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Toxicity of Rock Meal Based Fertilizers Towards Duckweed (*Lemna minor*)

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Abstract

The rock meal based fertilizers were evaluated for their toxicity towards duckweed (*Lemna minor*). *Lemna minor* is a standard ecotoxicological object for testing aquatic plant toxicity of chemicals including pesticides, biocides, heavy metals and variable industrial substances and mixtures. In recent years so called rock meal based fertilizers gain popularity in organic and standard agriculture. They prove to be effective, easy to produce and available for farmers. Some of them can express additional induced system resistance activity or even direct fungicidal or insecticidal action. Although fertilizers are considered to be safe for most the aquatic organisms, they can be toxic under certain circumstances, especially due to the fact they are used often in large amounts nearby water basins. The conducted research reveals that in registered concentrations, all of the tested products do not cause any harmful effects on *lemna minor*. Some of them were completely safe for the plants even in 7-fold increasing concentrations. However, some of the tested fertilizers were founded to be more toxic for duckweed in much less increasing of their concentrations.

1. Introduction

Duckweed (*Lemna minor*) is a standard ecotoxicological object for testing aquatic plant toxicity of chemicals including pesticides, biocides, heavy metals and variable industrial substances and mixtures. The plant is worldwide spread, and can be found in almost every climate except entirely waterless deserts and perhaps the tundra. Have a voracious grower that can double its mass within a week under optimum growing conditions (**Landolt, 1975; Hillman and Culley, 1978; Axtell *et al.*, 2003**). The plant express and excellent ability for bioremoval of heavy metals from aquatic ecosystems (**Rahmani and Sternberg, 1999; Khellaf and Zerdaoui, 2009; Bokhari *et al.*, 2016**). Duckweed has shown strong potential for the phytoremediation of organic pollutants, heavy metals, agrochemicals, pharmaceuticals and personal care products, radioactive waste, nanomaterials, petroleum hydrocarbons, dyes, toxins, and related pollutants (**Ekperusi *et al.*, 2019**). *Lemna minor* can have numerous applications as medicinal herba as antipruritic, antiscorbutic, astringent, depurative, diuretic, febrifuge andsoporific. It was also used in the treatment of colds, measles, oedema and difficulty in urination. Duckweed formulations were used in the treatment of rheumatism, liver diseases, and thyroid diseases. As external application, duckweed formulations were used in the treatment of abscesses, chronic wounds, and furuncles In China, the herb was used internally for body temperature regulation (reduce high fever) and swellings (edema). Externally it was applied as a remedy for various skin ailments, such as rash, eczema, measles, and insect bites (**GÜLÇİN *et al.*, 2010; Al-Snafi, 2019; González-Renteria *et al.*, 2020**). Duckweed can be used even in bioassay in allelopathy as remedy (**Einhellig *et al.*, 1985**). The plant is a typical test organism for measurement of the effects of fertilizers on aquatic ecosystems (**Bekcan *et al.*, 2009; Radić *et al.*, 2013**). and industrial wastewater (**Singh and Singh, 2006**). Duckweed is also excellent biofilter of nitrogen and phosphate in fish ponds (**Ferdoushi *et al.*, 2008**). There is a potential use of *Lemna minor* for the phytoremediation of isoproturon and glyphosate (**Dosnon-Olette *et al.*, 2011**). The plant is a classical test oblect for bioassays for contamination of aquatic systems with pesticides and biocides too (**Frankart *et al.*, 2002; Frankart *et al.*, 2003; Munkegaard *et al.*, 2008; Tagun and Boxall, 2018**). During recent years so called rock meal based fertilizers gain a popularity in the organic and standard agriculture. They prove to be effective, easy for production and available for farmers (**Hendronursito *et al.*, 2019; Leonardos *et al.*, 2000; Burbano *et al.*, 2022**). Even more, some of them were proved to express additional ISR (Induced System Resistance) activity in the plants (**Reuveni and Reuveni, 1998; Valchev and Valcheva, 2019; Kovacevik and Mitrev, 2019**) or direct pesticide action both fungicidal and insecticidal (**Ganchev, 2021; Ganchev, 2022**). Some of tested fertilizers in this research contain copper which was found to be toxic to *Lemna sp* due to inhibition of photosynthetic activity (**Megateli *et al.*, 2009; Oros, 2013; Perreault *et al.*, 2014**). Some studies also found that even a wood ash used as fertilizer can be toxic towards *Lemna minor* due to the elemental composition of the ash, its alkaline character, and possible interactions between these two properties (**Jagodzinski et al., 2018**).

2. Materials and methods

A strain of *Lemna minor* fronds naturally inhabited river "Nevolja" in village of Vasil Levski, Plovdiv district, Bulgaria,

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were obtained for testing and were grown in the same river water in laboratory conditions. Plastic cups from chemically inert material with 200 ml volume were used. In each cup 150 ml of tested fertilizer in the given concentration was placed. Each test variant consisted of 5 plastic cups with duckweed (replicates). In all variants only distilled water was used and in each cup colonies consisting of 2 to 4 visible fronds were transferred from the inoculum culture and randomly assigned to the test vessels under aseptic conditions. The number of fronds and colonies was the same in each test vessel. The test was terminated 7 days after the plants are transferred into the test vessels and frond numbers appearing normal or abnormal were determined every 3 days from the beginning of the test. The control variant consisted only with pure distilled water. Changes in plant development, e.g. in frond size, appearance, an indication of necrosis, chlorosis or gibbosity, colony break-up or loss of buoyancy, and in root length and appearance were visually observed. For each test fertilizer, lethal concentrations in which: 5 %, 25 % and 50 % phytoxocity symptoms appeared were determined. Mathematical manipulations of received test data were performed with R language for Statistical Computing (**Team, R. C, 2000**), drc packge (**Ritz *et al.,* 2016**).
In the current research, several inorganic / organic rock meal based fertilizers with ISR activity and complex content were tested produced by Panamin CO Ltd.:

- Panamin Suspension – with content of inorganic chemicals: $CaCO_3$ – 38.11 %; $MgCO_3$ – 4.76 %; SiO_2 – 16.8 %; K_2O – 0.91 %; P_2O_5 – 0.01 % and Fe_2O_3 – 0.52 %. pH = 8.8
- Panatop Immuno Active - with content of inorganic chemicals: CaO – 16.5 % and MgO – 10.2 %. pH = 6.8
- Panatop Immuno Active Plus with content of inorganic chemicals: CaO – 13%, MgO – 8 % and Cu – 5.5 %. pH = 6.8
- Panatop Immuno Safe - with content of inorganic chemicals: K_2O – 13.2 %; SiO_2 – 26.4 % and Cu – 0.4 %. pH = 12.1
- Panatop Fulvic Max - with content of inorganic and organic chemicals: potassium fulvate – 23 %; fulvic acids – 11.5 %; humic acids – 9.2 %; K2O – 2.3 %; amino acids – 3.5 % and algae extract – 5.8 %, Fe – 0.24 %; Mn – 0.12 %; Zn – 0.08 %; B – 0.08 %; Cu – 0.03 % and Mo – 0.01 %. pH = 7.5
- Panatop Alga Max - with content of inorganic and organic chemicals: seaweed extract – 53.5 %, amino acids – 10.07 %. Microelements: Fe – 0.14 %; Mn – 0.06 %; B – 0.04 %; Zn – 0.04 %, Cu – 0.02 % and Mo – 0.004 %. pH = 4.8
- Panatop Fulvic Start - with content of inorganic and organic chemicals: fulvic and humic acids – 2.32%, P_2O_5 – 5.8 % and l-amino acids – 5.8 %. Microelements: Zn – 0.58 %, Mn – 0.58 %, Cu – 0.2 3% and Mo – 0.06 %. pH = 6.5
- Panatop Fulvic D – with of inorganic and organic chemicals: $CaCO_3$ – 31.28 %; $MgCO_3$ – 20.71%; fulvic acids – 2.36 %; humic acids – 2.36 %; K_2O – 1.65 %. Microelements: B – 0.1 %; Cu – 0.03%; Fe – 0.26 %; Mn – 0.14 %; Zn – 0.08 % and Mo – 0.01 %. pH=8
- Panatop Amino D – with of inorganic and organic chemicals: l-amino acids –22.8 %; $CaCO_3$ –3.7%; $MgCO_3$ –2.5 %. Microelements: B – 0.02 %; Cu – 0.01 %; Fe – 0.07 %; Mn – 0.03 %; Zn – 0.02 % and Mo – 0.02 %. pH = 6.3
- Panatop Protect DSC – with of inorganic and organic chemicals: $CaCO_3$ – 29 %; $MgCO_3$ – 10.31%; SO_3 – 11.86 % and Cu – 12.15 %. pH = 7.7

3. Results

In the figure below (Figure.1) are presented results obtained from test with: Panamin Suspension, Panatop Imunno Active, Panatop Imunno Active Plus and Panatop Imunno Save

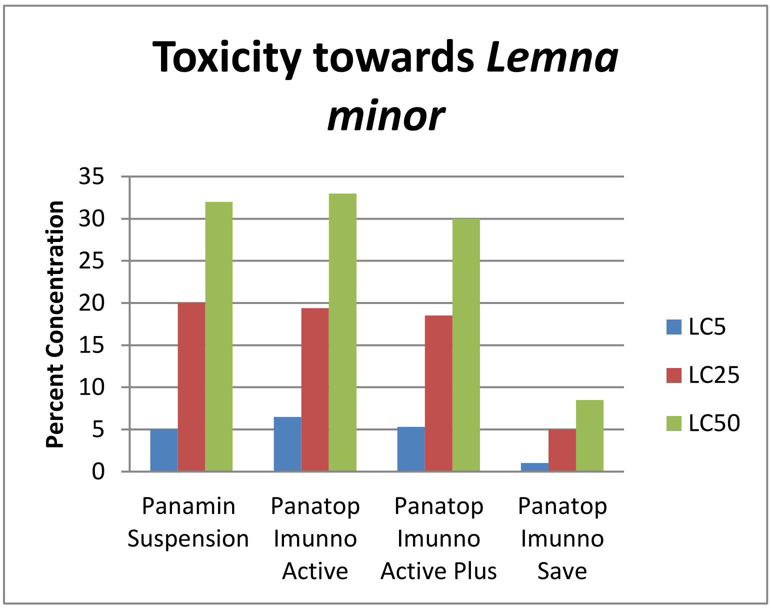

Figure 1 Toxicity of Panamin Suspension, Panatop Imunno Active, Panatop Imunno Avtive Plus and Panatop Imunno Safe towards duckweed (*Lemna minor*).

Panamin Suspension, Panatop Imunno Active and Panatop Imunno Active Plus expressed almost the same (very low) toxicity towards duckweed, while Panatop Imunno Safe was much more toxic.

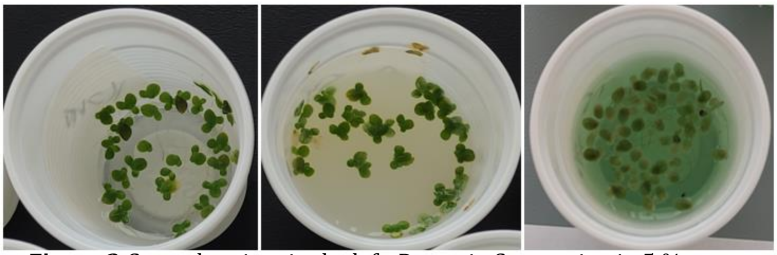

Figure 2 Control variant in the left; Panamin Suspension in 5 % concentration in the middle. Panatop Imunno Save in 8 % in the right.

The next figure (Figure.3) present toxicity of fertilizers: Panatop Fulvic Max, Panatop Alga Max, Panatop Fulvic Start**,** Panatop Fulvic D, Panatop Amino D and Panatop Protect DSC towards *Lemna minor.*

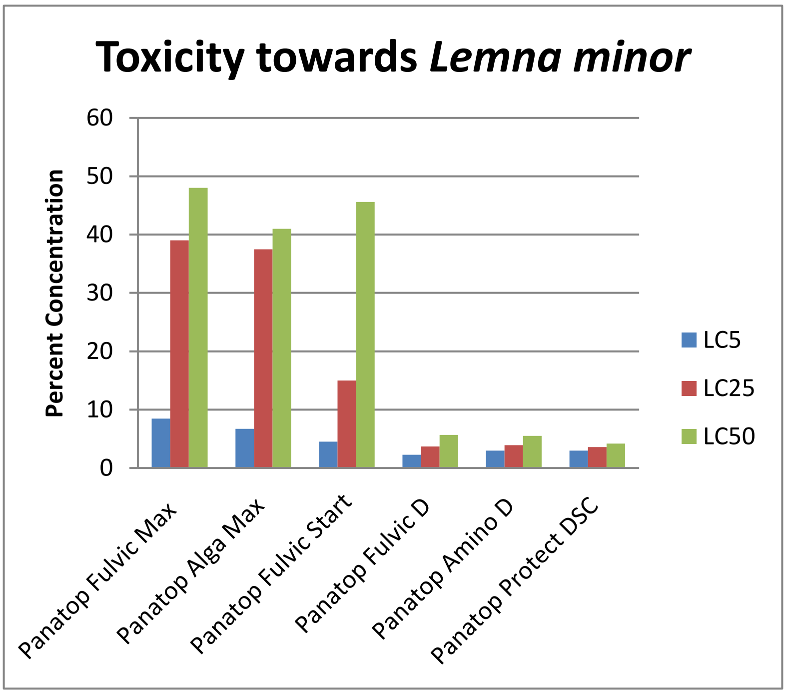

Figure 3 Toxicity of Panatop Fulvic Max, Panatop Alga Max, Panatop Fulvic Start, Panatop Fulvic D, Panatop Amino D and Panatop Protect DSC towards duckweed (*Lemna minor*).

Panatop Fulvic Max, Panatop Alga Max, Panatop Fulvic Start just like Panamin Suspension, Panatop Imunno Active and Panatop Imunno Active Plus show very low, similar toxicity towards *Lemna minor*. However Panatop Fulvic D, Panatop Amino D and Panatop Protect DSC have expressed much higher toxicity.

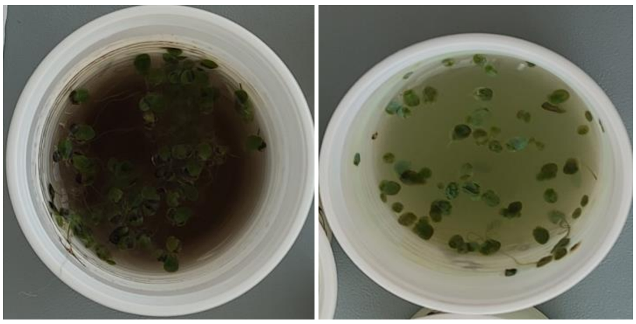

Figure 4 Panatop Amino D in 3 % concentration in the left and Panatop Protect DSC in 4 % concentration in right.

4. Discussion

The received results show the extremely low toxicity of tested fertilizers: Panamin Suspension, Panatop Imunno Active and Panatop Imunno Active Plus towards *Lemna minor*. Established LC50 was approximately 30 % and up to 5 % concentration they do not cause any phytotoxic damage on plants, although, pH of Panamin Suspension is 8.8 and Panatop Immuno Active Plus contain copper at 5.5 %. However, Panatop Imunno Save was far more toxic with established LC50 = 8.5 %. Although this product contains much less copper than Panatop Imunno Active Plus (only 0.4 %), has pH = 12.1 which causes this increased toxic effect. Panatop Imunno Save is registered to be used at 0.5 – 1 % concentrations. In 0.5 % concentration, no phytotoxic symptoms were observed on tested plants. In 1 % concentration 5 % mortality on lemna minor was found. From the Figure 2, can see the low toxicity of Panatop Fulvic Max, Panatop Alga Max and Panatop Fulvic Start. The established LC50 = 41- 48 % were even higher than Panamin Suspension, Panatop Imunno Active and Panatop Imunno Active Plus. Although, pH of Panatop Alga Max was relatively low = 4.8, the product does not cause any phytotoxic damage to plants even at 5.5 % concentration. Panatop Fulvic Max was absolutely safe for Lemna minor even at 7.8 % concentration. However, the tested fertilizers: Panatop Fulvic D, Panatop Amino D and Panatop Protect DSC showed significantly increased toxicity with the highest values observed in Panatop Protect DSC which has LC50 towards Lemna minor = 4.2 %. However, the registered concentrations of the fertilizers are 0.2 – 0.3 % and up to 2.5 % concentration no phytotoxic damage on plants were established. The other to products: Panatop Fulvic D and Panatop Amino D have similar toxicity although their similar content to Panatop Fulvic Max, Panatop Alga Max and Panatop Fulvic Start and pH = 6.3 – 7. These three products however contain CaCO3 and MgCO3 unlike Panatop Fulvic Max, Panatop Alga Max and Panatop Fulvic Start. From all tested products, Panatop Protect DSC has the highest copper content = 12.15 %, although pH is neutral (7.7). Even in this case Panatop Protect DSC express lower toxicity towards *lemna minor* than Panatop Imunno Save, despite that other researches shows copper inhibited *Lemna minor* growth at concentrations equal or higher than 0.00003 % concentration **(Kellaf and Zardoui, 2010)** and at 0.00048 % concentration, the spots from the fronds became green brownish and at the higher concentrations intense and extended chlorosis appears, the plants are white color with reddish spots **(Oros and Tudoran, 2004)**. The major content of Panatop Amino D is l-amino acids (22.8 %). The product was more toxic than others, tested in this study, but it still can be classify as low toxic (LC50 = 55000 ppm) although amino acids (canaline-urea cycle amino acids) were found to be extremely toxic to duckweed at a concentration of 5 μM **(Rosenthal et al., 1975)**. Other studies which evaluate toxicity of 55 non-protein amino acids, in terms of their effect on growth of *Lemna minor* reveal a wide diversity in their growth-inhibiting ability. Replacement of the sulphur atom by selenium in protein amino acids and certain fluoro-substitution was toxic. Addition of a methyl or phenyl group to the carbon skeleton of an individual protein amino acid did not affect *Lemna minor* growth **(Gulati et al., 1981)**. Panatop Alga Max has the major content seaweed extract (53.5 %) and was established to be one of the less toxic in the current research towards duckweed although, some studies reveal that seaweeds extracts can toxic to the plant **(Manilal et al., 2011)**. Although, different studies established that humic acid can reduce toxicity of chemicals towards *Lemna minor* **(Vinitnantharat et al., 2008; Castro et al., 2018; Lakshmikanthan and Chandrasekaran, 2022)**. Panatop Fulvic D with 2.36 % content of humic acids shows significantly higher toxicity towards duckweed in compassion to the other product with no humic acids content. However Panatop Fulvic Max with higher humic acids content from all tested products (9.2%) and Panatop Fulvic Start were with extremely low toxicity towards *Lemna minor*.

5. Conclusion

Conducted trials show that all of the tested fertilizes do not cause any damage on *lemna minor* in their registered concentrations. Panamin Suspension, Panatop Imunno Active, Panatop Imunno Active Plus, Panatop Fulvic Max, Panatop Alga Max and Panatop Fulvic Start shows extremely low toxicity towards tested plants. However, other fertilizers such as Panatop Fulvic D and Panatop Amino D although their similar to Panatop Fulvic Max, Panatop Alga Max and Panatop Fulvic Start and the presence of fulvic acids, humic acids and l-amino acids express significantly higher toxicity. The main difference in their content is the presence of $CaCO_3$ and $MgCO_3$, which chemicals in combination with fulvic, humic amd L-amino acids probably cause increased toxicity of the products towards the tested plants. Expectantly the fertilizer with the highest copper content was one of the most toxic towards *Lemna minor*. However the most toxic product was Panatop Imunno Save which although has much lower copper content (0.4 %) was with the highest pH level = 12,1.

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Declaration of interest
The author report no conflicts of interest.

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