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Full Length Research Paper

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

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The present work is aimed at the study of Artea (a systemic fungicide) effects on durum wheat (*Triticum durum* L. CV. *Hard GTA*). Seeds were grown in a medium containing 25, 50, 75 and 100 ppm of Artea under controlled conditions. Roots of eight day old were used to determine the enzymatic activities of catalase, ascorbate-peroxydase and guaïacol-peroxydase. Root respiratory activity was also determined using a polarographic method (Clarck electrode). The results after treatment with Artea show an enhancement of respiratory activity and increased levels of antioxidative enzymes 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). Modulations in respiratory metabolism and antioxidant system could probably be the result of Artea induced toxicity which could cause an oxidative stress state.

Key words: Toxicity, respiratory activity, antioxidant system, azole fungicides, catalase, Artea, ascorbate-peroxydase, guaïacol-peroxydase, *Triticum durum*.

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 an advanced defence system against ROS involving enzymatic and non-enzymatic means. Catalase, ascorbate-peroxydase and guaïacol-peroxydase 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 yields 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 as fungus membranes destructors. 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 septoriosis.

Despite the undoubted effectiveness of systemic fungicides in controlling plant diseases and improving crops yield, many studies have underlined their toxic effects on plant. They may induce a decrease in growth as well as modulate the metabolic balance. Morphological effects of azoles molecules on plants include reduced root elongation and trichom length, increases 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 of the increasing number of studies on azole induced oxidative stress, little is known about the effects of Artea on plant 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

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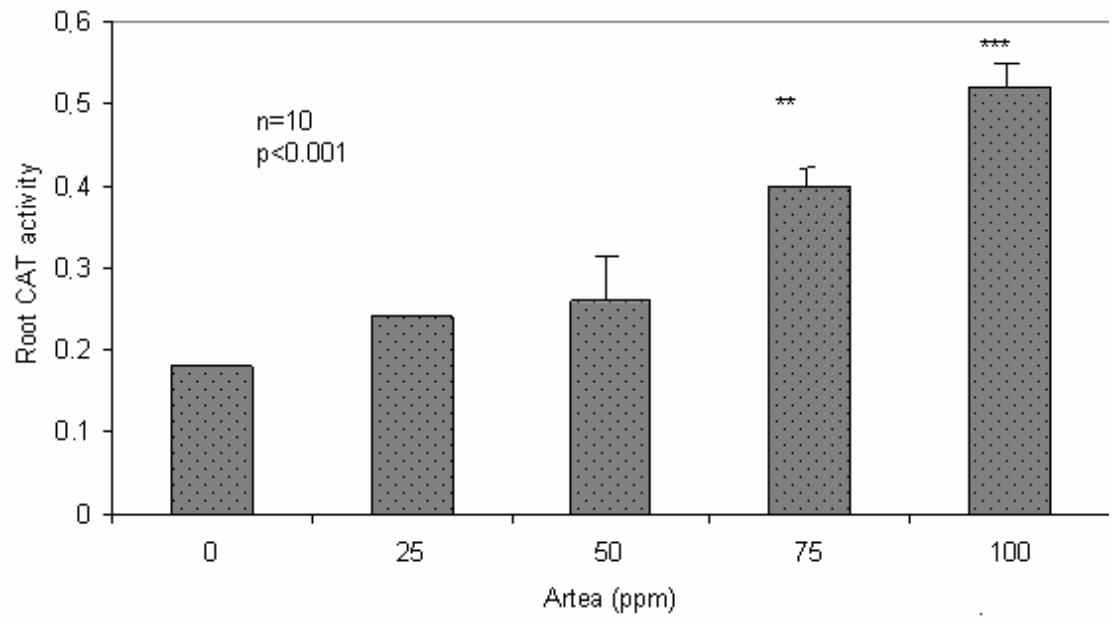

Figure 1. Effects of Artea on durum wheat root catalase (CAT) activity (nmole/min/mg protein).

treatment with Artea.

MATERIAL AND METHODS

Plant cultivation

Seeds of *Triticum durum* L. CV. GTA hard 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 roots tips were homogenised 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 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 catalase (CAT), the decrease in absorbance at 240 nm due to addition of H_2O_2 was monitored (Cakmac and Horst, 1991). For guaïacol-peroxydase (GPX), the increase in absorbance due to tetraguaïacol formation was recorded at 470 nm (Cakmac and Horst, 1991). For ascorbate-peroxydase (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 an adapted Clark electrode (Djebar and Djebar, 2000).

RESULTS AND DISCUSSION

Figure 1 shows the effects of Artea on catalyse (CAT) 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 guaïacol-peroxydase (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 a significant increase in ascorbate-peroxydase (APX) level which reach 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-peroxydase and ascorbate-peroxydase levels along with 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 acute molecules on plants, primarily resulting in growth decrease and other toxic effects (Williams et al., 1998; Siddiqui et al., 2001; Blokhina et al., 2003; Kuciel and Mazurkiewicz, 2004). A

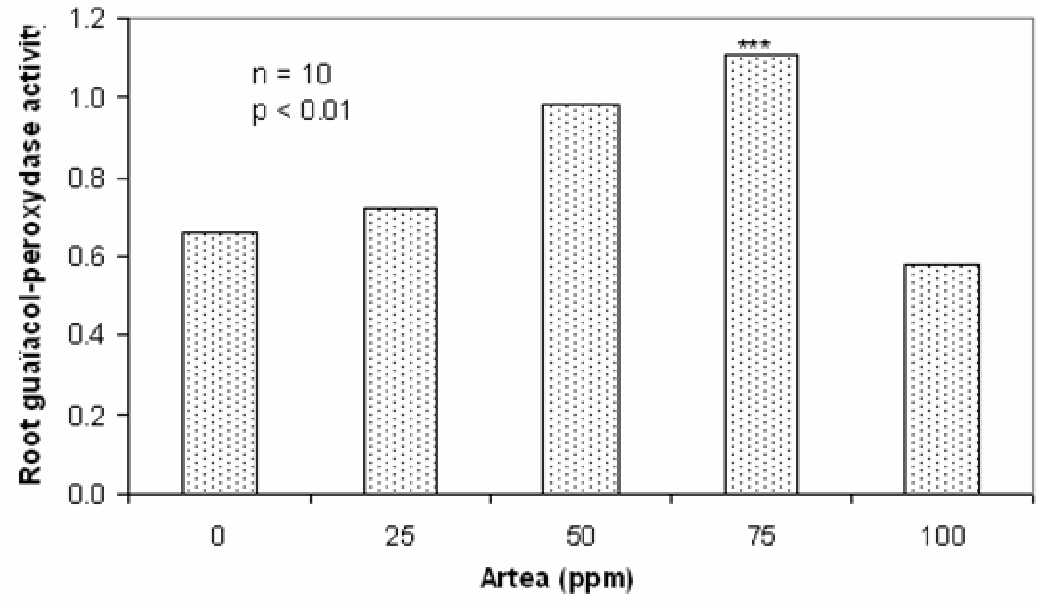

Figure 2. Effects of Artea on durum wheat root guaïacol-peroxydase (GPX) activity (nmole/min/mg protein).

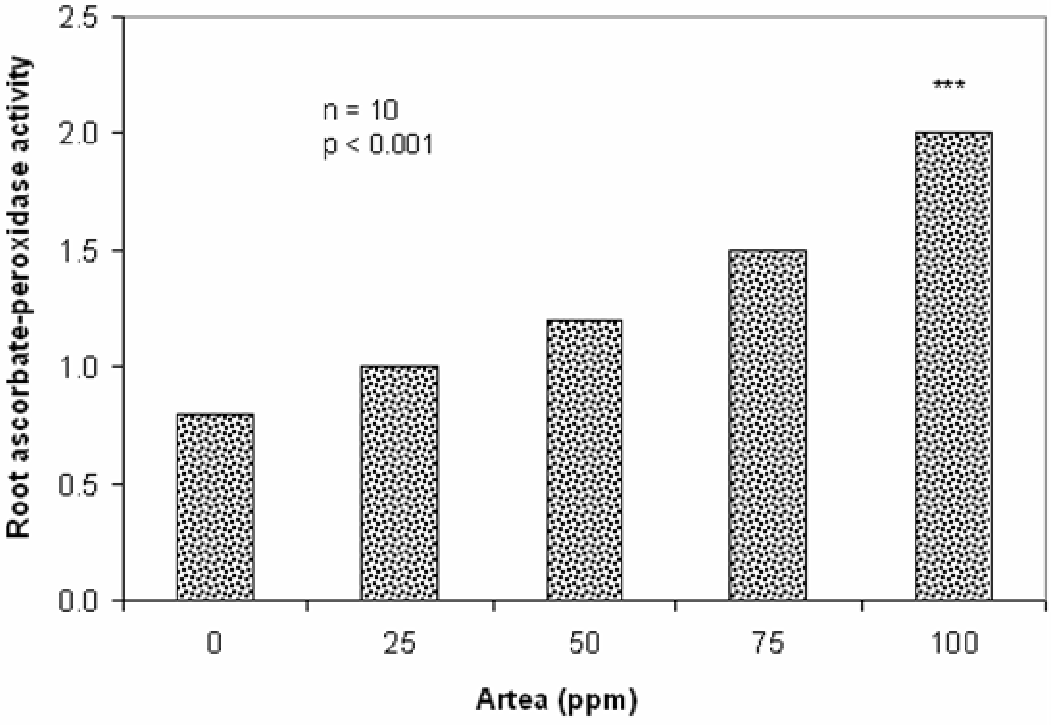

Figure 3. Effects of Artea on durum wheat root ascorbate-peroxidase (APX) activity (nmole/min/mg protein).

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 mechanism 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

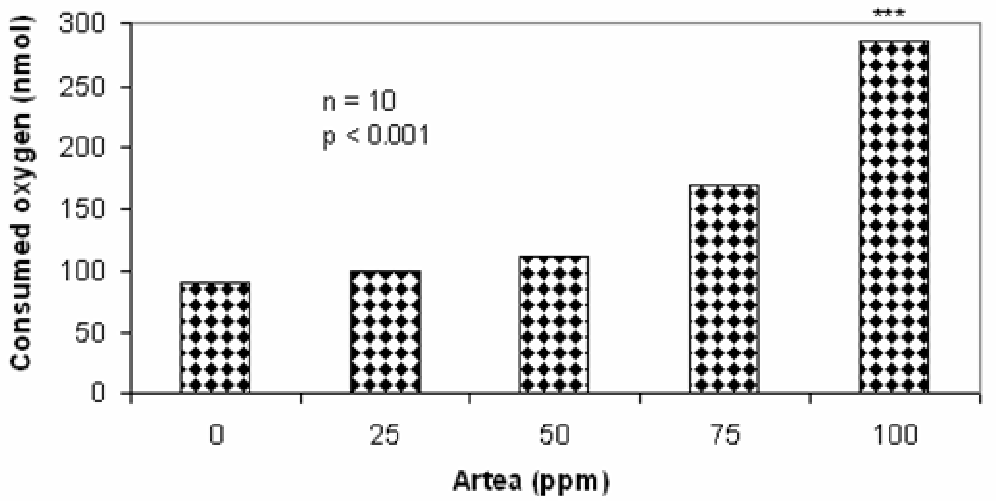

Figure 4. Effects of Artea on durum wheat root oxygen consumption activity (nmole/min/g FW).

produce more antioxidant enzymes to cope the 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 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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