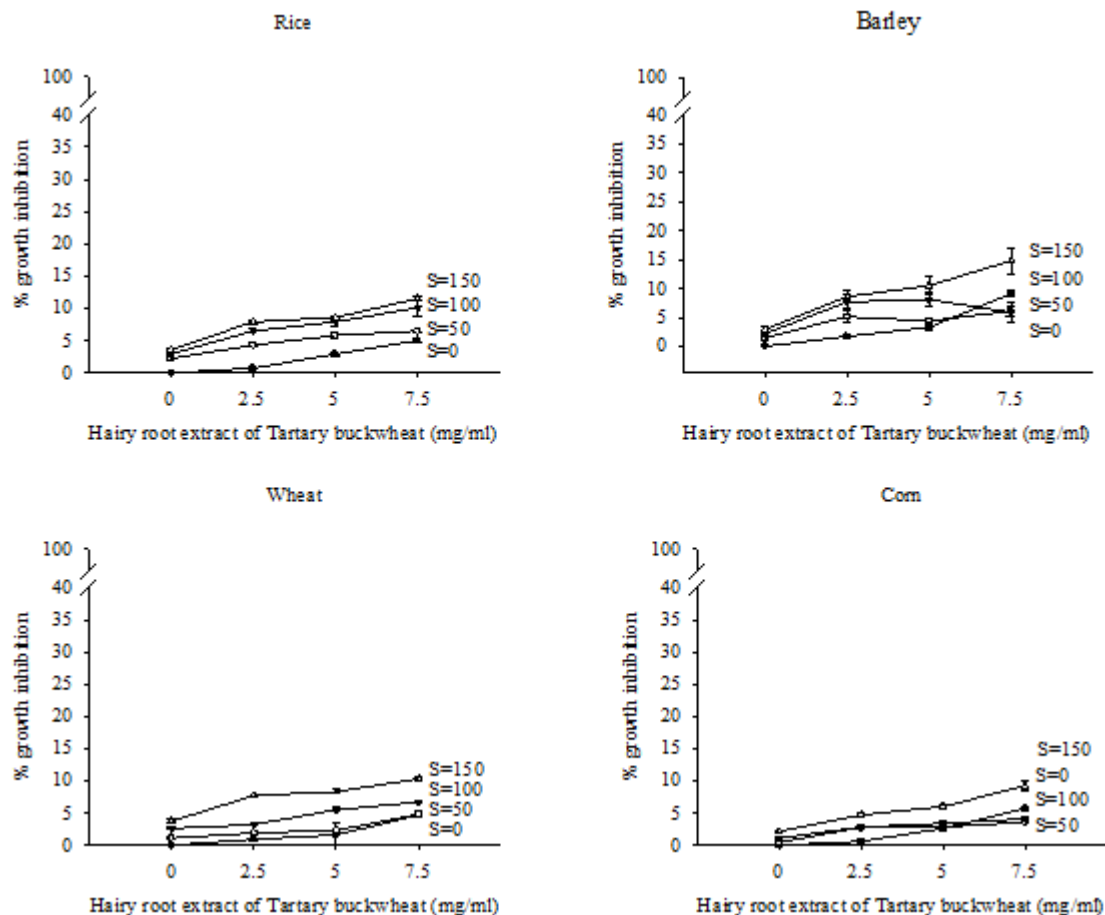


Fig 3. Combined effect of foliar application of sorgoleone and hairy root extract of *Fagopyrum tataricum* on growth inhibition of cereal crops. S=0, Sorgoleone (0 µg/ml); S=50, Sorgoleone (50 µg/ml); S=100, Sorgoleone (100 µg/ml); S=150, Sorgoleone (150 µg/ml).

the solution was then filtered through a poly filter (pore size, 0.45 µm). The filtrate was diluted 4-fold with methanol prior to HPLC analysis. HPLC quantification of sorgoleone was performed using a Futecs NS-4000 HPLC system (Futecs Co. Ltd., Daejeon, Korea) with a C₁₈ column (250 × 4.6 mm; particle size 5 µm; RStech, Daejeon, Korea). The mobile phase was 75% acetonitrile + 25% acidified water. Water was acidified with glacial acetic acid (97.5:2.5 v/v). After injection of 20 µl of the soluble methanol crude extract, sorgoleone was detected at 280 nm with a Waters tunable absorbance detector. The column flow rate was 1 ml min⁻¹ with a 40-min total run time for each sample. Samples were run in triplicate. Sorghum root extracts consists of a mixture of sorgoleone derivatives with various degrees of unsaturation (Kagan et al., 2003) as well as an equivalent amount of lipophilic resorcinols analogues (Dayan et al., 2009 b). Sorgoleone levels in the extracts were calculated on the basis of a standard curve obtained from a purified sample. The sorgoleone standard was provided by Franck Dayan, United States Department of Agriculture-Agricultural Research Service (USDA-ARS), Natural Products Utilization Research Unit.

General procedures to establish hairy root cultures of *F. tartaricum*

The establishment and maintenance of hairy root cultures

were performed as described by Kim et al. (2009). Young stems of *F. tataricum* were taken from plants grown *in vitro* and were cut at the ends into 7 mm lengths. Excised stems were dipped into *Agrobacterium rhizogenes* R1000 culture in liquid inoculation medium for 10 min, blotted dry on sterile filter paper, and incubated in the dark at 25°C on agar-solidified MS (Murishage and Skoog) medium. After two days of co-cultivation, the explant tissues were transferred to a hormone-free medium containing MS salts and vitamins (0.5 mg/l nicotinic acid, 0.5mg/l pyridoxine-HCl, 0.1 mg/l thiamine-HCl, and 2.0 mg/l glycine), 30 g/l sucrose, 500 mg/l cefotaxime, and 8 g/l agar. Numerous hairy roots were observed emerging from the wound sites of explants within two weeks. The hairy roots were separated from the explant tissue and subcultured in the dark at 25°C on agar-solidified MS medium. After repeated transfer to fresh medium, rapidly growing hairy root cultures were obtained. Isolated roots (0.5 g/l) were transferred to 30 ml of MS liquid medium, containing 30 g/l sucrose, in 100 ml flasks. Root cultures were maintained at 25°C on a gyratory shaker (100 rev/min) in a growth chamber under standard cool white fluorescent tubes with a flux rate of 35 µmol s⁻¹m⁻² and a 16-h photoperiod. After 21 d of culture, hairy roots were harvested. Dry weight and phenolic compound contents were determined. Three flasks were used for each culture condition and experiments were performed in duplicate.

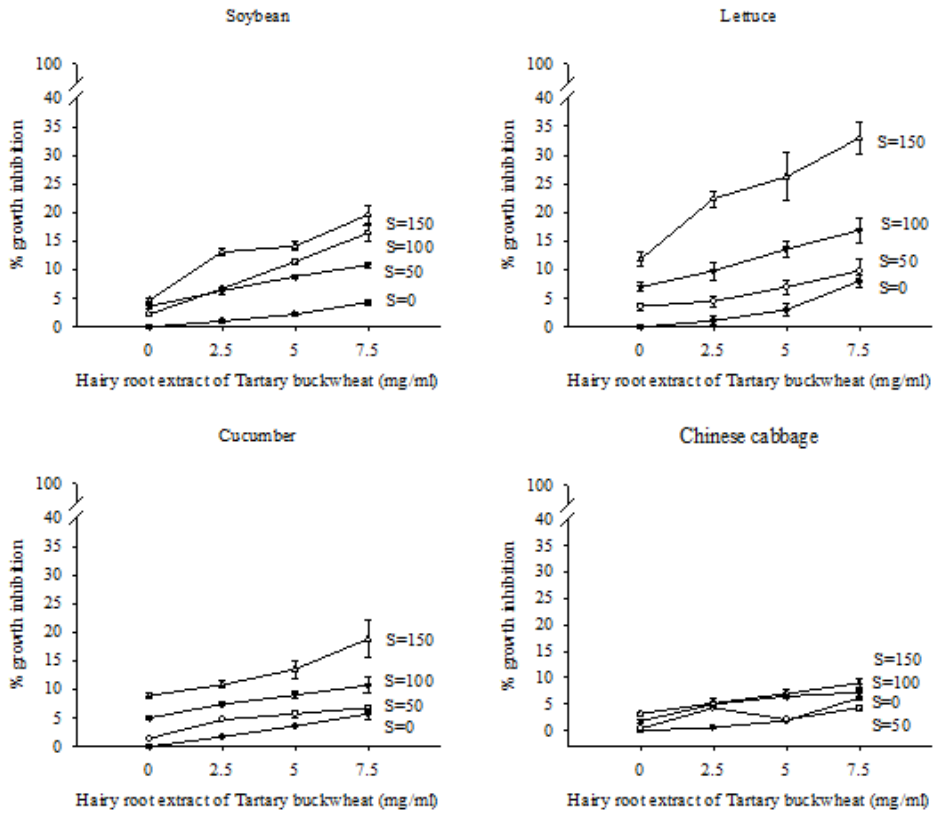


Fig 4. Combined effect of foliar application of sorgoleone and hairy root extract of *Fagopyrum tataricum* on growth inhibition of non cereal crops. S=0, Sorgoleone (0 $\mu\text{g/ml}$); S=50, Sorgoleone (50 $\mu\text{g/ml}$); S=100, Sorgoleone (100 $\mu\text{g/ml}$); S=150, Sorgoleone (150 $\mu\text{g/ml}$).

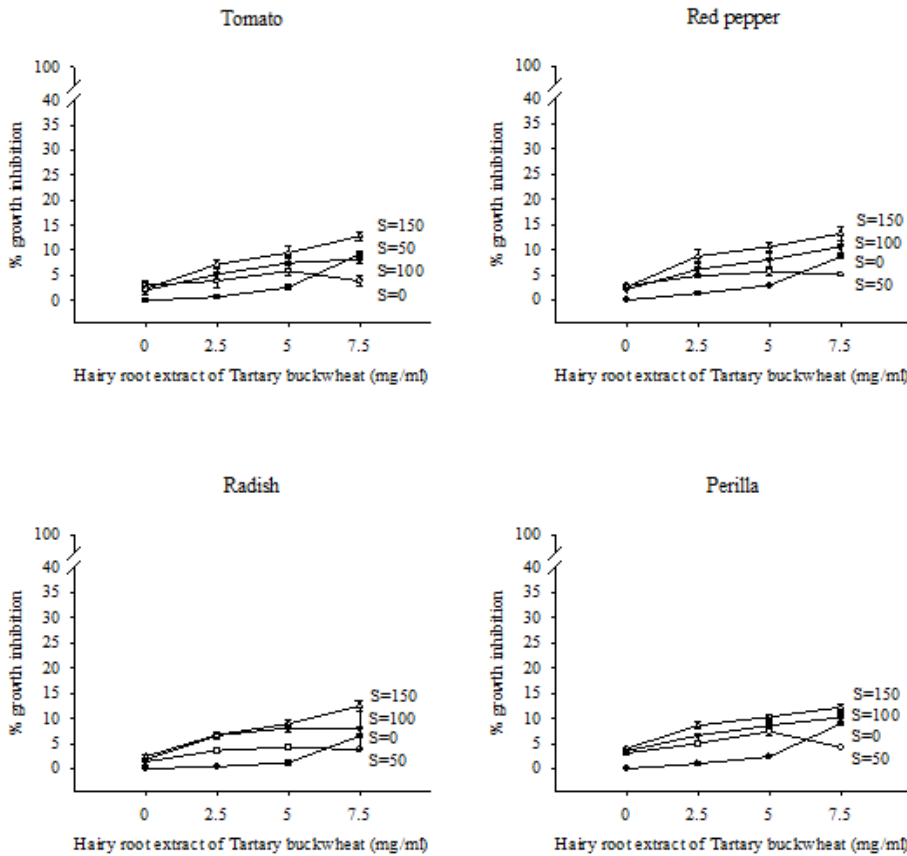


Fig 4 Continued. S=0, Sorgoleone (0 $\mu\text{g/ml}$); S=50, Sorgoleone (50 $\mu\text{g/ml}$); S=100, Sorgoleone (100 $\mu\text{g/ml}$); S=150, Sorgoleone (150 $\mu\text{g/ml}$).

Extraction of hairy root cultures of *F. tataricum*

Hairy roots of *F. tataricum* were cultured in half-strength Schenk and Hildebrandt's (SH) medium containing indole-3-butyric acid (IBA) at 0.5 mg/L. Fresh samples were collected and stored in sealed clear polyethylene plastic bags at -80°C until further use. Collected samples were freeze-dried at -80°C for at least 48 h and then ground into a fine powder with a mortar and pestle. Dried samples were extracted with methanol for 1 h at room temperature. The extract was filtered and evaporated under vacuum. The crude extract was stored at -80°C until further use.

Combined application of sorgoleone and hairy root extract of tartary buckwheat

A completely randomized experimental design with a factorial arrangement of treatments was used. Factor 1 was sorgoleone at 4 different concentrations: 0, 50, 100, and 150 µg ml⁻¹. Factor 2 was hairy root extract of *F. tataricum* at 4 different concentrations: 0, 2.5, 5 and 7.5 mg ml⁻¹. A postemergence test was performed on various grass weed species (*Echinochloa crus-galli*, *Digitaria sanguinalis*, *Setaria viridis* and *Poa annua*) and broadleaf weed species (*Rumex japonicus*, *Galium spurium*, *Erigeron canadensis*, *Plantago asiatica*, *Portulaca oleracea*, *Eclipta alba*, *Amaranthus retroflexus*, and *Aeschynomene indica*) to determine the combined effect of post-emergence application of sorgoleone and hairy root extract of tartary buckwheat. Ten seeds of each the following weeds, *E. crus-galli*, *D. sanguinalis*, *P. annua*, *E. canadensis*, *P. asiatica*, *A. retroflexus*, and *A. indica* (>80% germination) and 20 seeds each of *S. viridis*, *R. japonicus*, *G. spurium*, *P. oleracea* and *E. alba* (40-60% germination) were grown in plastic pots (15 × 12 cm). Two-thirds of the pot was filled with soil (sandy loam); the weed seeds were planted and then covered by filling the pot with soil. Seedlings were raised in the greenhouse at 25°C ± 5°C for three weeks. Extracts of sorgoleone and hairy root of tartary buckwheat dissolved in methanol were mixed with water. Twenty-one days after germination, the combined solution was applied to the foliage of weed seedlings after emergence. Finally, Tween 20 was added as a surfactant at a final concentration of 0.1% using a knapsack CO₂ sprayer at a concentration of 1000 L ha⁻¹. Controls were treated with the same water solution containing 1% methanol and 0.1% Tween 20. Three weeks after treatment, the shoot of each weed species was collected to determine biomass. The efficacy of the combined natural product application was measured based on the dry matter yield of each weed species. The inhibition was calculated using the following formula.

$$\% \text{ inhibition} = \frac{\text{Dry weight of control treatment} - \text{Dry weight from mixture of both extract treatment}}{\text{Dry weight of control treatment}} \times 100$$

Crop selectivity

To determine the combined effect of post-emergence application of sorgoleone and hairy root extract of tartary buckwheat, a postemergence test was performed on 12 different crop species i.e. rice (*Oryza sativa*), barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), corn (*Zea mays*), soybean (*Glycine max*), radish (*Raphanus sativus*), red pepper (*Capiscicum annum*), tomato (*Lycopersicon esculentum*), cucumber (*Cucumis sativus*), Chinese cabbage (*Brassica rapa*), and perilla (*Perilla frutescens*). Ten seeds of each crop species were seeded in plastic pots (15 cm × 12

cm). Seedlings were raised in the greenhouse (as described above) for three weeks. At 21 days, post-emergence application of sorgoleone combined with hairy root extract was performed using a knapsack CO₂ sprayer (as described above). Controls were treated with the same water solution containing 1% methanol and 0.1% Tween 20. Three weeks after treatment, the shoot portion of each crop was collected to determine the biomass. Efficacy of the combined application of sorgoleone and hairy root extract was measured based on dry matter of crop species.

Statistical analysis

All experiments were conducted two times using completely randomized design with three or four replications. Data from both experiments were pooled because no significant differences between experiments were observed, following to an analysis of variance (ANOVA) with sums of squares partitioned to reflect trial effects using the SAS Software release 9.2 (SAS, 2010) and means were separated by Fisher's protected LSD test (P < 0.05).

Conclusion

The present study revealed that application of mixtures of sorgoleone and hairy root extract of tartary buckwheat performed better than separate application of each product and that the mixtures of these natural products had a synergistic effect. The strong weed suppressive ability of these two combined natural products offers interesting possibilities for effective bio approaches to weed management.

Acknowledgments

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References

- Baerson SR, Dayan FE, Rimando AM, Nanayakkara NPD, Liu CJ, Schröder J, Fishbein M, Pan Z, Kagan IA, Pratt LH, Cordonnier-Pratt MM, Duke SO (2008) A functional genomics investigation of allelochemical biosynthesis in *Sorghum bicolor* root hairs. *J Biol Chem.* 283: 3231–3247
- Berenbaum M, Neal JJ (1985) Synergism between myristicin and xanthotoxin, a naturally cooccurring plant toxicant. *J Chem Ecol.* 11: 1349–1358
- Cook DD, Rimando AM, Clemente TE, Schröder J, Dayan FE, Nanayakkara NPD, Pan Z, Noonan BP, Fishbein M, Abe I, Duke SO, Baerson SR (2010) Alkylresorcinol synthases expressed in *Sorghum bicolor* root hairs play an essential role in the biosynthesis of the allelopathic benzoquinone sorgoleone. *Plant Cell* 22: 867–887
- Czarnota MA, Paul RN, Dayan FE, Nimbale CI, Weston LA (2001) Mode of action, localization of production, chemical nature, and activity of sorgoleone: a potent PSII inhibitor in *Sorghum* spp. root exudates. *Weed Technol.* 15: 813–825
- Czarnota MA, Rimando AM, Weston LA (2003) Evaluation of seven sorghum (*Sorghum* sp.) accessions. *J Chem Ecol.* 29: 2073–2083
- Dayan FE, Cantrell CL, Duke SO (2009a) Natural products in crop protection. *Bioorg Med Chem.* 17: 4022–4034
- Dayan FE, Howell JL, Weidenhamer JD (2009b) Dynamic root exudation of sorgoleone and its *in planta* mechanism of action. *J Exp Bot.* 60: 2107–2117

- Dayan FE, Owens DK, Duke SO (2012) Rationale for a natural products approach to herbicide discovery. *Pest Manag Sci.* 68: 519–528
- Dayan FE, Rimando Agnes M, Pan Z, Baerson Scott R, Gimsing AL, Duke SO (2010) Sorgoleone. *Phytochem.* 71: 1032–1039
- Dayan FE, Watson SB, Nanayakkara NPD (2007) Biosynthesis of lipid resorcinols and benzoquinones in isolated secretory plant root hairs. *J Exp Bot.* 58: 3263–3272
- Duke SO, Dayan FE, Rimando AM, Schrader KK, Aliotta G, Oliva A, Romagni JG (2002) Chemicals from nature for weed management. *Weed Sci.* 50: 138–151
- Einhellig FA (1995) Allelopathy: current status and future goals. In *Allelopathy. Organisms, Processes and Applications*; Inderjit, Dakshini, K. M. M., Einhellig, F. A., Eds.; American Chemical Society: Washington, DC, 1995; pp 1–24
- Fujii Y, Golisz A, Furubayashi A, Iqbal Z, Nasir H (2005) Allelochemicals from buckwheat and tartary buckwheat and practical weed control in the field. Proceedings the 20th Asian-Pacific Weed Science Society Conference. Ho Chi Minh City, Vietnam, 227–233
- Gealy DR, Wailes EJ, Estorninos LE, Chavez RSC (2003) Rice cultivar differences in suppression of barnyardgrass (*Echinochloa crus-galli*) and economics of reduced propanil rates. *Weed Sci.* 51: 601–60
- Green JM, Jensen JE, Streibig JC (1995) Models to assess joint action of pesticide mixtures. *Aspects Appl Biol.* 41: 61–68
- Guo X, Zhu K, Zhang H, Yao H (2007) Purification and characterization of the antitumor protein from Chinese tartary buckwheat (*Fagopyrum tataricum* Gaertn.) water-soluble extracts. *J Agric Food Chem.* 55: 6958–6961
- Inderjit, Duke SO (2003) Ecophysiological aspects of allelopathy. *Planta* 217: 529–539
- Kagan IA, Rimando AM, Dayan FE (2003) Chromatographic separation and *in vitro* activity of sorgoleone congeners from the roots of *Sorghum bicolor*. *J Agric Food Chem.* 51: 7589–7595
- Kim YK, Li X, Xu H, Park NI, Uddin MR, Pyon JY, Park SU (2009) Production of phenolic compounds in hairy root culture of tartary buckwheat (*Fagopyrum tataricum* Gaertn.). *J Crop Sci Biotech.* 12:53 – 58
- Netzly DH, Butler LG (1986) Roots of sorghum exude hydrophobic droplets containing biologically active components. *Crop Sci.* 26: 775–778
- Olofsdotter M, Jensen LB, Courtois B (2002) Improving crop competitive ability using allelopathy - an example from rice. *Plant Breed.* 121: 1–9
- Rice EL (1984) *Allelopathy*, 2nd edn. Academic Press, Orlando, FL, USA
- SAS (2010) *The little SAS Book for Enterprise Guide 4.2*. Cary, NC: Statistical Analysis Systems Institute Inc. 294–295
- Uddin MR, Park KW, Kim YK, Park SU, Pyon JY (2010) Enhancing sorgoleone levels in grain sorghum root exudates. *J Chem Ecol.* 36: 914–922
- Weston LA (1996) Utilization of allelopathy for weed management in agroecosystems. *Agron J.* 88: 860–866
- Yao Y, Shan F, Bian J, Chen F, Wang M, Ren G (2008). D-chiroinositol-enriched tartary buckwheat bran extract lowers the blood glucose level in KK-Ay mice. *J Agric Food Chem.* 56: 10027–10031