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The Roles of Antitoxin of Enzymes in Rice Plant, Grown on Acid Sulfate Soils, Mekong Delta

Huy BL[1*], Thanh LT[2], Van NT[3], Xuan HN[4] and Tan HD[5]

[1,4,5]Department of Environment science, Hochiminh City University of Food Industry (HUFI), Vietnam

[2]Department of Environment Science, Kien Giang University, Vietnam

[3]Department of Environment Science, Hochiming City University of Technology (Hutech), Vietnam

***Corresponding author:**

Ba Le Huy,
Department of Environment science,
Hochiminh City, University of Food Industry (HUFI), Vietnam ,
E-mail: lhuyba@gmail.com

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1. Abstract

Assessing the resistance, determining the nature of the resistance of the rice plant through the activity of three enzymes in rice: Catalase, Peroxydase and Phosphatase in the leaves of rice plants (the most active leaves of rice) rice plants) [2]. In addition, consider the role of Phosphatase in the fluctuations and the ability to accumulate P2O5 in soil and plants to see its role in rice's poison resistance in acid sulphate soil [4]. In other hand, Results shows that- High concentrations of toxins in the soil environment strongly inhibited the activity of Phosphatase enzymes in the roots, in the indicator leaves, and especially for rice, especially for plants that are less resistant to poisoning. But, Phosphatase enzyme in leaves has the effect on the providing energy for the plants to recover from poisoning. The activity of this enzyme is inversely correlated with the content of Al^{3+} in soil and Al^{3+} in plants. This proves that the antagonism between enzymes and toxins is evident, especially with Al^{3+} toxins. In other side, has highly toxic in the acid sulphate soil environment inhibits enzyme activity Peroxydase in rice roots. Under these conditions, peroxydase in the root plays a small role in helping the plant recover.

We have sufficient basis to confirm that the enzyme Peroxydase in leaves plays an important role, against the harmful effects of toxins. When the toxin in the soil, in plants increased activity of this enzyme also increased higher than the control variant. Especially, when the toxicity increases, the Peroxydase enzyme in the rice leaves will also increase to resistant to toxicity (+280% (compared to the control); while the toxicity- non -resistant variety plants did not increase (compared to the control). When the toxicity decreased, Peroxydase activity in toxic-resistant plants was also higher than toxic non-resistant: This increased at +643% for toxic-resistant rice compared to the control. While, in the weekly- resistant toxic- variety only increased +314% (compared to the control).

2. Introduce

Research of Le Huy Ba et al carry-out by [1-3] a series of experiments including: Step 1, Group testing of rice varieties including 15 varieties; In step 2, experiment comparing varieties to select alum tolerant varieties. Step 3, After identifying alum-tolerant varieties: Ca Dung variety (local) strong resistance to toxicity. Oppositely, IR38 variety is weakly to toxicity resistant. On other hand, they have found that, the toxic ions in acid sulphate soil environment (ex: Al^{3}, $Fe^{2+}Fe^{3+}$and SO_4^{2-}) there are coplicatly dynamics [1-4].

At this point, the new question that needs to be answered is the effect of toxins on the enzymes in plants. and vice versa, the role of the enzyme has no role to resist poison (how effect on this activity of enzymes in rice leaves). That means, what role of this enzyme to help rice plant to resistant influence of the toxicity. So that, we carry out some experiences (in vitro, in green house, and in the yield experiment together with the practical survey).

3. Materials and Research Methods

3.1. Material

Whole acid sulphate soil (not disturbed by cultivation): Heavy alkaline soil with pH = 3,6– 4,1; Al^{3+} = 500 - 800 ppm, although the plants do not die but the toxicity is also very heavy, from which people choose resistant alum varieties.

Vietnamese rice varieties

- Select a selection of 15 new rice varieties including new varieties (IR38) and local varieties (Ca Dung)
- Compare and select toxity-tolerant varieties: including good alum-tolerant rice varieties, weak alum-resistant rice varieties (rice varieties living on alum soil during the research process).
- The indicator tree is also a control plant: Elocharis Dulcis: take normal growing plants, transplant into pots with alum soil.
- With the control variant of soil: Neutral alluvial soil (Tien river) is not alum or toxic.

So we have 2 control variant: With plants: Good alum-tolerant rice transplantation compared to poor alum-resistant rice and compared with indicator plants.

The toxicity and tolerance test of rice increased the tox icity levels (Fe2 +, Al3 +, SO42- content increased gradually compared to the control (alluvial soil: non-toxic).

Experimental arrangement in pots placed in a greenhouse

- Arrangement in pots (diameter r = 30cm, height: 50cm, porcelain and porcelain materials), put in greenhouses (Poly Etylen)
- Experiment with 5 treatments, 4 replications, sequentially according to latin cell, the level of toxicity: increasing, with treatments:
- With Al^{3+}

  1. Al $^{3+}$ = 0ppm, 2. Al^{3+} = 135 ppm, 3. Al $^{3+}$= 500, 4. Al^{3+}=800ppm and 5. Al^{3+}=1000ppm,
- With Fe^{2+}

  1. Fe2 + = 0 ppm, 2. Fe2 + = 600 ppm; 3. Fe2 + = 800 ppm; 4. Fe2 + = 900 ppm; 5. Fe2 + = 1000 ppm.
- Maintaining sufficient alkaline water to flood the soil (5-7 cm)

3.2. Arranging Field Experiments

- Rice plants: On Nhi Xuan acid sulphate soil, plot: 25m2, soil kept unchanged, no plowing, arranged Experiment 4 times, according to random sequential plot.
- Location: Whole alum soil belongs to Nhi Xuan Farm, Nhi Xuan commune, Binh Chanh district, Southwest, Ho Chi Minh City.
- Maintaining alum toxicity by adjusting the natural alum water in the area
- Adjust the toxicity through 2 periods: 1: high and 2- low to identify the reaction of rice physiology and biochemistry: the activity of 3 enzymes.

3.3. Tracking Criteria

- Growth and development of rice, especially 3-4 stages of true leaves (roots, stems, leaves, ...)
- Monitoring the development and toxicity of control acid sulphate soil eco-indicator plant (Eleocharis Dulcis)
- Analysis of activity enzyme of rice plant: Take the second leaf (strongest activity leaf)

Analysis criteria:

- Soil and water environment: pH, Fe^{2+}, Al^{3+}, SO_4^{2-}, $PO_4^{3-}$by standr method (TCVN 2013)
- Activation of enzymes in functional leaves in rice: Amylase, Catalase, Peroxydase, Phosphatase Bio- analysis Method (Standar VN 2009.)

4. Results

4.1. The Effect of Aluminum Toxicity (Al^{3+}) and Iron (Fe^{2+}) In Soil on the Growth 0f Rice

Al^{3+} can be toxic even at low concentrations of 1-2ppm (D. Dent-1986, Le Huy Ba-1982) observed and analyzed on acid sulfate soil, said on the field when dry soil has a layer of $Al_2(SO_4)^3$ emerges in white, light and porous masses; When it is wet, it will contain mucus which contains 4,26% (Al^{3+}) and 38,4% (SO_4^{2-}). Al^{3+} has symptoms that are not always easy to recognize. For rice, the symptoms are expressed right at the roots (deformed, dented and brittle roots, although the color of the roots is not as black as iron poisoning), if the aluminum toxicity is high, the short rice roots will lose their hairs. and die.

Le Huy Ba's research showed that at a concentration of Al^{3+}at 135 ppm begening effect. While in the yield experiment, concentration of $Al^{\#+}$ at 500 ppm will effect on young rice plant (at rice plant have 3 real leaf). Thus, influence of toxic ion is depending on environmental condition

In addition, when Al^{3+} and Fe^{2+} exist simultaneously, the toxicity of soil also increases much more than normal [3].

In acid sulphate soils, Fe^{2+}, Fe^{3+} combine with S^{2-} to form FeS compound which attaches tightly to rice roots, impeding nutrient uptake in the soil as well as affecting respiration, causing rice roots to die.

4.1.1. The Effect of Al^{3+} on Young Rice Plants

Al^{3+} is a dangerous poison in the true tricuspid phase: at the concentration of Al^{3+}=135ppm in nutrient Konsac solution has begun to have an effect, especially for poorly tolerated varieties, and at 150 ppm there is a sign of death. and up to 600 ppm die a lot. At 1,000 ppm in 20 days, the rice dies as softly as it is boiled water. It is worth noting that the roots are poisoned although the hairs are shed a lot, the roots are atrophied but the color is still white.

4.1.2. The Influence of Fe^{2+} on Young Rice Plants

At Fe^{2+}=150 ppm, the effect has begun to affect and at 600ppm, it is lethal to the less poisonous young rice varieties. However, manifestations in black roots and high mortality rates. Good poison tolerant varieties have a higher tolerance level, but if Fe^{2+} is at 1,000 ppm, the mortality will occur in both rice varieties very quickly. When the rice plant has Fe^{2+} poisoning, it affects the roots (the number of root hairs is reduced) Unlike Al poisoning, iron root poisoning is not white but becomes black, as a result of H_2S has an effect with Fe to form FeS, attaching to the outer layer of the rice roots, Fe^{2+} has decreased but still hinders domestic exchange processes. Moreover, the tips of the roots often become parrots in one piece, while the leaves have many spots similar to the disease of fire injection.

4.1.3. Compare the Effects of Al^{3+} and Fe^{2+} Toxins

At Fe^{2+} = 600ppm, Al^{3+}=135ppm rice was damaged. On the other hand, when the concentration of Fe^{2+}=600 ppm, Al^{3+}=135ppm, the harmful effects become many times greater than each individual toxin. This is evident in all indicators and in turn goes from less poisoned to medium tolerant varieties to fairly toxic ones.

4.2. Effect of Toxic Concentration in Acid Sulphate Environment on Enzyme Activity of Catalase Enzyme and Indicator

The results in (Figure 1) indicate that the activity of this enzyme depends primarily on the growth period: They tend to decrease in both acid sulphate soils and in alum soil; Then came the influence of soil environmental toxins. On the other hand, we see that in acid sulfate environment, enzyme activity of Catalase is always lower than that on non-acid alluvial soil (average data of 8 varieties). This comment is at the confidence level with t=4,936 and $t_{0,99}$= 2,977. This effect is more significant when we compare between alum-resistant varieties like Ca Dung and alum-resistant varieties like IR38. (Figure 1) also with 95% confidence.

While on alluvial soil, the enzyme activity of Enzyme Catalase in IR38 is always higher than that of Ca Dung, whereas in acid sulphate soil, the opposite is true: when toxic concentration increases (days 35 and 102), activity decreases (toxin). in soil, it is higher Al^{3+}=800-900ppm, SO_4^{2-}=0.3-0.35%, pH_{H2O} = 3.5-3.6) (Figure 2).

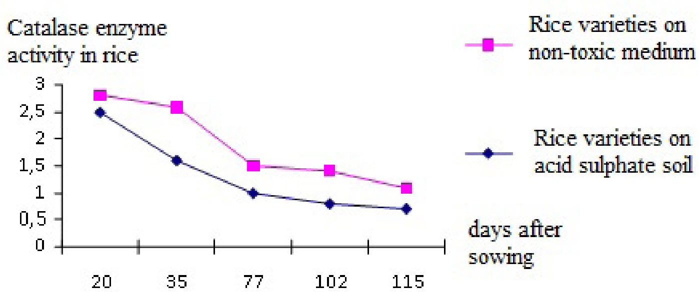


Figure 1: Effects of toxic substances in the soil on the activity enzyme Catalase compares between non-toxic soils and acid sulphate soils.

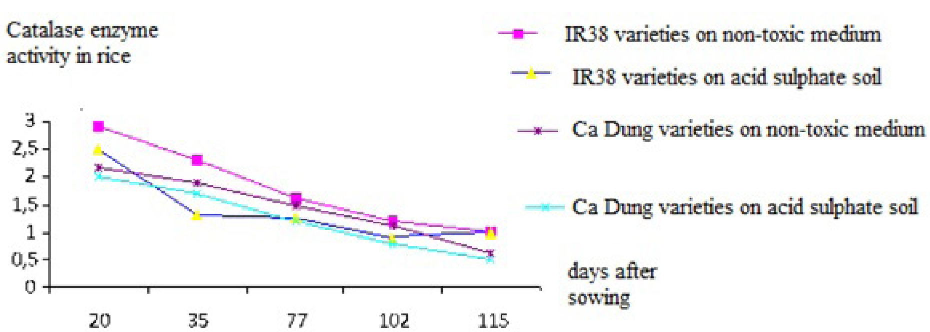


Figure 2: Effect of toxic concentration in acid sulfate environment to enzyme activity of Catalase enzyme in indicator (*Eleocharis dulcis*) and rice plants.

4.3. Effect of Toxic Substances in Acid Sulphate Soil Environment on Enzyme Activity Peroxydase in Indicator Leaves (Eleocharis Dulcis) and Rice Plants

Unlike Catalase, when the toxicity in acid sulphate environment increased (at 21, 60 days after sowing), the activity of the Peroxydase enzyme in rice leaves increased again (Figure 3). This rule is confirmed with high confidence $t= 3,400>t_{0,99}=2,977$.

The study results showed that the activity of the enzyme Peroxydase in the leaves depends on the growth period of the lua: high in the middle stage (60-70 days). On the other hand, it also depends on the characteristics of plants and varieties: the Eleocharis dulcis and Ca Dung alum tolerant varieties are always more active and more clearly expressed against the toxin than the less tolerant varieties, alum IR38.

Compared with the activity in non-alum alluvial soil, when the toxicity increased, Ca Dung variety had this activity increased by + 280% (compared to the control), while the IR38 variety increased only +247% (compared to the control). This was data of Eleocharisdulcis - plant indicator for acid sulfate soil, supplemented and confirmed with absolute confidence (99%). That makes us appreciate, the right role of Peroxydase with alum poison resistance (Table 1).

In contrast, in the case of leaves, the peroxydase enzyme in plant roots living on acid sulfate soil was strongly inhibited of toxins in the soil environment (Figure 4). That is, when the toxicity in the soil increases, this enzyme activity in the roots of the leaves is less resistant to alum and alum tolerance, including in the grass roots. On the other hand, the enzyme activity in the root plays a small role: releasing energy, providing for lua, after being poisoned: for example, on the 87th day when the toxin decreased, this enzyme activity increased by +63% with Ca Dung and +40,1% with IR38.

Thus, according to the above result, the Enoxydasa enzyme in leaves plays a role in promoting Nang and Lua plants to fight against invading toxins. However, this role only appears to be within a certain limit of the level of toxicity in the soil environment and the characteristics of different varieties (Table 2).

Data in Table 2. show that the activity of this enzyme is significant in the limit of toxins in the soil environment:

- For Nang grass indicator plants that can tolerate toxic substances in soil at the highest level with:

  $Al^{3+}\leq 950 \pm 31$ ppm, $Fe^{2+}\leq 907 \pm 44$ ppm, $SO_4^{2-}\leq 3209\pm 48$ ppm.

- For Ca dung varieties, resistant to alum well, at the level:

  $Al^{3+}\leq 890$ ppm, $Fe^{2+}\leq 842$ppm, $SO_4^{2-}\leq 0{,}26\%$

- For poorly resistant alum like IR38, it is only at level:

  $Al^{3+}\leq 750$ ppm, $Fe^{2+}\leq 676$ppm, $SO_4^{2-}\leq 0{,}28\%$.

Correlation between toxins and P2O5 in the soil environment and enzyme activity Peroxydase in plants.

The toxic effect on this enzyme activity and the interaction between them is partly reflected through the correlation coefficient (R).

In the above correlation coefficients, it is worth noting: the positive correlation between this Enxime activity in the leaves of Nang grass and R = +0,8432. At the same time, we also found a positive correlation between the activity of the leaves of Ca Dung alum-tolerant alum with the concentration of Al3 + in the soil environment with R = +0,802. On the other hand, it should be noted the inverse correlation between this enzyme activity in alum-tolerant luu (IR38) with Al3 + content in soil environment with R = -0,859. It is the characteristics of alum-resistant plants different from the alum-resistant plants (Table 3).

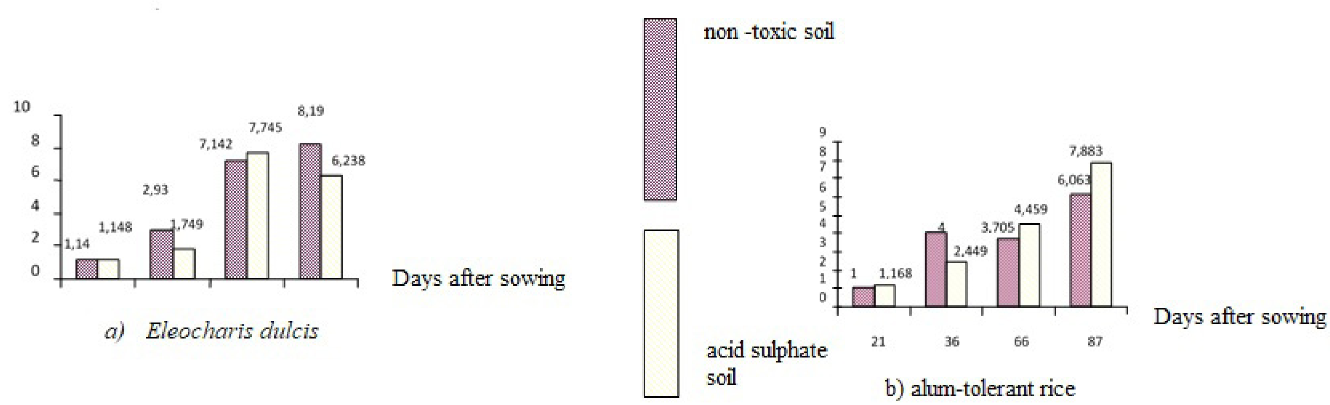
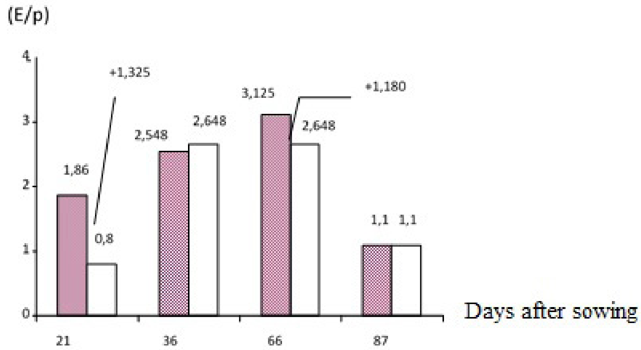
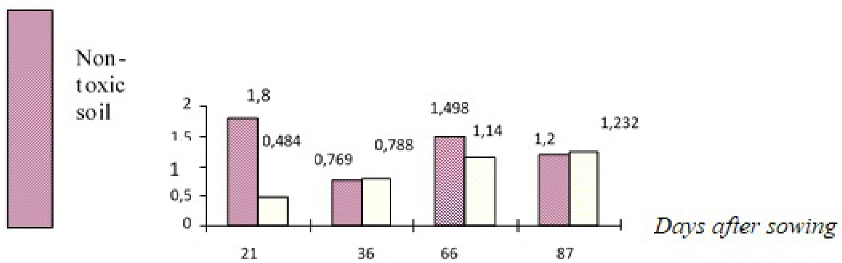


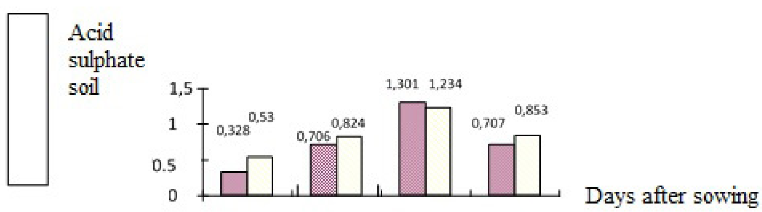
Figure 3: Effect of toxins in soil environment on Peroxydase activity in rice roots and indicator plants



a) *Eleocharis dulcis*



b) Ca Dung on acid sulfate soil



c) IR 38 on acid sulphate soil

Figure 4: Effects of toxins in soil environment on Peroxydase activity in rice roots (Ca Dung, IR38, *Eleocharis dulcis*)

Table 1: Toxic in acid sulphate soil environment

Toxic in alum soil environment	high	low
Al^{3+} (ppm)	805	465
Fe^{2+} (ppm)	898	609
SO_4^{2-} (%)	0.32	0.13

Table 2: Limiting the toxic effect of Enoxydase enzyme in plant leaves to toxic substances in the environment, on the 25th day after planting.

(Enzyme activation unit: E / p)

Total concentration of toxic substances in the environment (ppm)				Plant type		
Variant	Al3+	Fe2+	SO42-	*Eleocharis dulas*	Ca Dung	IR38
1	100 ± 5	135 ± 4	1000 ± 18	1,65 ± 0,07	1,26 ± 0,07	1,10 ± 0,08
2	450 ± 12	467 ± 18	1500 ± 47	2,47 ± 0,08	1,38 ± 0,04	1,15 ± 0,06
3	750 ± 16	676 ± 24	2108 ± 86	3,28 ± 0,10	1,62 ± 0,05	1,25 ± 0,05
4	890 ± 24	842 ± 32	2606 ± 58	3,30 ± 0,04	1,92 ± 0,10	0,62 ± 0,04
5	950 ± 31	907 ± 44	3209 ± 48	3,85 ± 0,14	1,64 ± 0,06	0,13 ± 0,00
6	1085 ± 525	1050 ±58	3693 ± 92	1,67 ± 0,12	0,10 ± 0,0	0,00 ± 0,00
7	Non-toxic soil environment (control)			1,51 ± 0,04	1,45 ± 0,03	1,00 ± 0,05

($t_{0,95}$ = 2,447)

Table 3: Correlation coefficient (R) between the concentration of P_2O_5 toxins in the soil environment and Enxime Peroxydase (M) activity in the plants.

Correlate	M - SO_4^{2-}		M - Fe^{2+}		M - Al^{3+}		M - P_2O_5	
In the tree	leaves	roots	leaves	Roots	Leaves	Root	Leaves	Root
Eleocharis Dulcis	+0,7204	-0,2015	+0,6344	-0,2104	+0,8432	-0,3215	-0,3467	+0,5241
Cà Dung	+0,6843	-0,2254	+0,6278	-0,2447	+0,8023	-0,3254	-0,3670	+0,4632
IR38	+0,3452	-0,4568	+0,4312	-0,5649	+0,6174	-0,8592	-0,2891	+0,3434

Note: M is the activity of Enzyme Peroxidase

$R_{0,95}$ = 0,754

4. 4. Toxic Effect in Acid Sulphate Soil Environment on Enzyme Phosphatase Activity in Indicator and Rice Plants

Influence Phosphatase in indicator leaves and rice plants.

Toxicity in the acid sulphate environment not only limits the activity of enzyme Catalaza, Peroxydase in the roots, but also limits the activity of Phophatase enzyme in the leaves of the indicator plants and the lua. This is shown when the toxicity increased twice, this enzyme activity decreased at -21,2% and -53,6%, with Ca Dung variety; -40,7% and -69,6% for the IR38 variety, while for the Nang grass indicator plants, there is a decrease of -45% and -18%.

Equally interesting is the discovery of the role of Peroxydase in the leaves, where this Phophatase enzyme plays an important role in helping the plant recover after poisoning.

It is confirmed that this rule depends on the type and variety characteristics. They become significant in the case of Ca Dung alum and indicator plants. For example, on day 87 when the toxicity in the soil decreased from low to high, this activity in the grass indicator increased by +59,9% and especially in alum-tolerant plants Ca dung increased by +643%, while the same was tolerated. alum also increased but lower, at only +314% compared to the control. Another feature to note is that this activity also depends on the growth time of the later stages than the previous period.

Affects the Phophatase in rice roots

Unlike the leaves, the enzyme Phophatase in roots has very weak activity (Table 4). A comparison between the two varieties shows that this enzyme activity in the roots of Nang grass is a poison-tolerant adaptive wild plant that has a greater activity than alum-tolerant lua (Ca Dung). Alum tolerant lua root is more active than alum-resistant lua (IR38).

This is manifested in the inequality:

M(Eleocharis Dulcis) > M(Ca Dung) >M(IR38)

(M: Phosphatase enzyme activity in roots)

The data in (Table 5) show that there is a negative and tight correlation between the concentration of Al^{3+} toxin in the soil and the Phophatase activity in the leaves, especially for the alum tolerant variety less than IR38. This proves that plants have mobilized Peroxydase into the body to support the vitality of trees. On the other hand, Phosphatase is inversely and tightly correlated with the amount of Al^{3+} in plants. This proves that there is an inverse and opposite reaction that inhibits each other between the toxin and Perory dase and phosphatase. Particularly noteworthy is the correlation between this enzyme activity and the accumulation of free phosphorus of Ca Dung alum tolerant variety (R =+0,6441), with 99% confidence.

That allows us to deduce that phosphorus and phophatase play a large role in the mechanism of plant poison resistance to toxins in the soil environment.

(***: Eleocharis dulcis, A: phosphatase activity)

Table 4: The influence of toxic substances in acid sulphate soil environment on the enzyme activity Phophatase in the roots indicates acid sulphate-resistant and acid-resistant rice plants.

The day after sowing	Toxic in soil environment	Acid sulphate soil			Non toxic soil		
		Eleocharis Dulcis	Type of rice plants		*Eleocharis Dulcis*	Type of rice plants	
			Cà Dung	IR38		Cà Dung	IR38
21	high	1.29	1.29	0.96	0.14	3.5	2.21
36	low	0.42	0.34	small	0.42	0.3	0.41
66	high	small	small	small	Streaks	Streaks	Streaks
87	low	small	small	small	Streaks	Streaks	Streaks

Table 5: Correlation (R) between toxic concentration in soil environment and activity (A) of Phosphatase enzyme in alum-resistant and alum-resistant plants.

Plants	A - SO_4^{2-}	A -Fe^{2+}	A - Al^{3+}	A - P_2O_5
Cà Dung	-0.3648	-0.5601	-0.6462 ***	+0.6441***
IR38	-0.5001	-0.6239	-0.8972	0.4427

5. Conclusion

Thus, based on the results of experiments of many years of research from 1979 up to now, it is possible to come to the conclusion:

- High concentrations of toxins in the soil environment strongly inhibited the activity of Phosphatase enzymes in the roots, in the indicator leaves, and especially for rice, especially for plants that are less resistant to alum.
- Enzime Phosphatase in leaves has the effect of providing energy for the plants to recover from poisoning. The activity of this enzyme is inversely correlated with the content of Al3 + in soil and Al3 + in plants. This proves that the antagonism between enzymes and toxins is evident, especially with Al3 + toxins.
- Highly toxic in alum soil environment inhibits enzyme activity Peroxydase in rice roots. Under these conditions, peroxydase in the root plays a small role in helping the plant recover.
- We have sufficient basis to confirm that the enzyme Enoxydase in leaves plays an important role, against the harmful effects of toxins. When the toxin in the soil, in plants increased activity of this enzyme also increased higher than the control.

When the toxicity increases, the Peroxydase in the alum tree leaves will also increase +280% (compared to the control); while the alum-resistant plants did not increase (compared to the control).

When the toxicity decreased, Peroxydase in alum-resistant plants was also higher than alum-resistant: +643% for alum-resistant rice compared to the control. The poorly resistant alum variety only increased +314% (compared to the control).

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