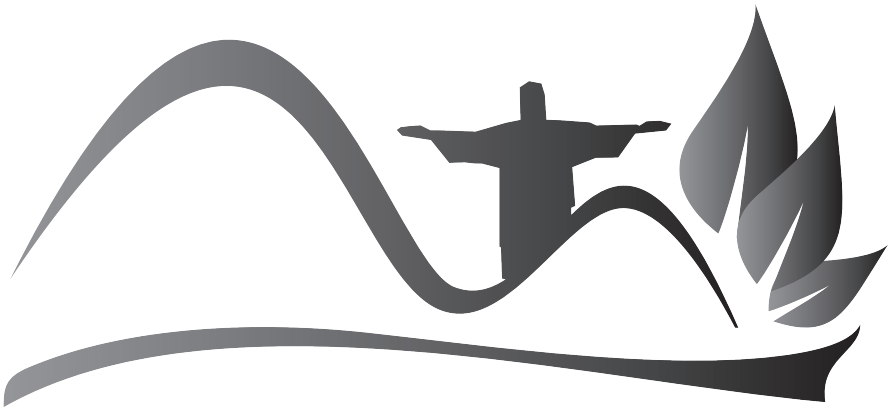
16th WORLD FERTILIZER CONGRESS OF CIEC

TECHNOLOGICAL INNOVATION FOR A SUSTAINABLE TROPICAL AGRICULTURE

PROCEEDINGS

International Scientific Centre of Fertilizers (CIEC)



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PROCEEDINGS

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NATURAL PHENOLIC ALDEHYDES AS PLATFORMS FOR THE DEVELOPMENT OF NOVEL UREASE INHIBITORS

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Introduction

Urea is one of the most common nitrogen fertilizers used in agriculture worldwide due to its high nitrogen content, relatively low price and easy management. Ureases are key enzymes for the global nitrogen cycle, occurring in plants, fungi and bacteria. This type of hydrolase speeds up by one-hundred-trillion-fold the hydrolysis of urea to yield ammonia and carbon dioxide (Krajewska et al. 2009). Thus, ureases from soil microbiota rapidly hydrolyzes the urea applied to crop fields, causing significant nitrogen losses due to ammonia volatilization when this process occurs on soil surface (Follmer, 2008; Artola et al., 2011).

Urease inhibitors can be used as an alternative strategy to slow down urea hydrolysis, increasing the chances of incorporation of this nitrogen fertilizer to soil either by rain, irrigation or mechanical operations (Junejo et al., 2011). In this context, urease inhibitors have received considerably attention by their ability to modulate ammonia formation in soil from urea (Artola et al., 2011; Kawakami et al., 2012).

Nature is unequivocally a great source of bioactive compounds that exhibit a wide variety of benefits to human beings and animals as well (Silva et al., 2014; de Fátima et al., 2014). Indeed, the investigation of the potential of plant-derived natural products as urease inhibitors can be valuable for the discovery and development of new urease inhibitors of agricultural interest. The focus of the work presented here was to obtain four compounds derived from the plant natural products protocatechuic aldehyde, syringaldehyde and vanillin as prototypes and test them for the potential to inhibit (*in vitro* and in soil) ureases and improve plant growth in the presence of urea as fertilizer.

Methods

Tested Compounds

The compounds used in this study were synthetized in high purity grade in one step, in which **PAD1** and **PAD2** are derived from protocatechuic aldehyde, **VD** from vanillin and **SD** from syringaldehyde according to Modolo et al. (2013).

In vitro Assays

In vitro tests were performed with jack bean urease in reactions containing urea 10 mM in presence or absence of **PAD1**, **PAD2**, **VD** and **SD** at 1.6 mM. The inhibition mode exhibited by the compounds tested was investigated from reaction with jack bean urease by using compounds at concentrations in the range of 200-1600 μM. Ammonium production was determined using the indophenol method (Krajewska and Ciurli, 2005).

Soil and Soil-Plant System Assays

The concentration of phenolic aldehydes derivatives necessary to inhibit the activity of soil microbiota ureases by 50% (IC_{50}) was determined by incubating topsoil (0.5 g), classified as Haplustox (Brazilian cerrado region) in the presence of 72 mM urea and different concentrations of compounds-test. The relative growth rate was determined according to the formula RGR = [ln(Ht2)−ln(Ht1)]/T, where Ht2 and Ht1 are the final and initial height, respectively, and T is is the number of days between the initial and final measurements, i.e. 26 and 55 days, respectively (Gunaratne et al., 2011).

The best urease inhibitor was further tested in the system soil-*Pennisetum glaucum* to evaluate its effect on plant growth supplemented with urea as nitrogen fertilizer.

Results and discussion

The compounds **PAD1** and **PAD2** were the most potent urease inhibitors in *in vitro* assays, among the compounds tested *in vitro* (enzyme inhibition higher than 96%). Compounds **VD** and **SD** were still found as promising inhibitors as they negatively affected urease activity by 55% and 69%, respectively.

The jack bean urease kinetics was affected by all compounds tested as described in Table 1. The plant-phenolic aldehyde derivatives were found to be mixed inhibitors as they affected both K_m for substrate and enzyme V_{max} (Table 1).

The IC_{50} values for **PAD1** and **SD** in experiments with topsoil were 3 mM. We have failed to determined the IC_{50} value for **PAD2** due to great variations in the results obtained from distinct experiments. Then, it is believed that this compound might be biotransformed by the soil microbiota in varied extents as it occurs for the commercial urease inhibitor *N*-(butyl)thiophosphoric triamide (**NBPT**) (Kawakami et al., 2012). The maximum inhibition of soil microbiota ureases was 16% when **VD** was applied at concentrations equal or higher than 50 µM. The system top soil-*Pennisetum glaucum* (millet) was then used to assess the effect of compound **PAD2** on plants. The growth rate of millet plants upon treatment with urea plus 0.5% or 1.0% **PAD2** (w/w) was found to be higher than that of plants supplemented with urea solely (Fig. 1).

Conclusions

The results indicated that the plant phenolic aldehydes derivatives **PAD1**, **PAD2**, **VD** and **SD** inhibited the urease activity in both *in vitro* and soil assays at different extents. The compounds function as mixed inhibitors and the use of **PAD2** in soil-millet system supplemented with urea improved the growth of such crop.

Acknowledgements

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Table 1. Effect of plant-phenolic aldehyde derivatives on jack bean urease kinetics.

Compound	Concentration (μM)	K_m (μM)	V_{max} (μmol min^{-1} mg $prot^{-1}$)
PAD1	0	6.3	3.1
	200	7.4	2.4
	400	10.0	2.1
PAD2	0	5.7	2.2
	400	7.8	1.1
	800	8.7	0.7
VD	0	4.8	3.1
	800	7.2	2.4
	1600	6.1	2.0
SD	0	5.6	4.2
	800	6.8	2.7
	1600	7.1	2.0

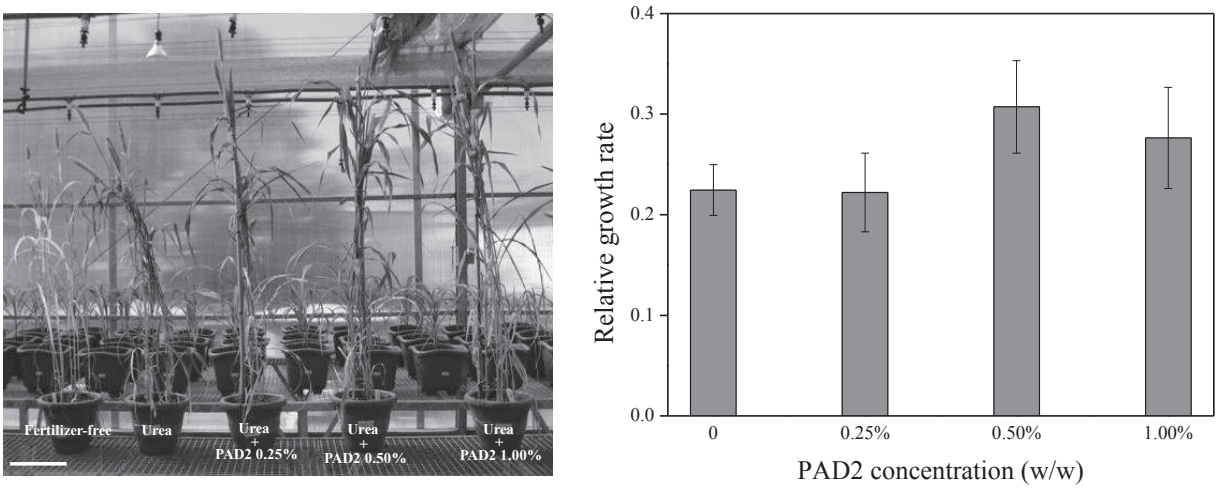

Figure 1. Effect of compound PAD2 on the growth of millet plants supplemented with urea. Values represent means + standard error of experiments performed with four replicates.