INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
17–1350/2018/20–5–1027–1032
DOI: 10.17957/IJAB/15.0594
http://www.fspublishers.org

Full Length Article

Chiral 3-dichloroacetyl Oxazolidines Induced the Activity of Enzymes in Maize for the Detoxified Herbicide

Fei Ye[1], Hai-Feng Cao[1], Li-Na Wei[1], Chen Wang[1], Ying Fu[1], Chun-Yan Li[2] and Shuang Gao[1*]
[1]*College of Science, Northeast Agricultural University, Harbin, P.R. China*
[2]*College of Resources and Environment, Northeast Agricultural University, Harbin, China*
[*]For correspondence: gaoshuang@neau.edu.cn

Abstract

The expression of detoxifying enzyme glutathione S-transferase (GST) and antioxidant enzyme activities in maize seedlings were studied in response to three potential herbicide safeners (3-dichloroacetyl oxazolidine and its two optical isomers). The protective effect of chiral herbicide safeners on detoxifying to chloroacetanilide herbicides butachlor in maize was evaluated. All safeners increased the expression levels of herbicide detoxifying enzymes, including GST, catalase (CAT) and peroxidase (POD) to reduce chloroacetanilide herbicide phytotoxicity in maize seedlings. Our results indicate that the R-isomer of 3-(dichloroacetyl)-2,2,5-trimethyl-1,3-oxazolidine can induce glutathione (GSH) content, activity of GST, and the affinity for 1-chloro-2,4-dinitrobenzene (CDNB) substrate in maize, which can protect maize from injury by chloroacetanilide herbicide butachlor. The information of antioxidative enzymes activation was obtained to discuss the role of chiral safener in overcoming oxidative stress caused by the herbicide in maize.

Keywords: Chiral safener; Biological activity; Antioxidative enzyme activity; Protection mechanism; Butachlor

Introduction

Butachlor is a selective pre-emergent acetanilide herbicide. It is extensively used to control perennial grasses and a variety of broad leaf weeds of rice, corn, soybeans, and peanuts crops (Liu *et al*., 2011). Butachlor is absorbed mainly by germinating plant shoots, and secondly by roots. It appears to affect the formation of long chain fatty acids in susceptible plants (Cao *et al*., 2016). Previous studies have shown that butachlor harmed crops more often in conditions that soil containing relatively many water within two weeks of application (Hausman *et al*., 2013; Rasool *et al*., 2014). Butachlor is safe for soybean and peanut, so it applies to intercrop a corn field with soybean and peanut. The phytotoxic effects of butachlor on corn are often manifested as the inability of leaves to separate from the spiral. Affected by phytotoxicity, plant roots may become abnormally short, branches distort and the leaves turn dark green (Alla *et al*., 2008).

Herbicide safeners are applied with herbicides to protect crops against their injury, without affecting weed control efficacy of the herbicides (Elmore *et al*., 2016). Herbicide safeners are particularly effective in protecting monocot crops by increasing herbicide detoxification (Mhlanga and Chauhan, 2016; Bartucca *et al*., 2017). The current studies suggested herbicide safener appeared to induce a set of enzymes and improve herbicide metabolism (Stoilkova and Yonova, 2010; Buono and Ioli, 2011). It was found that the ability of safeners to protect maize from herbicide damage was related to the induction of the activity of GST (Fu *et al*., 2011; Li *et al*., 2017). The main detoxification pathway represents the conjugation of chloroacetanilide and thiocarbamate sulfoxides herbicides with glutathione. (Jo *et al*., 2011; Ye *et al*., 2016). However, the effects of chloroacetanilide safeners on herbicide detoxification pathway were rarely reported. Safeners naphtbalic anhydride and dichlormid could increase crop tolerance to herbicide butachlor by increasing the content of GSH and enhancing the activity of GST (Scarponi *et al*., 2006; Kraehmer *et al*., 2014). It was also found that dichloromethyl-dioxolane safener protected maize by enhancing the activity of GST on promote the conjugation of glutathione with chloroacetanilide herbicide butachlor in the metabolic detoxification (Rezaei *et al*., 2013).

Some reports indicated that 3-dichloroacetyl-substituted oxazolidines containing one chiral center usually have certain bioactivity (Sriharsha and Shashikanth, 2006; Zhao *et al*., 2015). Among the most widely used dichloroacetamide safeners in maize, (R,S)-3-dichloroacetyl-2,2,5-trimethyl-1,3-oxazolidine (R-29148) was particularly effective at protecting maize against herbicides. It could protect corn effectively by enhancing the expression of GST enzymes which involved in herbicide detoxification (Li *et al*., 2009). Activities of some antioxidative system enzymes including CAT and POD, are responsible for alleviating the oxidative stress caused by

To cite this paper: Ye, F., H.F. Cao, L.N. Wei, C. Wang, Y. Fu, C.Y. Li and S. Gao, 2018. Chiral 3-dichloroacetyl oxazolidines induced the activity of enzymes in maize for the detoxified herbicide. *Int. J. Agric. Biol*., 20: 1027–1032

herbicides, heavy metal and salt. Herbicide was found to cause oxidative stress in plants, which responded by an increase in the activity of antioxidative enzymes such as POD and CAT in the root tissues (Farombi *et al*., 2008; Martins *et al*., 2011; Bajpai and Srivastava, 2015; Obermeier *et al*., 2015). However, there is no report on the action principle of chiral safeners (Jablonkai, 2013). Chiral R-29148 and 3-dichloroacetyl substituted oxazolidines were successfully synthesized in previous report (Gao *et al*., 2012). Therefore, the present study aimed to ascertain the mechanism by which the raceme R-29148 and its chiral isomers protect maize from injury caused by butachlor and verify the supposition that detoxification of herbicide can be enhanced by safeners. The protective effects of chiral safener were examined on maize-seedling growth, GSH levels, GST, POD, and CAT enzyme activities, and to prove chiral safeners' potential to promote herbicidal metabolism on the expression of detoxifying and antioxidative enzymes activity.

Materials and Methods

Materials and Chemical Reagents

Butachlor emulsifiable concentrate (60%) was purchased from Zhongshi Pharmaceutical Co., Ltd (Shandong, China). Butachlor standards were provided by Aladdin Chemistry (Shanghai, China). 5, 5'-dithiobis-(2-nitrobenzoic) acid (DTNB), 1-chloro-2, 4-dinitrobenzene (CDNB) and GSH were purchased from Sigma (Shanghai, China). Other chemical solvents were obtained from Aladdin Chemistry (Shanghai, China). Racemic R-29148, R-isomer, and S-isomer were synthesized in our laboratory, with the purity levels more than 99.0% (Table 1) (Fig. 1). The maize seeds, named 'Dongnong 253' (*Zea mays* L.), were germinated and raised in a growth chamber at the Pesticide Chemistry Laboratory, Northeast Agricultural University.

Plant Material and Growth Conditions

Before sowing, seeds were soaked in treatment solutions containing safener (0, 1, 5, 10, 25, 50, 100 $mg \cdot L^{-1}$) at 26.5°C for 12 h. The controls were soaked in distilled water instead of safer solution. The seeds were germinated at 26.5°C for 24 h after soaking and then were sown in paper-cups (8 cm × 12 cm) directly, 6 seeds per cup. Each cup contained 150 mL quartz sand which was prewashed with solution and treated for sterilized. Quartz sand was sterilized by sodium hypochlorite solution (5%, w/v) after being washed with hydrochloric acid (10%, v/v). 60 mL but achlor solution (100 $mg \cdot L^{-1}$) was added to the sand quartz, and water-holding capacity in each cup was controlled at 60%. Water was used for the treatment on the control. The seedlings were cultured in the growth chamber with a relative humidity of 75% at 26.5±1°C and with a 12 h light photoperiod.

Samples from maize shoot and root under each treatment were collected at 8 d, and liquid nitrogen was used to frozen samples immediately. Frozen samples were stored at −80°C. Frozen tissues were then grounded in liquid nitrogen until determination of enzyme activities (GSH, GST, POD, and CAT). Length and fresh weight (FW) of shoot and root were also determined. All experiments were performed in triplicate. The optimal safener concentrations were determined by calculating the recovery rate of maize growth index. Recovery rate of growth index was calculated by the formula followed:

$$\text{Recovery rate (\%)} = \frac{\text{Growth index of maize treated by safener and herbicide - Growth index of maize treated by herbicide}}{\text{Growth index of maize untreated - Growth index of maize treated by herbicide}}$$

GSH Content Assay

GSH level was measured in accordance with Ismaiel and Papenbrock (2014). Homogenizing was carried out in sulfosalicyclic acid (5%, w/v). After being centrifuged at 15 000 × g for 20 min at 4°C, supernatant of the homogenates was immediately used for the GSH contents assays. GSH contents were determined spectrophotometrically at 412 nm with the DTNB reagent.

Extraction and Activity Determination of GST

Extraction and activity determination of GST was performed according with Buono and Ioli (2011). 200 mg frozen maize seedling tissue was used to measure the GST activity. After being ground into powder with the attendance of liquid nitrogen, the tissue was homogenized in 1 mL QB buffer (potassium phosphate buffer 100 mM pH 7.8, with EDTA 1 mM and polyvinylpyrrolidone at 5% w/v) at 4°C. The homogenate was centrifuged at 15 000 × g for 20 min at 4°C. The final assay mixture consisted of 50 mM phosphate buffer (pH 6.5), 1 mM CDNB, 1 mM GSH, and 0.5 mM EDTA. The increase in absorbance at 340 nm was recorded after adding the root extract for 180 s (60 s intervals). GST activity was defined as the amount of herbicide reaction with GSH catalyzed by 1mg GST in unit time ($nmol \cdot s^{-1} \cdot mg^{-1}$ protein).

To determine the activity of GST in vitro against butachlor, HPLC assays were carried out according with Scarponi *et al*. (2006). The GST enzyme extraction was mixed with GSH and butachlor standard solution. After being incubated for 2 h, 10 µL 3.6 M HCl was added into the reaction mixture to stop the reaction, and the mixture was added into methanol for extracting and then collected for HPLC analysis. By comparing the concentrations of butachlor before and after reaction, the GST activity was measured. GST activity was defined as the amount of butachlor reaction with in one minute with unit mass of GST ($nmol \cdot min^{-1} \cdot mg^{-1}$ protein).

GST Kinetic Parameters Assay (CDNB)

The procedure described by Buono *et al.* (2006) was followed for measuring kinetic parameters of GST with modification. According to double reciprocal plots, a linear regression analysis of 1/*V* vs. 1/*S* was used to determine the kinetic parameters V_{max} and K_M. The GST activity was determined over a range of 1-chloro-2, 4-dinitrobenzene (CDNB) concentration (1.0-32.0 mM) at a single GSH concentration of 5 mM.

CAT Activity Assay

Decomposition rate of H_2O_2 was determined at 240 nm to evaluate the total activity of CAT according with Hemanth Kumar (2016). The reaction mixtures containing 1.9 mL H_2O, 0.1 mL enzyme extract and 1 mL hydrogen peroxide (0.3% ,v/v) were determined spectrophotometrically for 3 min. The CAT activity was determined as μmol H_2O_2 $min^{-1} \cdot g^{-1}$ FW.

POD Enzyme Assay

To investigate the effect of safener to target, POD activity was determined based on a modified method from Obermeier *et al.* (2015). The mixture used for determination consisted of 1 mL 50 mM sodium phosphate buffer (pH 7.0), 2 mL 0.3% of hydrogen peroxide and 0.95 ml 0.2% guaiacol 0.01 mL enzyme extract was added to reaction mixtures. The mixture was determined spectrophotometrically at 470 nm for 5 min. The change in absorbance was recorded with the addition of H_2O_2. The peroxidase activity was determined as $mmol \cdot min^{-1} \cdot g^{-1}$ FW.

Statistical Analysis

SPSS statistic program (Version 16.0) software was used for data analysis. Differences between the treatments were evaluated by the least significant difference using the grouped mean and Duncan multiple range test.

Results

Growth Index of Maize

Growth index inhibition rate of maize treated by butachlor decreased significantly. Safener solutions with different concentration were used to test for the ability to decrease the injury, which was caused by butachlor, so as to obtain a suitable treatment regime. The protective effects of three safeners at different concentrations were recorded for R-29148 at the concentration 25 $mg \cdot L^{-1}$, R-isomer at the concentration 5 $mg \cdot L^{-1}$, and S-isomer at the concentration 50 $mg \cdot L^{-1}$. The protective effects of three safeners at different concentrations were assayed, and the results showed that the inhibition caused by butachlor could be significantly decreased after treated by all the three chiral safeners. The recovery rates of maize seedling treated by R-isomer and racemic R-29148 were higher than by S-isomer. The growth index recovery rates of maize were shown in Fig. 2.

GSH Content

The safener's ability on detoxification was related to the conjugation level of glutathione in maize (Taylor *et al.*, 2013). In this evaluation, the increasing of GSH level in root and shoot of maize which was treated by safeners were greater than those treated by butachlor (Table 2). Similarly, GSH level in the seedling tissue treated by the R-isomer-butachlor was greater than those treated by the butachlor. After treated by R-isomer, the GSH contents in the root and shoot increased observably. The results indicated that there was a relationship between the enhanced GSH level in the maize seedlings and the protective activity of herbicide safener. The increase of GSH induced by safener has also been reported previously (Jablonkai, 2013).

GST Activity

Safeners had the ability to motivate the activity of GST and toxicity could be effectively reduced by enzymecatalyzed conjugation of GSH with the herbicide. It was found that the activity of GST in the root of maize seedling which was treated by butachlor increased compared with the control. Treatment with safener and butachlor also caused increase in the *in vivo* activity of GST (Table 3). The in vitro activity of GST was also increased attributed to the chiral safeners when butachlor was used as the substrate instead of CDNB (Table 3). The data indicated that difference in protecting effects of safener in the maize root might attribute to the different activity degree of GST on CDNB or butachlor substrate. Among the three safeners studied, R-isomer showed effectively induction on the activity of GST.

Kinetic Parameters of GST

Enzymatic extracts from maize roots were used to determine the kinetic parameters of GST in maize (Table 4). After treated by butachlor, V_{max} decreased while K_M increased. Compared with the untreated control, V_{max} increased after treated by R-isomer and racemic R-29148, while K_M decreased by 23 and 8%, respectively. Table 4 showed the obvious influence of R-isomer to induction and dynamics of GST activity. Other safeners also induced the affinity of GST to substrate in the conjugated reaction. It was similar with the results of GSH content and GST activity.

POD and CAT Activity

It was reported that POD and CAT could protects crops from the stress caused by herbicide because their activities were connected with metabolizing the oxidative stress caused by high herbicide doses (Obermeier *et al.*, 2015).

Table 1: Chemical name of test safeners

Safener	Chemical name
R-29148	(R,S)-3-dichloroacetyl-2,2,5-trimethyl-1,3-oxazolidine
R-isomer	(R)- 3-dichloroacetyl-2,2,5-trimethyl-1,3-oxazolidine
S-isomer	(S)- 3-dichloroacetyl-2,2,5-trimethyl-1,3-oxazolidine

Table 2: Effect of safeners and butachlor on GSH content in maize

Treatment	GSH content in root ($\mu g \cdot g^{-1}$)	GSH content in shoot ($\mu g \cdot g^{-1}$)
Control	4.319 ± 0.121 d	12.757 ± 0.092 e
Butachlor	5.092 ± 0.174 c	13.876 ± 0.166 d
R-isomer + Butachlor	7.862 ± 0.202 a	17.387 ± 0.353a
S-isomer + Butachlor	6.428 ± 0.216 b	16.340 ± 0.145c
R-29148 + Butachlor	6.775 ± 0.122 b	16.791 ± 0.184 b

Mean ± standard deviation. Values sharing same letters differ non-significantly different (P>0.05). The values correspond to averages of three replicate

Table 3: Effect of safeners and butachlor on activity of GST in maize

Treatment	GST activity *in vivo* ($\mu mol \cdot min^{-1} \cdot mg^{-1}$ protein)	Treatment	GST activity *in vitro* ($nmol \cdot min^{-1} \cdot mg^{-1}$ protein)
Control	18.64 ± 0.92 a	Control	75.36 ± 0.95 c
Butachlor	13.86 ± 0.24 b	Butachlor	——
R-isomer + Butachlor	20.92 ± 1.61 a	R-isomer	96.71 ± 2.15 b
S-isomer + Butachlor	14.71 ± 0.50 b	S-isomer	75.63 ± 3.36 c
R-29148 + Butachlor	14.61 ± 0.88 b	R-29148	88.69 ± 2.07 a

Mean ± standard deviation. Values sharing same letters differ non-significantly different (P>0.05). The values correspond to averages of three replicates

Table 4: Effect of safeners and butachlor on kinetic parameters of GST in maize

Treatment	V_{max} ($nmol \cdot min^{-1} \cdot mg^{-1}$ protein)	K_m ($mmol \cdot L^{-1}$)
Control	14.87 ± 0.30 c	0.52 ± 0.042 a
Butachlor	12.71 ± 0.44 d	0.51 ± 0.027 a
R-isomer	23.82 ± 0.23 a	0.39 ± 0.019 c
S-isomer	12.73 ± 0.67 d	0.50 ± 0.043 a
R-29148	17.54 ± 0.35 b	0.48 ± 0.037 b

Mean ± standard deviation. Values sharing same letters differ non-significantly different (P>0.05). The values correspond to averages of three replicates

Table 5: Effect of safeners and butachlor on POD and CAT activity of maize

Treatment	CAT Activity ($\mu mol\ min^{-1}\ g^{-1}$ FW)	POD Activity ($mmol\ min^{-1}\ g^{-1}$ FW)
Control	2.09 ± 0.06 e	1135 ± 2.28 e
Butachlor	35.90 ± 1.35 a	1574 ± 5.20 c
R-isomer + Butachlor	20.60 ± 0.83 b	1206 ± 3.11 d
S-isomer + Butachlor	6.60 ± 0.24 d	2039 ± 6.65 b
R-29148 + Butachlor	12.70 ± 0.17 c	2807 ± 4.06 a

Mean ± standard deviation. Values sharing same letters differ non-significantly different (P>0.05). The values correspond to averages of three replicates

The effect of safeners and butachlor on POD and CAT activity were determined to investigate the protective effectiveness of chiral safeners (Table 5). Compared with the control, a significant increase could be observed on POD activity in the roots of maize seedling which was treated by butachlor alone. After being treated by R-isomer, activity of POD decreased. The decreased activities of POD indicated that it might play essential roles in detoxifying herbicide under chiral safener treatment.

The activities of CAT could also protect crops and reduce the stress caused by herbicide by participating in metabolizing the oxidative stress. In this case, activity of CAT increased after being treated by butachlor compared with control. The decreasing on CAT activities after treatment with three safeners was shown in Table 5.

Discussion

Marked acceleration of glutathione conjugation responsible for herbicide resistance in plant had been well-documented (Ismaiel and Papenbrock, 2014). GST activity could be stimulated by safeners and enhance the conjugation of GSH with butachlor catalyzed by GST, which led to effectively detoxify (Scarponi *et al*., 2009; Jablonkai, 2013). This meant that the detoxification ability of safener could be decided by the degree of conjugation of glutathione in maize to some extent. Overall, the R-isomer could enhance content of GSH and stimulate activity of GST to promote conjugation of glutathione with butachlor in the maize seedlings, and then protect maize from chloroacetanilide herbicide injury. Compared with those treated by butachlor,

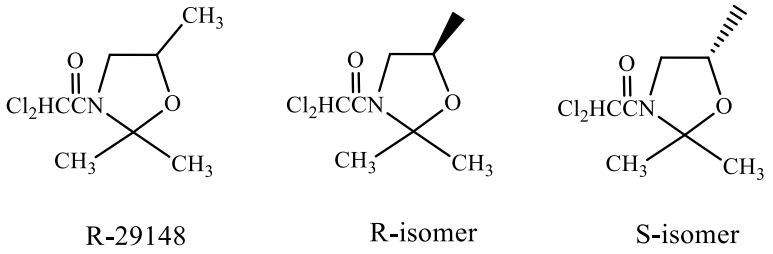

Fig. 1: Structure of safeners used for test

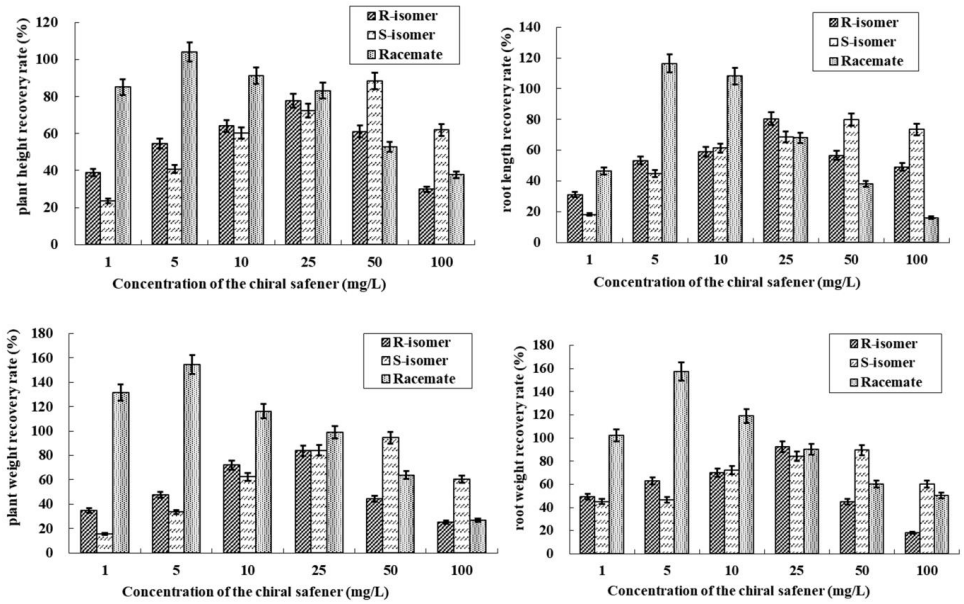

Fig. 2: Recovery rate of growth level of maize treated with butachlor and safener

significant increasing could be observed on dynamics of GST activity toward CDNB in case treated by safener (Ye *et al*., 2016). Results indicated that the R-isomer altered the kinetic parameter V_{max} and K_M significantly. In this case, it was shown that different chiral safeners could induce GST to accelerate the conjugation of butachlor and GSH，which was responsible for the detoxification.

In our experiment, the antioxidative defense system consisting of POD and CAT was investigated. POD and CAT were involved in the mechanism of oxidative stress which was caused by herbicides. It could protect plants from the stress generated by herbicide overdoses. Increasing in POD and CAT activities in several plant species during herbicide exposure was reported (Bajpai and Srivastava, 2015; Sytykiewicz, 2015). Both herbicide and safener induced different trends of antioxidative enzymes activities and antioxidative stress response. In this study, further suggestion could be generated that the activities of POD and CAT in maize decreased after treated by R-isomer, which indicated resistance to oxidative stress in which the chiral safener played a certain role in maize. However, there was no significant change on POD activity after treated by racemic R- 29148 and S-isomer. The activities of the two enzymes decreased after treated by R-isomer, which were in contrast to their response to herbicide. It could be concluded that R-isomer had the ability to reduce oxidative stress caused by butachlor in maize. This mechanism could be an important approach for the explanation of chiral safener detoxification in maize.

Conclusion

Based on data obtained in this study, it can be concluded that the effects of racemic R-29148 and its chiral isomers on growth and enzymes activity of maize could protect maize against injury from chloroacetanilide herbicide butachlor. The growth level of maize and GST activity were inhibited by butachlor significantly, which could be tempered by adding the R-isomer. The data indicated that the safener could enhance the GST activity, and the enhanced GST activity facilitated maize seedling survival at low butachlor concentrations. There are indications that the R-isomer can affect POD and CAT activity, which detoxified the plant from the effects of the

butachlor. Moreover, further studies are still needed to determine the exact mechanism of chiral safener to protect maize from injury by chloroacetanilide herbicide.

Acknowledgements

This research was funded by the National Nature Science Foundation of China (No. 31572042), the Natural Science Foundation of Heilongjiang Province (No. ZD2017002, C2015013, C2015014), the Research Science Foundation in Technology Innovation of Harbin (No. 2017RAQXJ017), and the National Students' Innovation and Entrepreneurship Training Program (No. 201710224002).

References

Alla, M.M.N., A.M. Badawi and N.M. Hassan, 2008. Effect of metribuzin, butachlor and chlorimuron-ethyl on amino acid and protein formation in wheat and maize seedlings. *Pestic. Biochem. Phys.*, 90: 8–18

Bajpai, J. and A. Srivastava, 2015. *In vitro* response of black gram genotypes to herbicide stress and elevation of antioxidative defence system. *Acta Physiol. Plant*, 37: 182

Bartucca, M.L., S. Celletti, S. Astolfi, T. Mimmo, S. Cesco, I. Panfili and D.D. Buono, 2017. Effect of three safeners on sulfur assimilation and iron deficiency response in barley (Hordeum vulgare) plants. *Pest. Manag. Sci*., 73: 240–245

Buono, D.D., M. Micheli, L. Scarponi and A. Standardi, 2006. Activity of glutathione S-transferase toward some herbicides and its regulation by benoxacor in non-embryogenic callus and *in vitro* regenerated tissues of *Zea mays*. *Pestic. Biochem. Phys*., 85: 61–67

Buono, D.D. and G. Ioli, 2011. Glutathione S-Transferases of Italian Ryegrass (*Lolium multiflorum*): Activity toward some chemicals, safener modulation and persistence of atrazine and fluorodifen in the shoots. *J. Agric. Food Chem*., 59: 1324–1329

Cao, C.Y., Q.W. Wang, F. Jiao and G.N. Zhu, 2016. Impact of co-exposure with butachlor and triadimefon on thyroid endocrine system in larval zebrafish. *Exp. Toxicol. Pathol*., 68: 463–469

Elmore, M.T., J.T. Brosnan, G.R. Armel, J.J. Vargas and G.K. Breeden, 2016. Herbicide Safeners Increase Creeping Bentgrass (Agrostis stolonifera) Tolerance to Pinoxaden and Affect Weed Control. *Weed Sci.*, 30: 919–928

Farombi, E.O., Y.R. Ajimoko and O.A. Adelowo, 2008. Effect of Butachlor on Antioxidant Enzyme Status and Lipid Peroxidation in Fresh Water African Catfish, (Clarias gariepinus). *Inter. J. Environ. Res. Pub. Hea*., 5: 423–427

Fu, Y., M.W. Yang, F. Ye and S. Gao, 2011. Glutathione S-transferase, acetolactate synthase and safener: a study to reduce herbicidal pollution. *The 5th International Conference on Bioinformatics and Biomedical Engineering*, 1: 707–709

Gao, S., Y. Fu, L.X. Zhao, Z.Y. Xing and F. Ye, 2012. Synthesis and Crystal Structure of Novel Chiral N-dichloroacetyl-2-substituted-5-methyl-1,3-oxazolidines. *Heterocycles*, 85: 903–910

Hausman, N.E., P.J. Tranel, D.E. Riechers, D.J. Maxwell, L.C. Gonzini and A.G. Hager, 2013. Responses of an HPPD Inhibitor-Resistant Waterhemp (Amaranthus tuberculatus) Population to Soil-Residual Herbicides. *Weed Technol*., 27: 704–711

Hemanth Kumar, N.K., M.S. Kumari, M.V. Singh and S. Jagannath, 2016. Oxidative stress and antioxidant metabolic enzymes response of maize (*Zea Mays* L.) seedlings to a biotic stress (Alachlor) condition. *Int. J. Agric. Biol*., 8: 2227–2231

Ismaiel, A.A. and J. Papenbrock, 2014. The effects of patulin from Penicillium vulpinum on seedling growth, root tip ultrastructure and glutathione content of maize. *Eur. J. Plant Pathol*., 139: 497–509

Jablonkai, I., 2013. *Herbicide Safeners: Effective Tools to Improve Herbicide Selectivity, Hungary*. Available at: http://dx.doi.org/10.5772/55168 (Accessed: 17 December 2014)

Jo, H.J., J.J. Lee and K.H. Kong, 2011. A plant-specific tau class glutathione S-transferase from Oryza sativa with very high activity against 1-chloro-2,4-dinitrobenzene and chloroacetanilide herbicides. *Pestic. Biochem. Phys*., 101: 265–269

Kraehmer, H., B. Laber, C. Rosinger and A. Schulz, 2014. Herbicides as Weed Control Agents: State of the Art: I. Weed Control Research and Safener Technology: The Path to Modern Agriculture. *Plant Physiol*., 166: 1119–1131

Li, D.Z., L. Xu , S. Pang, Z.Q. Liu, K. Wang and C.J. Wang, 2017. Variable levels of glutathione s-transferases are responsible for the differential tolerance to metolachlor between maize (*Zea mays*) shoots and roots. *J. Agr. Food Chem*., 65: 39-44

Liu, W.Y., C.Y. Wang and T.S. Wang, 2011. Impacts of the herbicide butachlor on the larvae of a paddy field breeding frog (Fejervarya limnocharis) in subtropical Taiwan. *Ecotoxicology*, 20: 377–384

Obermeier, M., C.A. Schroder, B. Helmreich and P. Schroder, 2015. The enzymatic and antioxidative stress response of Lemna minor to copper and a chloroacetamide herbicide. *Environ. Sci. Pollut*., 22: 18495–18507

Martins, P.F., G. Carvalho, P.L. Gratão, M.N. Dourado, M. Pileggi, W.L. Araújo and R.A. Azevedo, 2011. Effects of the herbicides butachlor and metolachlor on antioxidant enzymes in soil bacteria. *Process Biochem*., 46: 1186–1195

Mhlanga, B. and B.S. Chauhan, 2016. Thierfelder Christian. Weed management in maize using crop competition: A review. *Crop Prot*., 88: 28–36

Rasool, N., Z.A. Reshi and M.A. Shah, 2014. Effect of butachlor (G) on soil enzyme activity. *Eur. J. Soil Biol*., 61: 94–100

Rezaei, M.K., Z.S. Shobbar, M. Shahbazi, R. Abedini and S. Zare, 2013. Glutathione S-transferase (GST) family in barley: Identification of members, enzyme activity, and gene expression pattern. *J. Plant Physiol*., 170: 1277–1284

Scarponi, L., E. Quagliarini and D.D. Buono, 2006. Induction of wheat and maize glutathione S-transferase by some herbicide safeners and their effect on enzyme activity against butachl or and terbuthylazine. *Pest. Manag. Sci*., 62: 927–932

Scarponi, L., D.D. Buono, E. Quagliarini and R.D. Amato, 2009. Festuca arundinacea grass and herbicide safeners to prevent herbicide pollution. *Agron. Sustain.*, 29: 313–319

Sriharsha, S.N. and S. Shashikanth, 2006. Synthesis and antimicrobial activity of novel 1, 3-oxazolidine nucleoside analogs. *Heterocycl Commun.*, 12: 213–218

Stoilkova, G. and P. Yonova, 2010. Safeners for chlorsulfuron onmaize (*Zeamays* L.). *Qual. Assur. Saf. Crop*, 2: 28–35

Sytykiewicz, H., 2015. Transcriptional responses of catalase genes in maize seedlings exposed to cereal aphids' herbivory. *Biochem. Syst. Ecol*., 60: 131–142

Taylor, V.L., I. Cummins, M. Brazier-Hicks and R. Edwards, 2013. Protective responses induced by herbicide safeners in wheat. *Environ. Exp. Bot.*, 88: 93–99

Ye, F., H.F. Cao, Y. Fu, L.X. Zhao and S. Gao, 2016. The safener effect of chiral derivatives of 3-dichloroacetyl oxazolidine against haloxyfop-P-methyl-induced toxicity in maize. *Zemdirbyste*, 103: 29–34

Zhao, L.X., H.T. Qu, Y. Fu, S. Gao and F. Ye, 2015. Alleviation of injury from chlorimuron-ethyl in maize treated with safener 3-dichloroacetyl oxazolidine. *Can. J. Plant. Sci*., 95: 897–903

(Received 28 November 2017; Accepted 28 December 2017)