African Journal of Biotechnology Vol. 8 (17), pp. 4128-4130, 1 September 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Short Communication

Effects of artea, a systemic fungicide, on the antioxidant system and the respiratory activity of durum wheat (*Triticum durum* L.)

Youbi Mustapha[1]*, Djebar Med Réda[1], Berrebbah Houria[1] and Hennouni Nacira[2]

[1]Laboratoire de Toxicologie Cellulaire, université de Annaba, B.P. 23000, Algérie.
[2]Centre Universitaire d'El-Tarf, B.P. 36000, Algérie.

Accepted 6 May, 2006

The present work aimed at the study of the effects of Artea, a systemic azole fungicide, on durum Wheat (*Triticum durum* L. cv. GTA dur). Seeds were grown in a medium containing respectively 25, 50, 75 and 100 ppm of Artea under controlled conditions. Roots of eight-day-old plants were used to determine catalase, ascorbate-peroxidase and guaïacol-peroxidase enzymatic activities. Root respiratory activity was also determined using a polarographic method (Clark electrode). Treatment with Artea resulted in an enhancement of respiratory activity and increased antioxidative enzymatic levels in durum wheat roots. Activities of catalase, ascorbate-peroxydase and guaïacol-peroxydase increased proportionally and were more meaningful at high concentrations (75 and 100 ppm) compared with controls. Modulations in respiratory metabolism and antioxidant system could probably be the result of Artea induced toxicity which could lead to an oxidative stress state. The present study enhances previous works relevant to the toxic effects induced by azole fungicides on plants.

Key words: Toxicity, respiratory activity, antioxidant system, azole fungicides.

INTRODUCTION

Reactive oxygen species (ROS) are produced in both stressed and unstressed plant and are mainly issued from oxygen metabolism in mitochondria (Alscher et al., 2002). Plants have well developed defense system against ROS involving enzymatic and non enzymatic means. Catalase, ascorbate-peroxidase and guaïacol-peroxidase are antioxidant enzymes which play a capital role in keeping H_2O_2 levels harmless and therefore contribute to protecting plant from ROS damages.

Cultivated plants are often subject to a variety of toxic substances leading to important yield reductions (Ezzahiri, 2001). Azole fungicides are systemic substances which were developed to control fungal diseases affecting both plants and animals. Propiconazole and cyproconazole are both azole molecules well known for causing fungus membranes destruction. They are used as active ingredients to fabricate several systemic fungicides. Recently, a new propiconazole-cyproconazole fungicide; Artea ec 330, was brought into the market. It is used for limiting damages caused to cereal crops by a number of diseases such as rust and septoriose.

Despite the undoubted effectiveness of systemic fungicides in controlling plant diseases and improving crops yield, many studies have underlined their toxic effects on plant in the sense they may induce a decrease in growth as well as modulating the metabolic balance. Morphological effects of azoles molecules on plants include reduced shoot elongation and trichom length, inc-

*Corresponding author. E-mail: youbi_mustapha@yahoo.fr

Abbreviations: APX, Ascorbate-peroxidase; **CAT**, catalase; **GPX**, guaiacol-peroxidase; **SOD**, super-oxide dismutase; **ROS**, reactrive oxygen species.

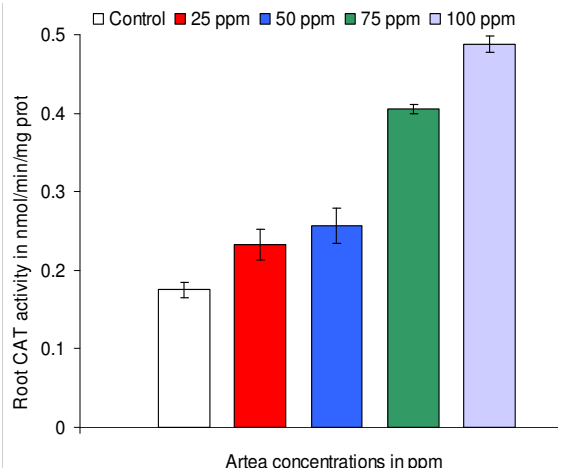

Figure 1. Effects of Artea on durum wheat root catalase (CAT) activity.

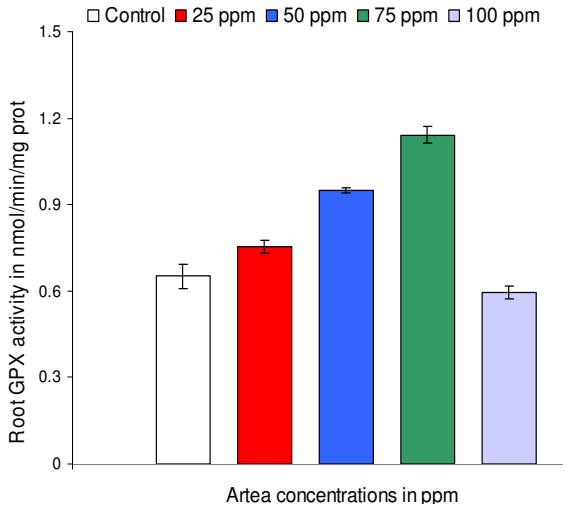

Figure 2. Effects of Artea on durum wheat root guaïacol-peroxidase (GPX) activity.

reased epicuticular wax and larger chloroplasts (Fletcher and Hofstra, 1988; Gao et al., 1988). Biochemical effects of azoles include increased levels of proline (Mackay et al., 1990), antioxidant enzymes (Seneratna et al., 1988) and chlorophyll content (Fletcher and Hofstra, 1988).

In spite the increasing number of studies on azole-induced oxidative stress, little is known about the effects of Artea on pant respiratory activity and antioxidant system. Thus, the aim of the present study was to determine the effects of Artea on durum wheat root after a short Artea treatment.

MATERIAL AND METHODS

Plant cultivation

Seeds of *Triticum durum* L. cv. GTA dur were sterilized with 5% sodium hypochlorite for 3 min. After being washed with distilled water several times, seeds were incubated in Petri dishes containing 25, 50, 75 and 100 ppm solutions of the systemic fungicide Artea. Experiment was performed in 9 cm diameter sterilized Petri plates containing filter paper soaked in fungicide solutions. 10 seeds were placed in each Petri plate separately. Untreated Petri plates served as control. Seeds were germinated under controlled conditions. Small amounts of respective fungicide's solutions were added when it was obvious that Petri dishes were beginning to dry out.

Enzyme assays

For extraction of antioxidative enzymes, eight-day-old root tips were homogenized with 0.1 M sodium phosphate buffer (pH 6.8) in a chilled pestle and mortar. The extraction was performed as described by Loggini et al. (1999). Enzyme activities in each extract were determined spectrophotometrically using a diode array spectrophotometer. Assays were conducted in a total volume of 3 ml at 25°C for 3 min and the results were repeated three times using 15–20 root tips. For CAT, the decrease in absorbance at 240 nm due to addition of H_2O_2 was monitored (Cakmak and Horst, 1991). For GPX, the increase in absorbance due to tetraguaiacol formation was recorded at 470 nm (Cakmak and Horst, 1991). For APX, the activity was followed as the decrease at 290 nm due to the consumption of ascorbate (Nakano and Azada, 1981). Proteins in each extract were assayed according to the method of Bradford (1976) using BSA as standard. Roots oxygen consumption was monitored polarographically using a Clarck type electrode (Djebar and Djebar, 2000).

RESULTS AND DISCUSSION

Figure 1 shows the effects of Artea on catalase content in durum wheat roots. CAT levels increase proportionally with fungicide concentration (about 150% at 100 ppm). At 25 ppm, the increase in CAT content is about 46%. The effects of Artea on root GPX content are shown in Figure 2. Although Artea triggers an increase in GPX levels up to 75 ppm (about 75%), a decrease in GPX is recorded at 100 ppm (about 10%). Figure 3 demonstrates that Artea treatment results in a significant increase in APX level which reached its maximum at 75 and 100 ppm (about 80% and 140%, respectively).

Relatively to oxygen consumption, Figure 4 indicates a significant increase at 100 ppm of Artea (about 400%). At 25 ppm, a slight non significant decrease (about 6%) is recorded.

Durum wheat treatment with Artea induced an increase in catalase, guaïacol-peroxidase and ascorbate-peroxidase levels along with a stimulation of respiratory activity particularly at high concentrations (75 and 100 ppm). The absorption of artea active ingredients (propiconazole and cyproconazole) by seeds subsequently to germination outbreak implicates their penetration into different root tissue cells. The stimulation of oxygen consumption indicates considerable respiratory metabolism in mitochondria which is an indication of an important ATP production (Bouraoui et al., 1998). Several studies have outlined the toxic effects of azole molecules on plants, primarily resulting in growth decrease and other

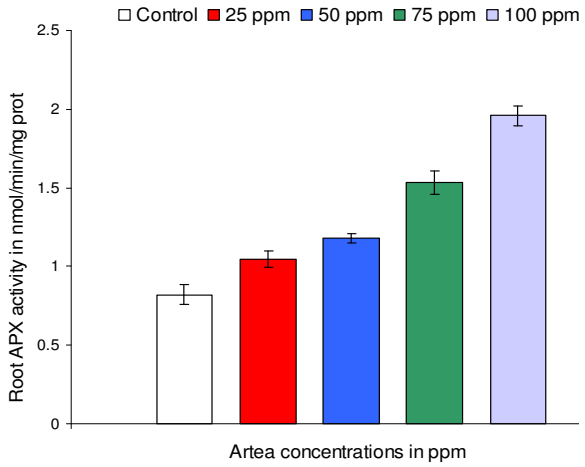

Figure 3. Effects of Artea on durum wheat root ascorbate-peroxidase (APX) activity.

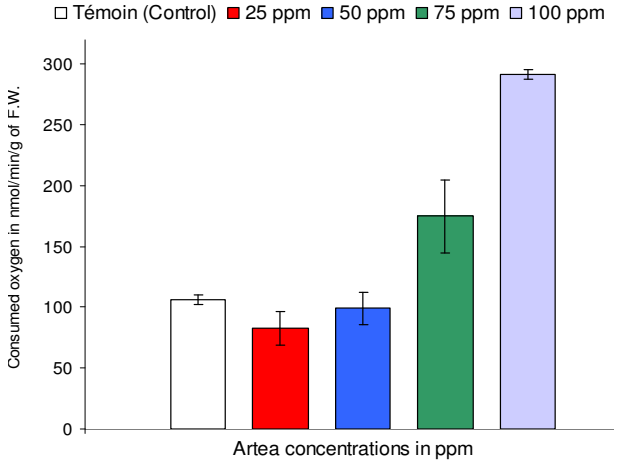

Figure 4. Effects of Artea on durum wheat root respiration average

Toxic effects (Siddiqui et al., 2001; Kuciel and Mazurkiewicz, 2004; Williams et al., 1998; Blokhina et al., 2003). A decrease in root number and length was also recorded after the treatment of durum wheat with similar Artea concentrations (data not shown). In response to cyproconazole and propiconazole toxic effects, root cells mobilizes a set of detoxifying mechanisms which are largely dependant on ATP in order to maintain a possible normal growth rate (Grene, 2002). As a result, ATP demand rises along with oxygen consumption.

Besides, respiratory metabolism stimulation is combined to a surplus production of reactive oxygen species (ROS) mainly in mitochondria (Grene, 2002; Kiss et al., 2003; Kuciel and Mazurkiewicz, 2004). This leads cells to produce more antioxidant enzymes to cope with damages caused by free radicals. CAT, GPX and APX would contribute to H_2O_2 dismutation issued by SOD which transforms O_2^- into H_2O_2 (Grene, 2002). Cells could thus limit damages caused by H_2O_2 which is indirectly issued from propiconazole and cyproconazole via respiration.

In conclusion, the treatment of durum wheat with the systemic fungicide Artea reveals that it could induce negative metabolic and biochemical changes which corroborate the toxic effects of azole fungicide on plant outlined by previous studies.

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