Herbicidal Effects and Crop Selectivity of Sorgoleone, a Sorghum Root Exudate under Greenhouse and Field Conditions

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온실과 포장조건에서 수수 추출물 Sorgoleone의 제초활성 및 작물 선택성
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ABSTRACT Weeds are known to cause enormous losses due to their interference in agro ecosystems. Because of environmental and human health concerns, worldwide efforts are being made to reduce the heavy reliance on synthetic herbicides that are used to control weeds. In this regard phytotoxicity of allelochemical sorgoleone, which is a major component of the hydrophobic root exudates of Sorghum bicolor was evaluated in different weed species and also its crop selectivity in greenhouse and field conditions. Sorgoleone strongly inhibited the growth of different weeds by pre-emergence and post-emergence applications both in greenhouse and field conditions. Post-emergence application of sorgoleone on 21-day-old weed seedlings had a greater inhibitory effect than the pre-emergence application. Again, broadleaf weed species were more susceptible than grass species to the application of sorgoleone at both stages of growth. Growth of broadleaf weed species was suppressed by greater than 80% for most of the weed species except a few species and among them the species Rumex japonicus and Galium spurium were completely suppressed at 200 µg ml⁻¹ sorgoleone. Like greenhouse trial, sorgoleone was more effective for broadleaf weed species followed by sedge and grass weed species in the field condition. The growth inhibition of weeds was slightly lower in field condition compared to greenhouse condition. The crop species like rice, barley, wheat, corn, perilla, tomato, soybean and Chinese cabbage were tolerant to sorgoleone while lettuce and cucumber were slightly susceptible to sorgoleone. Consequently, sorgoleone may be
applied to control weeds in organic farms without affecting the growth of crop.

Key words: allelochemical; crop selectivity; sorgoleone; weed control.

INTRODUCTION

Natural products (allelochemicals) may play a key role in suppressing weeds in crop fields without impairing the environment as they have no residual effects in the environment. Most allelochemicals reported in plants are secondary metabolites. Allelochemicals (e.g. phenolics, terpenoids, alkaloids, coumarins, tannins, steroids, and quinines) are released by the plant into the environment by root exudation, volatile emissions, leaching from the leaves and other aerial parts, and the decomposition of plant material (Weir et al. 2004; Xuan et al. 2005). Natural products, well known as a source of novel pharmaceuticals, are also a promising source of novel herbicides (Dayan 2000; Duke et al. 2002).

In plants, allelochemicals can be present in the leaves, bark, roots, root exudates, flowers, and fruits (Weir et al. 2004). Sorghum has been known to cause ‘soil sickness’ and affect the growth of other crops in rotation systems (Forney et al. 1985; Einhellig and Rasmussen 1989). Sorghum is sometimes used in integrated pest management systems as a green manure or as a cover crop to suppress weed populations (Weston 1996) or as a crop residue in no-tillage farming (Alsaadawi and Dayan 2009). Sorgoleone is an oily exudate secreted by the root hair of sorghum; it contains the lipid benzoquinone sorgoleone (2-hydroxy-5-methoxy-3-(8′Z, 11′Z, 8′, 11′, 14′pentadecatriene)-p-benzoquinone) a potent allelochemical (Netzly and Butler 1986; Inderjit and Duke 2003). Sorgoleone and its 1, 4-hydroquinone form account for 90% of the oily root exudate of sorghum root hairs. The remaining 10% contains several minor congeners varying in the substitutions in the aromatic ring, and/or in the number of carbons and the level of unsaturation in the tail (Rimando et al. 1998; Kagan et al. 2003). All these variants of sorgoleone appear to contribute to the overall allelopathic potential of sorghum (Kagan et al. 2003; Rimando et al. 2003).

Sorgoleone was first discovered by investigators searching for secondary metabolites involved in triggering the germination of the obligate parasitic plant Striga asiatica (witchweed) (Chang et al. 1986; Netzly et al. 1988). Early studies reported that several classes of water-soluble compounds, such as the phenolics, may be responsible for these allelopathic properties but these compounds are ubiquitous in plants and are not likely to be involved in the unique activity observed in sorghum (Lehle and Putnam 1983; Panasiuk et al. 1986).

Sorgoleone is phytotoxic to broadleaf and grass weeds at concentrations as low as 10 µM in hydroponic assays (Nimbal et al. 1996), and broad-leaf weed species are more susceptible than grass weed species in growth chamber bioassay (Uddin et al. 2009). The biological activity of sorgoleone is much greater than those of many phenolics, flavonoids, coumarins, and sesquiterpene lactones and can be compared to synthetic herbicides such as diuron, atrazine, and metribuzin that inhibit photosynthesis.

The objective of this study was to determine the herbicidal activity of sorgoleone in weed species and its crop selectivity under greenhouse and field conditions.
MATERIALS AND METHODS

Plant materials
Weed seeds were collected from the Experimental Farm of Chungnam National University, Daejeon and also from Rural Development Administration (RDA), Korea. Crop seeds were purchased from market.

Growing condition of sorghum for root collection
Sorghum seeds (cultivar Chalsusu, with high sorgoleone content (Uddin et al. 2009) were used in this study. Seeds were treated with benomyl (fungicide) for 4 h and then rinsed several times in distilled water. Seeds were grown in sterile Petri dishes (100×40 mm) on the surface of sterile Whatman #1 filter paper (90-mm diameter) for root collection. The dishes 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 (Uddin et al. 2010). Root samples were stored in sealed clear polyethylene plastic bags at -80°C until further use.

Sorghum root extraction for sorgoleone
Sorgoleone was extracted according to the procedures described by Netzly and Butler (1986) except that methanol was used as a solvent instead of methylene chloride (Uddin et al. 2010). Seedling roots were excised and dipped (30 s) in methanol (1:20 w/v) to extract sorgoleone. The crude extract was filtered and evaporated under vacuum. Sorgoleone was determined using HPLC.

Pre-emergence herbicidal activity under greenhouse condition
The efficacy of sorgoleone as pre-emergence against several weed species was evaluated. Sorgoleone was formulated at concentrations of 0, 25, 50, 100, 150 and 200 μg ml⁻¹ by initially dissolving the original crude extract in methanol and then mixing the solution with water. Methanol concentration in the test solution did not exceed 1%. Twelve weed species of grass (i.e. Echinochloa crus-galli, and Digitaria sanguinalis), sedge (i.e., Cyperus nipponicus), and broadleaf weeds (i.e., Rumex japonicus, Galium spurium, Erigeron candensis, Plantago asiatica, Portulaca oleracea, Eclipta alba, Amaranthus retroflexus, Polygonum hydropiper, and Chenopodium album) were grown in plastic pots (15×12 cm). Two-thirds of the pot was filled with soil, and each pot was seeded with 10 seeds each of E. crus-galli, D. sanguinalis, C. nipponicus, E. candensis, P. asiatica, and A. retroflexus (% germination was >80), and 20 seeds of R. japonicus, G. spurium, P. oleracea, E. alba, P. hydropiper, and C. album (germination was 40-60%). Then, the remainder of the pot was filled with soil. Afterwards, the pots were watered, and ~3 h later sorgoleone was applied using a knapsack CO₂ sprayer at a concentration of 1000 L ha⁻¹. Seedlings were grown in the greenhouse at 25±5°C for a 5-wk period. Treatments were replicated 4 times and arranged according to a completely randomized design in the greenhouse. Five weeks after sorgoleone treatment, the shoot portion of each weed species was collected. The efficacy of sorgoleone was measured from the dry matter content of each weed species.

Efficacy of post-emergence application in the greenhouse
To determine the effect of post-emergence-applied sorgoleone, a post-emergence test was performed on the same weeds used in the pre-emergence assays. Seedlings were raised in the greenhouse as described above for a 3-wk period. At 21 d after emergence, sorgoleone was applied to the foliage of
weed seedlings at concentrations of 0, 25, 50, 100, 150 and 200 µg ml⁻¹. Finally, Tween 20 was added as a surfactant at a final concentration of 0.1%. Sorgoleone was applied using a knapsack CO₂ sprayer at a concentration of 1000 L ha⁻¹. Three weeks after treatment, the shoot portion of each weed species was collected to determine the biomass.

**Efficacy of post-emergence application in the field**

A post-emergence test of sorgoleone was performed on an upland non-cultivated field. At 2-3 leaf stage, sorgoleone was applied to the foliage of weed seedlings at concentrations of 0, 25, 50, 100, and 200 µg ml⁻¹. Sorgoleone was applied using a knapsack CO₂ sprayer at a concentration of 1000 L ha⁻¹. Three weeks after treatment, the shoot portion of each weed species was collected to determine the biomass.

**Crop selectivity test with sorgoleone in the greenhouse as pre-emergence application**

To see the effect of sorgoleone to crop species a pre-emergence test was evaluated. Assays were performed as same as the pre-emergence application of sorgoleone to weed species. Sorgoleone concentration was 0, 25, 50, 100, 150 and 200 µg ml⁻¹. Twelve different crop species i.e., rice, barley, wheat, corn, soybean, lettuce, radish, red pepper, tomato, cucumber, Chinese cabbage and perilla were grown in plastic pots (15×12 cm). Pots were seeded with 10 seeds of each crop species. Five weeks after sorgoleone application, the shoot portion of each crop species was collected. Efficacy of sorgoleone was measured based on the dry matter yield of each crop species.

**Crop selectivity test with sorgoleone in the greenhouse as post-emergence application**

With the same sorgoleone concentrations and same crop species, a post-emergence application was performed to see their tolerance to sorgoleone. Seedlings were raised in the greenhouse as described above for a 3 wk period. At 21 d of age, a post-emergence application of sorgoleone was tested at concentrations of 0, 25, 50, 100, 150 and 200 µg ml⁻¹. Finally, Tween 20 was added as a surfactant at a final concentration of 0.1%. Sorgoleone was applied to leaves of crops using a knapsack CO₂ sprayer at a concentration of 1000 L ha⁻¹. Three weeks after treatment, the shoot portion of each crop species was collected to determine the biomass.

**Statistical analysis**

The data collected were subjected to analysis of variance technique using the SAS Software release 9.2 (SAS Institute Inc., Cary, NC, USA). Standard deviations are also provided to show variations associated with particular means.

**RESULTS**

**Pre-emergence effect of sorgoleone on weed control in the greenhouse**

To evaluate herbicidal efficacy, a bioassay was done against a number of weed species with sorgoleone at concentrations ranging from 25 to 200 µg ml⁻¹. After a 5-wk growth period, the presence of allelochemical sorgoleone resulted in an effective reduction in weed shoot biomass. The growth inhibition started at the lowest applied concentration (25 µg ml⁻¹). The broadleaf weed species *R. japonicus*, *G. spurium* and *E. candensis* were the most susceptible, these showed greater than 80% growth inhibition with 200 µg ml⁻¹ sorgoleone (Fig. 1). The weed species *P. asiatica*, *P. oleracea*, *A. retroflexus* and *E. alba* displayed the next highest level of susceptibility at greater than 70% with 200 µg ml⁻¹.
sorgoleone. The grass species *E. crus-galli* and *D. sanguinalis* showed 50-55% of growth inhibition with 200 µg ml⁻¹ sorgoleone.

### Post-emergence herbicidal activity of sorgoleone under greenhouse condition

The post-emergence application of sorgoleone was evaluated 21 days after treatment when seedlings were 6 weeks of age and resulted in significant growth reduction, with the broadleaf weed species showing greater susceptibility than grass weed species. Post-emergence application of sorgoleone on 21-day-old seedlings had a greater inhibitory effect than the pre-emergence application to broadleaf weeds. But, the growth inhibition of grass was almost the same to the application of sorgoleone at both stages of growth. Most of the broadleaf weed species growth was suppressed by greater than 80% with 200 µg ml⁻¹ sorgoleone. A growth reduction of 100%, 96.6%, 85.1%, 87.3%, 82.8%, 81.5% and 80.6% were found in the weed species of *R. japonicus*, *G. spurium*, *E. candensis*, *P. asiatica*, *P. oleracea*, *E. alba* and *A. retroflexus*, respectively with 200 µg ml⁻¹ sorgoleone (Fig. 2).

### Post-emergence herbicidal activity of sorgoleone under field condition

The efficacy of sorgoleone in the field condition was evaluated by application of a post-emergence at 2-3 leaf stage of several weed species in a non-cultivated upland field. The results were evaluated 21-day after sorgoleone treatment. A total of twelve weed species were found at the time of harvest for shoot biomass. Among them, 5, 4 and 3 weed species were from broadleaf, sedge and grasses, respectively. Broadleaf weed species showed greater susceptibility than grass weed species. The growth inhibition was 83.3%, 82.5%, 82.1%, 76.0% and 75% for the broadleaf weed species of *Rorippa indica*, *E. alba*, *Ammannia coccinea* and *Ludwigia prostrata*, respectively (Fig. 3). The sedge weed species displayed slightly greater inhibition than...
Fig. 3. Effect of post-emergence application of different concentrations of sorgoleone on control of weeds by species in field condition. Values are presented as mean ± SD.
Ea, Eclipta alba; Po, Portulaca oleracea; Lp, Ludwigia prostrata; Ac, Ammannia coccinea; Ri, Rorippa indica; Ci, Cyperus iria; Cn, Cyperus nipponicus; Cd, Cyperus difformis; Fm, Fimbristylis miliacea; Ec, Echinochloa crus-galli; Ds, Digitaria sanguinalis; Ei, Eleusine indica.

Fig. 4. Effect of pre-emergence application of different concentrations of sorgoleone on inhibition of crop growth. Values are presented as mean ± SD.
Ri, Rice; Ba, Barley; Wh, Wheat; Co, Corn; So, Soybean; Le, Lettuce; To, Tomato; Rp, Red pepper; Ra, Radish; Pe, Perilla; Cu, Cucumber; Cc, Chinese cabbage.

Fig. 5. Effect of post-emergence application of different concentrations of sorgoleone on inhibition of crop growth. Values are presented as mean ± SD.
Ri, Rice; Ba, Barley; Wh, Wheat; Co, Corn; So, Soybean; Le, Lettuce; To, Tomato; Rp, Red pepper; Ra, Radish; Pe, Perilla; Cu, Cucumber; Cc, Chinese cabbage.

grasses species in the field condition. Cyperus difformis and D. sanguinalis were the most susceptible species among sedge and grass weed species, respectively.

Crop selectivity test with sorgoleone in the greenhouse as pre-emergence
The growth inhibition of crop species as measured by shoot biomass was very low. Most of the crop species were tolerant to sorgoleone showing a shoot growth reduction lesser than 10% with 200 µg ml⁻¹ sorgoleone. Among them, two crop species i.e., lettuce and cucumber showed slightly susceptible to sorgoleone. A growth inhibition of 17.9% was found in lettuce with 200 µg ml⁻¹ sorgoleone, followed by 13.4% in cucumber (Fig. 4).

Crop selectivity test with sorgoleone in the greenhouse as post-emergence application
Growth inhibition in crop species was slightly greater in post-emergence application of sorgoleone than pre-emergence application. The crop species like rice, barley, wheat, corn, soybean, tomato, perilla and Chinese cabbage were very tolerant to sorgoleone. Among the crop species studied here, lettuce and cucumber showed susceptibility in post-emergence application of sorgoleone showing greater than 20% growth inhibition at 200 µg ml⁻¹ sorgoleone (Fig. 5).
DISCUSSION

The common allelochemicals from crop plants are generally secondary metabolites. These include phenolics, terpenoids, alkaloids, coumarins, tannins, flavonoids, steroids and quinines (Einhellig and Leather 1988). An increasing number of studies have shown that for managing weeds, allelopathic interactions can be utilized directly or indirectly through the use of allelochemicals as alternatives to herbicides (Dayan et al. 2000; Duke et al. 2002).

From our study, significant growth reduction as measured by shoot growth in several broadleaf and grass species was observed after both pre-emergence and post-emergence applications of sorgoleone, with the greatest phytotoxicity and growth reduction noted for the broadleaf weeds. In particular, *R. japonicus*, *G. spurium*, *E. candensis*, *P. asiantica*, and *A. retroflexus* were greatly inhibited (Fig. 2). In a growth chamber study Uddin et al. (2009) reported that broadleaf weed species were more susceptible to sorgoleone than grass weed species. Einhellig and Rasmussen (1989) also noted in field experiments in which grain sorghum residues were located the previous year that the inhibitory effects of grain sorghum were primarily on broadleaf weeds, with little activity observed on grass weeds. They observed these effects the following year, indicating that the allelopathic potential of the crop and or chemical(s) may persist. Putnam and DeFrank (1983) found that residues of sorghum reduce the number and biomass of common purslane and smooth crabgrass (*D. ischaemum* [Schr.] Muhl.) in the fields by 70 and 98%, respectively. Four- to 6-week-old sorghum plants were observed to suppress weeds without damaging large seeded legumes and 2- to 4-week herbage was more effective than the old (6 to 8 week) herbage. All parts of sorghum like roots, herbage and germinating seeds release phytoinhibitors reducing the growth of grass and broadleaf species such as green foxtail, velvetleaf, and smooth pigweed (Panasiuk et al. 1986, Hoffman et al. 1996). Panasiuk et al. (1986) reported that the interplanting of sorghum reduces the dry weight of barnyard grass, redroot pigweed, and red sorrel. Sorghum residues release sorgoleone, cyanogenic glycosides-dhurrin, and a number of breakdown products of phenolics that bring about weed suppression (Nicollier et al. 1983; Weston et al. 1989).

Post-emergence application of sorgoleone was evaluated 21 days after treatment when seedlings were 42 days of age and resulted in significant growth reduction in all the studies weed species. Affected species exhibited severe in case of post-emergence application. Burning and growth inhibition were visually observed 2-3 days after treatment in sensitive species, and significant stunting of growth measured by 21 days after treatment. Sorgoleone is phytotoxic to broadleaf weeds and grass weeds at concentrations as low as 10 µM in hydroponic assays and also reported that sorgoleone acted as PS II inhibitor (Nimbal et al. 1996), and broadleaf weed species are more susceptible than grass weed species in growth chamber bioassay (Uddin et al. 2009).

Most of the crop species i.e., rice, barley, wheat, corn, soybean, perilla, tomato, and Chinese cabbage were tolerant to sorgoleone, while the crop species lettuce and cucumber showed slight susceptibility to sorgoleone in both pre-emergence and post-emergence application in this study. Sorgoleone had strong herbicidal potential as growth inhibition of all weed species was found from greenhouse and field study (Fig. 1-3). But the growth inhibition was very low in case of crop; it means sorgoleone contained allelopathic compounds and that their phytotoxicity remains lower in case of crop species. From the study of Hill et al. 2007 showed that germination
and radicle growth of weeds significantly reduced by methanol and ethyl acetate extracts of hairy vetch extracts except for corn, tomato and cucumber. They also mentioned that corn and cucumber radicle elongation was stimulated at low concentration of the extracts. In a study Uddin and Pyon 2010 demonstrated that rotation crop residues especially hairy vetch and Chinese milkvetch have high herbicidal effects against weeds without inhibiting the growth of crop species which was found in our study.

The results of this study suggest that sorgoleone contains allelochemicals and the weed species were very susceptible to this allelochemicals but most crop species were tolerant to them. The strong weed suppressive ability of sorgoleone offer interesting possibilities for effective environment friendly approaches to weed management.

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LITERATURE CITED


