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Uptake and toxicological effects of pharmaceutical active compounds on maize

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A B S T R A C T

Contamination of the soil environment with pharmaceutical active compounds (PACs) is an emerging issue. An experiment was conducted to evaluate the effects and accumulation of PACs in maize (*Zea mays* L.). After screening a variety of maize hybrid seeds for tolerance and sensitivity to paracetamol, two maize hybrids, ICI 339 and Syngenta 7720 (PACs tolerant and sensitive, respectively) and were selected for this experiment. Five paracetamol solutions were applied in two splits with 500 ml of water containing 0, 0.31, 0.62, 0.93 and 1.24 g paracetamol l^{-1}. The application of paracetamol significantly ($P < 0.05$) decreased grains yield by up to 50%. Hybrid Syngenta 7720 accumulated 0.063 ng g^{-1} paracetamol in the grain, which was 8% more (0.058 ng g^{-1}) than the amount accumulated in hybrid ICI 339. Similarly, significant ($P < 0.05$) amounts of paracetamol (0.132 and 0.153 ng g^{-1} in ICI 339 and Syngenta 7720, respectively) were accumulated in the root. The accumulation of paracetamol in maize grain and root increased linearly when the dose of paracetamol was increased, but grain protein contents were not affected. The results indicate that under the current experimental conditions, edible parts of the crop plants are contaminated with paracetamol as a PAC and could have negative effects on consumers.

1. Introduction

Pharmaceutical compounds are biologically active chemicals which are used for the cure and treatments of different diseases (Marsoni et al., 2014). The main sources of introduction of pharmaceutical active compounds (PACs) into the environment are patient excretions, household, veterinary antibiotics and hospital wastes, discharge from pharmaceutical manufacturing industries, and improper waste disposal (Pena et al., 2010; Sharma et al., 2013; Kuppusamy et al., 2018). The active ingredients in pharmaceuticals are partially metabolized in humans and animals and then excreted into the sewage systems (Kim and Aga, 2007; Du and Liu, 2012). The most commonly detected pharmaceutical classes in waste water treatment plants (WWTPs) are antibiotics and non-steroidal anti-inflammatory drugs (NSAIDs) (Fatta-Kassinos et al., 2011; Collado et al., 2014). Paracetamol is currently the most widely used drug in the world (Valencia et al., 2016). In England in the year 2000, paracetamol was the most prescribed drug, with an estimated weight of more than 400 t (Sebastine and Wakeman, 2003); and a year later in 2001, China exported approximately 15,348 t of paracetamol. The presence of pharmaceutical residues has been detected in surface, ground and even drinking water (Arnold et al., 2013; Osorio et al., 2016). Decontamination processes in waste water treatment plants (WWTPs) are not efficient enough to completely remove or eliminate these compounds (De Cazes et al., 2014). Effluents from WWTPs are used for land irrigation in many developing countries (Fatta-Kassinos et al., 2011), and the biosolids produced may be used as fertilizer on agricultural land (Du and Liu, 2012). Similarly, animal manure, which may contain residues of veterinary medicine, is spread on arable land and may also be a source of introduction of PACs into the environment (Kumar et al., 2012; Knäbel et al., 2016). Due to its wide-

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spread production and usage, paracetamol has been found more frequently than NSAIDs and pain relievers. Estimates show that it appears in aquatic environments at the rate of 0.01–0.3 mg l^{-1} where it causes soil toxicity (Kasprzyk-Hordern et al., 2008; Miège et al., 2009). Paracetamol is a threat to non-target organisms as it causes nephrotoxicity, hepatic necrosis, and, in some cases, death to human and experimental animals when taken in overdoses (Olaleye and Rocha, 2008).

Pharmaceutical compounds can cause biochemical transformations of carbon and nitrogen in the soil (Westergaard et al., 2001; Du and Liu, 2012), producing indirect effects on plant growth by changing nutrient availability, root growth and development (Carvalho et al., 2014). A study by Minden et al. (2017) revealed that different PACs resulted in a significant delay in germination of *Brassica napus* and *Capsella bursa-pastoris*. Similarly, Pan and Chu (2016) observed that germination of carrots was severely affected and that root elongation decreased linearly with increasing concentrations of tetracycline.

The presence of pharmaceuticals in water and soil may affect agriculture by entering different crops like wheat, rice, maize, and soybean (Gottschall et al., 2012; Marsoni et al., 2014), but some plants have strong resistance to the pharmaceuticals (Minden et al., 2017). Studies by Carter et al. (2014) have demonstrated that the effects of PACs depend on plant species, plant organ, and the type and concentration of pharmaceuticals applied. Pharmaceuticals delay the time of germination (Minden et al., 2017), with herbs (*Apera spica-venti*) being more sensitive than grasses (*Triticum aestivum*). In addition, changes in plants' physiological traits, such as reduced photosynthesis, decreased transpiration rate (Redshaw et al., 2008), mitochondrial cytochrome C oxidase activity (Gomez et al., 2007), and synthesis of abscisic acid by the PACs, have been reported (Kasai et al., 2004; Werner et al., 2007). The results of these studies clearly show that pharmaceuticals in the soil can accumulate in plant tissues and have harmful effects on the physiological traits of the plant. In turn, accumulated pharmaceutical compounds in plants may be introduced into human food, as agriculture serves as an important route for food chain contamination by pharmaceuticals because of high demands for water and sewage sludge compost (Piel et al., 2012). The accumulation and transport of PACs in soil-crop systems show potential risks to the agro-ecosystem of soil-edible plant part systems such as common vegetables like carrots, potatoes, and radishes, and common crops like alfalfa, rice, and wheat. Pharmaceutical active compounds also show potential risks to soil ecosystems and the water quality of plant-based products (Du and Liu, 2012), and these compounds affect plant growth and yield (Goss et al., 2013). The presence of PACs in water, soil, and edible crop parts also presents some risk to human beings who utilize crops grown in affected areas (Boxall et al., 2006; Carter et al., 2014). Wu et al. (2013) investigated the uptake of compounds in common pharmaceuticals and personal care products by common vegetables and found that the compounds accumulate in the root and shoots.

The present study was conducted to assess the ecological risks of PACs on maize plants. Using maize as a model crop, we hypothesized that paracetamol, a common drug available both with and without a prescription, can inhibit germination and seedling growth and may accumulate in maize grains. Various levels of paracetamol were tested to assess its possible uptake, its buildup in the soil-plant-food chain continuum, and its potential toxicological effects given its frequent usage and abundance in the environment (Valencia et al., 2016).

2. Materials and methods

2.1. Screening of maize hybrid and PACs

2.1.1. Seed germination test

Initially, ten commercially available maize hybrids and three drugs were screened before starting the experiment. During this process maize hybrids seeds were sterilized with 2.5% sodium hypochlorite for 15 min and washed thoroughly with distilled water. Hydrated seeds were transferred to petri dishes (100 mm diameter) containing a Whatman filter and 5 ml of distilled water pH 7.1 (control). Solutions of 2.5 g l^{-1} of three commons PACs, paracetamol, ibuprofen and diclofenac, were used for screening the maize hybrids. The three PACs used for screening experiments were purchased from Wuhan Yuancheng Technology Development Co. Ltd., China, and each had a 99% purity guarantee.

Ten seeds of each hybrid were placed in petri dishes until germination. Seventy-two hours after germination, seeds were counted according to the APAT protocol (APAT, 2002). Seeds were considered germinated when root length reached 45 mm. This germination test was carried out for screening purposes to determine the maize hybrids that were most tolerant and most sensitive to PACs. Two hybrid seeds were ultimately selected for the experiment. Syngenta ICI-339 and 7720 were found to be the most paracetamol tolerant and sensitive respectively. Therefore, one compound (paracetamol), which accumulates in maize seedlings, was selected to analyze the effects of the PACs on growth, yield and accumulation in maize grain. The screening was done with the following germination characteristic of the plants.

2.1.2. Time to 50% emergence (E50) [days]

The time to 50% emergence of seedlings (E_{50}) was calculated according to the following equation of Coolbear et al. (1984) modified by Farooq et al. (2005)

$$E_{50} = t_i + \left[\frac{N/_2 - n_i}{n_j - n_i}\right](t_j - t_i) \quad (1)$$

where N is the final count of seeds at emergence and n_i and n_j are the cumulative number of seeds emerged at adjacent days t_i and t_j when $n_i < (N+1)/2 < n_j$.

2.1.3. Mean emergence time (MET)

Mean emergence time (MET) was calculated according to following equation (Ellis and Roberts, 1981),

$$MET = \frac{\sum Dn}{\sum n} \quad (2)$$

where n is the number of seeds that emerged on day D, and D is the number of days that were counted from the beginning of emergence.

Energy of germination was recorded on the 4th day after planting. It is the percentage of emerged seedlings 4 days after planting relative to the total number of seeds tested (Farooq et al., 2006).

$$EE\ (\%) = \frac{No.\ of\ seedlings\ emerged\ 4 days\ after\ sowing}{Total\ no.\ of\ seeds\ planted} \times 100 \quad (3)$$

2.1.4. Final emergence percentage

Final germination percentage was taken at the end of experiment. It represents the ratio, in percentage, of the number of emerged seedlings to the total number of seeds planted.

$$FGP\ (\%) = \frac{Final\ number\ of\ seedlings\ emerged}{Total\ no.\ of\ seeds\ planted} \times 100 \quad (4)$$

2.2. Experimental site and design

The experiment was carried out at the COMSATS Institute of Information Technology (30° N, 72.3° E), during 2015. The experimental soils contained 58% sand, 17% silt, and 25% clay with soil pH of 7.5 and 0.86% organic matter. The available inorganic nitrogen, phosphorus, and potassium were 0.52, 5.24, and 16.32 g kg^{-1}, respectively. The experiment was laid out in a completely randomized design (CRD) with factorial arrangement, having five replications.

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