https://doi.org/10.26651/allelo.j/2020-49-2-1260 0971-4693/94 Euro 20.00
Allelopathy Journal 49 (2): 141-150 (March, 2020) *International Allelopathy Foundation* 2020
Table: 2, Figs : 5

Allelopathic effects of scandent hop (*Humulus scandens* Lour) on barnyard grass (*Echinochloa crus-galli* L.)

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(Received in revised form: February 02 2020)

ABSTRACT

To find the allelopathic herbicidal plants, the herbicidal activity of ethanol extract from scandent hop was evaluated on the seed germination, seedling growth and defence enzymes activities of barnyard grass. The scandent hop inhibited the germination and seedling growth of barnyard grass and followed the order: root extract > leaf extract >complete shoot extracts > stem extracts. Seed germination and seedlings growth decreased with increase in concentrations from 0.02g/mL to 0.6g/mL. The GC-MS analysis detected 10-major compounds (acetol, pyran, myristaldehyde, vinyl propionate, ethyl ester, oleic acid, phytol, methyl ester) in *H. scandens*. The inhibitory activity of compounds followed the order : methyl linoleate > ethyl linoleate > phytol. The leaves extract at lower concentrations (0.02, 0.1g/mL) increased the activities of catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) but were decreased at higher concentrations(0.6g/mL). Thus scandens hop extracts were inhibitory to the seeds germination, seedling development and antioxidant enzymes activities in barnyard grass.

Key words: Allelopathic effects, barnyard grass, chemical compounds, defence enzymes activity, ethanol extract, GC-MS, germination, *Humulus scandens*, scandent hop, seed germination, seedling growth

INTRODUCTION

Barnyard grass (*Echinochloa crus-galli* L.) weed is main cause of yield losses and decreased quality in numerous crops including rice. In rice cultivation, weeds caused more losses than insects and diseases (11). Herbicides are used to control weeds, but these cause several problems viz., weed resistance to herbicides (9). All these side effects attracted researchers' attention to search the efficient and more eco-friendly products of biological origin, which are now emerging as component of integrated weed management (18). The allelopathy refers to the interactions between the organisms through the plants released allelochemicals. Some of the allelochemicals plays role in weed management, including phenols, terpenes, alkaloids and other secondary metabolites (14). However, only few biologically synthesized products are effective under field conditions (16). Hence, this study aimed to screen the herbicidal natural products from *Humulus* plant.

Scandent hop (*Humulus scandens* Lour, Moraceae) is perennial climbing herb and found in north and south China (except Xinjiang and Qinghai), Vietnam and Japan (23). It often grows in ditches, wasteland, ruins, poor soils and edge of forest. Researches on scandent hop have mainly focussed on the chemical constituents, physiological functions and pharmacological effects, but its allelopathic effects on other plants were less reported.

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Hence, this study aimed to screen the herbicidal natural products from *Humulus* plant. The results of this research may help to develop potent standard bio-herbicide from scandent hop against barnyard grass.

MATERIALS AND METHODS

The root, stem and leaves of scandent hop were collected on August 16, 2017 from uncultivated land in Changsha, Hunan Provence, China (N28°28' 21", E113°4' 52"). The mature seeds of barnyard grass were collected on June 10, 2018 from paddy fields in Changsha (N28°28'55", E113°4'53").

Scandent hop extracts preparation

Fresh scandent hop plants were cleaned by distilled water to remove dirt and soil particles. Two kg fresh scandent hop parts (root, stem, leaf and whole plant) were immersed in 8 L of 100 % ethanol for 24 h, and stirred every 4 h. Thereafter the material was filtered through vacuum (SHZ-DIII, Shanghai Giangqiang industrial development Co., LTD, China) and the resulting solution was collected and stored at 4°C. The leftover plant material was re-immersed twice in 16 L of 100% ethanol for 48 h and the mixture was stirred every 4 h. All these three batches of extracts were combined and the ethanol was evaporated on rotary evaporator (IKA RV10, Germany). The ethanol extracts residues were stored at 4°C.

Bioassays

(i). **Petriplate bioassay:** Twenty seeds of barnyard grass were sown equidistant in petri dishes (7-cm-dia) lined with filter paper. Each petriplate was applied 5 mL water or extract as per treatment. One mL water was added every other day, to keep the filter paper moist. The petri dishes were incubated [12 h photoperiod (100-150 $\mu mol.m^{-2}.s^{-1}$) 25/20 °C during the light/dark cycle]. The seeds were considered germinated, when their radicle protruded 0.5 cm from the seed coat (4). The number of germinated seeds were counted after 7 d**.**

(ii). **Plastic cup bioassay:** Seedling growth was evaluated by transplanting 6-seedlings per plastic cup (5-cm-dia) containing agarose. Each cup was applied 5 mL water or extract (0.1, 0.5, 1, 3 g). The treatments were replicated thrice. The plastic cups were incubated [12 h photoperiod (100-150 $\mu mol.m^{-2}.s^{-1}$) 25/20 °C during the light/dark cycle ingrowth chamber]. The seedlings fresh weight was recorded after 7 d. Based on the fresh weight data, herbicidal effect was calculated as under :

$$E = 100 \times (C-T) / C$$

Where, E: Herbicidal effect and C: Fresh weight of weeds shoot in control, T: Fresh weights of weeds shoot in treatment group.

Gas chromatography-mass spectrometry (GC-MS) analysis

The natural compounds in scandent hop (root, stem, leaf, whole plant) extracts were identified by GC-MS ITS-40 (GC-ITMS; Finnigan MAT, San Jose, CA) as per Al-Sohaibani and Murugan (1). The qualitative analysis was done by matching the MS spectra with spectra of NIST library, Medicinal and Aromatic Plant and Drug Research Centre (TBAM) Library and Wiley GC-MS Library (7). Relative percentage of extracts depended on the peak acreage of the total ion chromatograms (TIC) (24).

Bioassay with major compounds

The phytol, monomer of methyl linoleate and ethyl linoleate were purchased from Adamas Changsa. The 3-leaf-stage barnyard grass seedlings were sprayed with 10 mL water or test compounds (phytol, ethyl linoleate, methyl linoleate) at 0.001, 0.005, 0.01, 0.05, 0.1, 0.2g/mL concentrations. The barnyard grass seedling were growth under 12 h photoperiod (100-150 μmol.m^{-2}.s^{-1}) 25/20 °C during the light/dark cycle ingrowth chamber. After 7 days, fresh weight of barnyard grass seedling were recorded.

Defence enzyme activities

The 3-leaf-stage barnyard grass seedlings were sprayed with 5 mL water or aqueous extract (0.1, 0.5, 1, 3g mixed with 5mL water). After 7 days, barnyard grass leaves (0.5g per treatment) were used to measure the defence enzyme activities (CAT, POD and SOD). The crude extraction of CAT, POD and SOD was done as per enzyme assay kit (Nanjing Jiancheng Bioengineering Institute). The CAT, POD and SOD were determined by measuring their absorbance at 405 nm, 420 nm and 550 nm, respectively (22). The results were described as relative activity (%) comparing the last value with initial value.

Statistical analysis

The experimental data were statistically analyzed using one-way ANOVA, and determined the difference using the LSD test at the 5 % significance level by DPS V17.10 software (10).

RESULTS AND DISCUSSION

Seeds germination

The different parts of scandent hop ethanolic extracts had variable influences on the seed germination of barnyard grass(Fig.1). At 0.02 g/mL the stem aqueous extract caused 10% inhibition, the root extracts caused 35 % inhibition, while, leaf and whole plant extracts caused20 % inhibition. At 0.1g/mL, the root aqueous extract was most inhibitory than leaf and whole plant extracts and the stem extract was least harmful. At 0.2 g/mL, the inhibition rate of whole plant leaves, stems extracts was similar, but roots extract was most inhibitory. The root extract at 0.6 g/mL inhibited the weed seeds germination by 73 %, while, the extracts of stems, leaves and whole plant caused 60 % inhibition, indicating that the root extract was more inhibitory to barnyard grass seeds germination. The donor weed may have produced some ethanol-soluble toxins inhibitory to seed germination and seedlings vigour. The inhibitory effects of extracts on barnyard grass seeds germination followed the order: root ethanolic extracts > leaf ethanolic extracts > whole plants ethanolic extracts > stem ethanolic extracts. Our results are similar to other allelopathic study. The seed growth and germination of wheat, lettuce, radish and pepper increased at 2, 5, 10, 20 mg/mL of scandent hop extracts but decreased at 40, 100, 200 mg/mL (16). The study has proved that scandent hop had allelopathic effects on other plants seeds germination.

Seedling growth

The ethanolic extracts of scandent hop decreased the seedling growth of barnyard grass (Fig. 2). The alcohol extracts from leaf, stem and plant of *H. scandens* at 0.02 g/mL concentrations were little inhibitory (5 %) to the barnyard grass seedlings, but root extract caused 14.99 % inhibition. At 0.1 g/mL, the alcohol extracts from roots and leaves caused 25 % inhibition in barnyard grass seedlings, while, the alcohol extracts from stems and whole plants were less inhibitory (10%). The alcohol extracts from roots and leaves at 0.2 g/mL, caused 41.3% and 41.6 % inhibition, respectively. The inhibition rates of alcohol extracts from stems and whole plants on barnyard grass were 20.3 % and 25.7% at 0.2 g/mL, respectively. The inhibitory activity of scandent hop on seedling growth of barnyard grass followed the order: root (60.1%) > leaf extract (53.9%) > whole plant (42 %)>stem (36.8%). Previous studies also suggested that the various parts from the same plant have different inhibitory effects. The root secretions of *Stellera chamaejasme* suppressed the root tip of tall fescue (*Festuca arundinacea*), dahusian wild rye (*Elymus dahuricus*) and bluegrass (*Poa annua*) seedlings (8). The inhibitory effects of white top (*Cardaria draba*) and Syrian sage (*Salvia syriaca*) shoot extracts on different vegetable crops were concentration dependent and roots were more sensitive to extracts than shoots (19). Previous studies reported that roots were more sensitive to allelopathy than shoots (20).

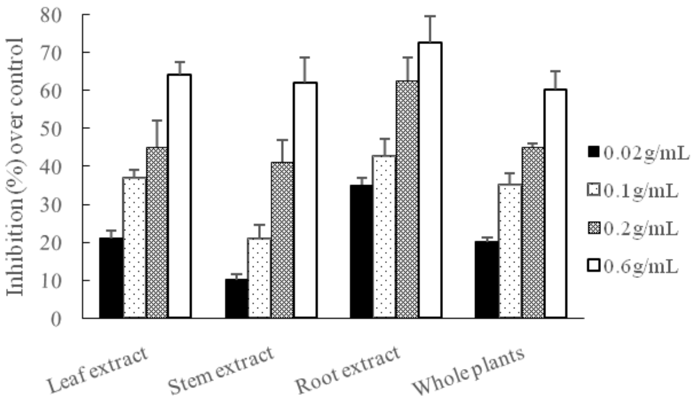

Figure 1. Effects of scandent hop ethanol extracts concentrations on seed germination of barnyard grass.

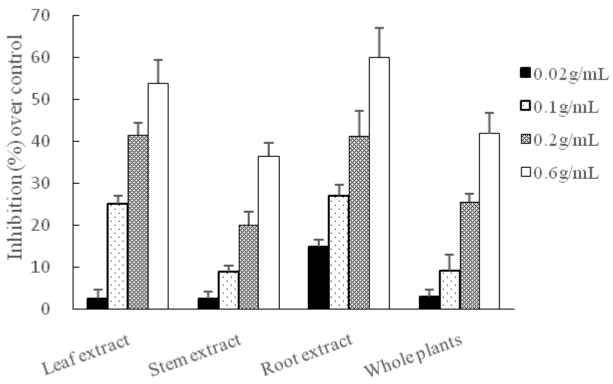

Figure 2. Effects of ethanol extracts of different parts of scandent hop on shoot weight of barnyard grass seedlings.

GC-MS analyses : Herbicidal activity of major compounds

The total lon chromatography of scandent hop ethanol extracts was obtained from the GC/MS chromatogram (Fig. 3). The relative percentage of phytochemicals identified and isolated in scandent hop are given in Table 1. The phytochemical contents were : 2-Propanone,1-hydroxy-Acetol (0.95%), 2,3-Dihydro-3,5-dihydroxy-6-methy1-4H-pyran-4-one (0.79 %), Tetradecanal, Myristaldehyde (0.89 %), Propanoic acid, ethenyl ester Vinyl propionater (0.56 %), Hexadecanoic acid, ethyl ester (11.19 %), Dodecanamide, N,N-bis(2-hydroxyethyl) (2.16 %), Phytol (9.63 %), Hexadecanoic acid, ethyl ester (0.77 %), Methyl linoleate (3.74 %), Ethyl linoleate (67.35 %).GC-MS revealed 4-main components (Ethyl linoleate, Hexadecanoic acid, Methyl linoleate and Phytol) in crude extract.

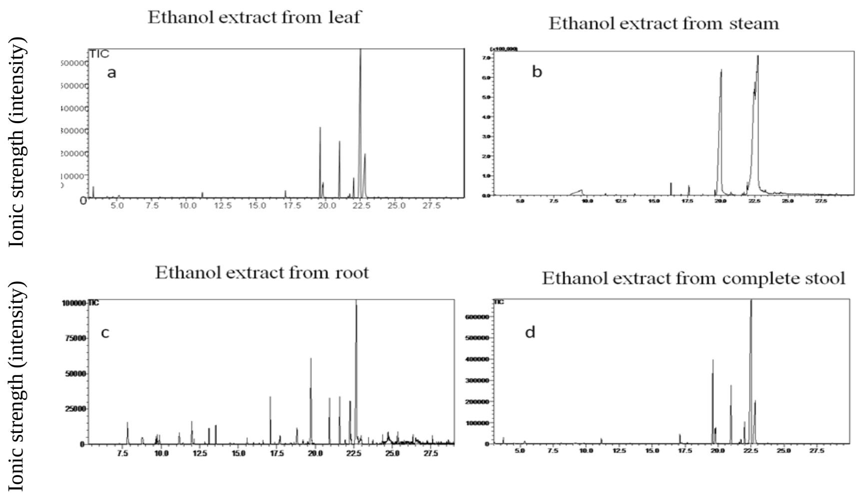

Figure 3. The total lon chromatography of the ethanol extracts of Leaf, Stem, Root and Whole plants of scandenthop from GC/MS chromatogram.

Table 1. Chemical compounds contents of GC-MS analysis of ethanol extracts from scandent hop.

Compounds	Molecular formula	Molecular weight	Plant part	RAP (%)
2-Propanone， 1-hydroxy-Acetol	$C_3H_6O_2$	74	Leaf, root, stem	0.95
2,3-Dihydro-3,5-dihydroxy-6-methy1-4H-pyran-4-one	$C_6H_8O_4$	144	Stem	0.79
Tetradecanal, Myristaldehyde	$C_{14}H_{28}O$	212	Full plants	0.89
Propanoicacid,etheny1ester Vinylpropionate	$C_5H_8O_3$	100	Full plants	0.56
Hexadecanoic acid， ethylester	$C_{16}H_{32}O_2$	284	Root	11.19
9-Octadecenoic acid(Z)-Oleicacid	$C_{18}H_{34}O_2$	282	Full plants	1.97
Dodecanamide,N,N-bis(2-hydroxyethyl)	$C_{16}H_{33}NO_3$	287	Full plants	2.16
Phytol	$C_{20}H_{40}O$	296	Root、leaf	9.63
Methy linoleate	$C_{19}H_{34}O_2$	294	Root	3.74
Ethyl linoleate	$C_{20}H_{36}O_2$	306	Root, leaf	67.35

With increasing dosage of methyl linoleate and ethyl linoleate, the inhibitory effects increased (Fig. 4 B,C).The phytol was slightly inhibitory to barnyard grass. The EC_{50} values of methyl linoleate, ethyl linoleate and phytol were 0.1880, 0.2998 and 0.4532 μg/mL, respectively (Table 2). The inhibitory effects of 3- compounds followed the order : methyl linoleate > ethyl linoleate > phytol.

Table 2. Regression formula and relative EC_{50} of Barnyard grass to major compounds in *H. scandens* ethanolic extracts

Compounds	Regression formula	Related coefficient	EC_{50} (μg/mL)	95% confidence limits
Methy linoleate	Y=5.7324+1.0090x	0.9941	0.1880	0.1546-0.8107
Phytol	Y=5.4154+1.2086x	0.9509	0.4532	0.2378-0.6239
Ethy linoleate	Y=5.5307+1.0144x	0.9801	0.2998	0.2063-0.6855

Y: Barnyard grass fresh biomass (g), x: compounds weight (g).

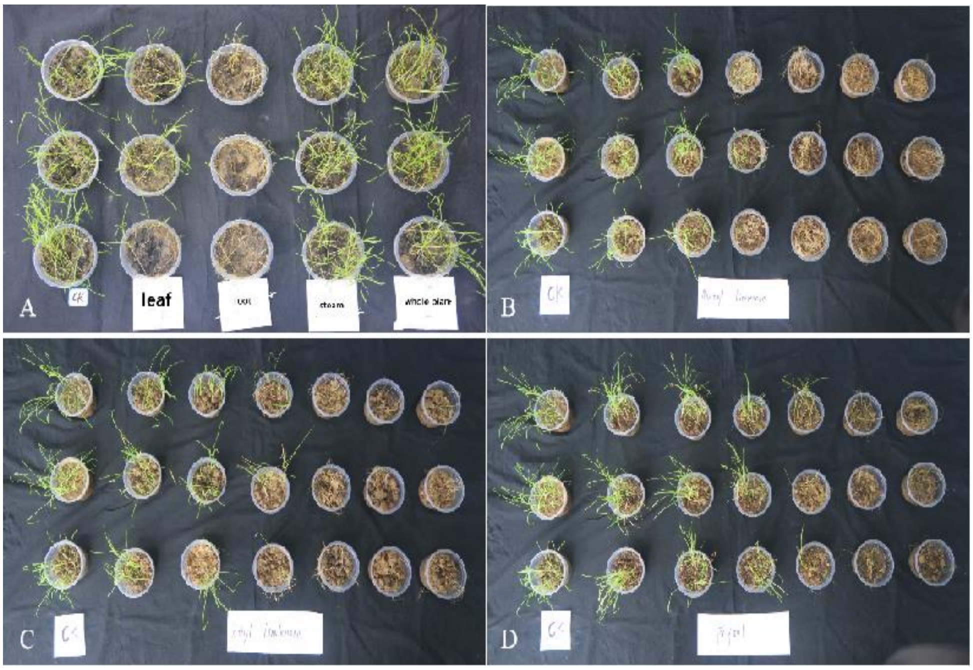

Figure 4. Toxicity test of alcohol extract from *Humulus scandens* on barnyard grass.
A. Determination of toxicity of alcohol extracts from different *Humulus scandens* parts to barnyard grass seedlings (Control, ethanol extract of leaves, roots, stems, alcohol extract of whole grass,0.1g/mL); B. Determination of toxicity of methyl linoleate with different concentration gradients to barnyard grass seedlings (0, 0.001, 0.005, 0.01, 0.05, 0.1, 0.2 g/mL); C. determination of toxicity of ethyl linoleate with different concentration gradients to barnyard grass seedlings (0, 0.001, 0.005, 0.01, 0.05, 0.1, 0. 2g/mL); D. determination of toxicity of phytol with different concentration gradients (0, 0.001, 0.005, 0.01, 0.05, 0.1, 0.2 g/mL) to barnyard grass seedlings.

The ethyl linoleate was main allelochemical and inhibited the barnyard grass growth. While hexadecanoic acid promoted the growth in eggplant (25). Octadecanoic acid and other herbicidal compounds were isolated from *Sapindus mukorossi* extract (17). It contained highest content of ethyl linoleate and confirmed the herbicidal potential of persian speedwell (*Veronica persica*) allelochemicals and its activity against four weeds (*E. crusgalli*, *Beckmannia syzigachne*, *Leptochloa chinensis* and *Lindernia procumbens* (13). Phytol has strong antioxidant effects, hence, it may have herbicidal effects. The increase in concentration of hexadecanoic acid promoted the height and stem diameter of eggplant. It also improves the rhizosphere environment of eggplant and promotes its growth (25). Thus these 3-compounds may play an important role in weed control.

The Pentafluorosulfonamide, Dioximer and Pyrimidoxime are ALS inhibitors, used as herbicides to control the barnyard grass with single target site ,high activity and easily produce resistance to drug (21). Hence, to delay the development of resistance in barnyard grass to ALS inhibitors, avoid the re-use of same type of pentafluorosulfonamide and dichloro ether to control resistant barnyard grass. Therefore, the allelochemicals of scandent hop can be used to develop herbicides to control barnyard grass. The alternative use of herbicides with different mechanisms not only reduce the use of chemical herbicides, but also controls the development of herbicide-resistant barnyard grass and delay or prevent the development of herbicide resistance. Besides, it can also provide raw materials for development of new bio-safe and efficient herbicides.

Defence enzymes activity

The activities of CAT, POD and SOD enzymes increased at lower concentration of extracts but decreased at higher concentrations (Fig 5).In seedlings treated with ethanol extracts of scandent hop in range of 0.02 g/mL to 0.2 g/mL, CAT activity increased from 56.34 μ/g FW to 83.81 μ/g FW. However at 0.6 g/mL, CAT activity decreased to 61.22 μ/g FW. Similar trends were observed in POD and SOD activity. At 0.1 g/mL, POD activity reached 50.29 μ/g FW, higher than 40.86 μ/g FW in 0.2g/mL treatment. SOD activity at 0.1g/mL was 271.99 μ/g FW and it was 208.37 % > 0.02 g/mL.

Catalase (CAT) activity Peroxidase (POD) activity Superoxide dismutase (SOD) activity

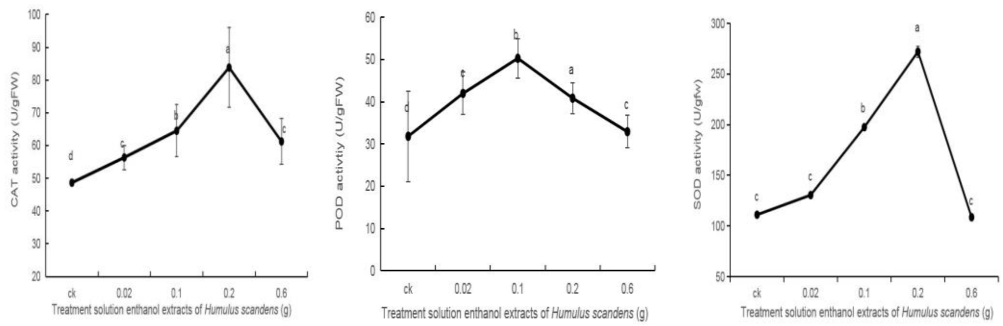

Figure 5. Catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) activity at 3- leaf stage in barnyard grass seedlings treated with 5 mL water or ethanol extracts of scandent hop (0.02, 0.1, 0.2 and 0.6 g/mL).

As important enzymes (SOD, CAT and POD) in defence system, complement each other in maintaining the stability of ROS level in barnyard grass cells. The antioxidant activity is harmful by-product of biochemical stress in plants, such as salt stress significantly promoted the activity of SOD and POD in rice leaves (12). The POD induction might be associated with oxidative reactions at the bio-membrane; number of intermediary metabolism enzymes stimulates the metal-sensitive photo-synthesis (2). Burns (3) found that some peculiar soil enzyme activity level was related to various biotic and abiotic factors, such as proliferating cells and soil aqueous phase. Glyphosate increases the antioxidant enzymes level (SOD, CAT, POD) and significantly reduce the chlorophyll content and growth of rubber bark tree (*Eucommia ulmoides*) (6). Fenoxaprop-p-ethyl enhances the level of SOD, POD, CAT and other antioxidant enzymes in *Microcystis aeruginosa*. High concentration of Fenoxaprop-p-ethyl can promote the apoptosis in two types of algae (5).

CONCLUSIONS

The different parts of scandent hop had variable inhibitory effects on seed germination and seedling development of barnyard grass. Seeds germination and seedlings growth decreased, with increase in extract concentrations (0.02-0.6 g/mL).GC-MS analysis detected10 major compounds in ethyl extract of scandent hop. The major compounds (methyl linoleate, ethyl linoleate and phytol) had herbicidal effects and can be used to develop herbicide. But whether these three compounds are also safe for crops remains to be seen. The leaves extract at lower concentrations (0.02, 0.1 g/mL) increased the CAT, POD and SOD activity but decreased at higher concentrations (0.2 and 0.6g/mL) in barnyard grass. Thus scandent hop extracts were allelopathic to barnyard grass seed germination, seedling growth and antioxidant enzyme. Hence its biomass may be potential resource to develop new efficient bio-herbicide to control the barnyard grass.

ACKNOWLEDGEMENTS

This work was supported by a Grant from the China Agriculture Research System(CARS-16-E19), Natural Science Foundation of China (31801754), Natural Science Foundation of Hunan (2018JJ3289), Scientific-Innovative of Hunan Agricultural Sciences and Technology (2019LS05, 2019LS06and 2019TD03)and the Central Government Directs Local Science and Technology Development Projects (2019CT5009).

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