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Reduction of Imidacloprid and Tebuconazole in *Oryza Sativa* Plantation Applying Strains of *Trichoderma spp*.

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The objective of this research was to assess the reduction of imidacloprid and tebuconazole in soil plantations of *Oryza sativa* applying strains of *Trichoderma* spp. Regarding the methodology, 3 treatments were used; strain 1 (TS1), 2 (T163+TS1) and 3 (T163), 19 pots of 15 x 15 cm with 3.5 kg soil were used. One pot was used for the control group (without treatment) and eighteen pots were used for experimental groups (6 pots for each strain). 5 seeds *Oryza sativa* of Esperanza variety (MQR78) were planted in each pot on 2 cm to the soil surface, irrigation was carried out using drench technique every 24 hours for 90 days. For *Trichoderma* spp inoculation it was added 40 ml of strain 1, strain 2 and strain 3 in 18 pots with *Oryza sativa* seeds, identifying that the root measured 2 centimeters (6 pots for each strain). Likewise, the fungus strains were inoculated after 45 days to nourish the soil and a soil sample was taken after 45 days to identify the absorption of tebuconazole (fungicide) and imidacloprid (insecticide), finally the height of the plant (cm) was measured in each experimental strain, the plant was measured from the ground level to the tip of the longest leaf. The results showed that strain 1 reduced 12.39 mg / kg of imidacloprid and 17.31 mg / kg of tebuconazole. Regarding the percentage of decrease efficiency, it was concluded that strain 1 is more efficient with 64.2% for imidacloprid and 64.5% for tebuconazole and finally, strain 3 showed greater height of the *Oryza sativa* plant with 79.8 cm.

1. Introduction

Increase temperature caused by climate change and anthropogenic activities provide uncertainty to agriculture, likewise in absence of nutrients, the use of chemical products, floods, droughts, soil salinity, among others, affect food security. (Shobhit et al., 2017). Plants are surrounded by microorganisms, within them we find bacteria, fungi, oomycetes, nematodes, insects and viruses, these organisms can have a negative impact on the plant, destroying up to 30% of the crops through diseases and decomposition processes. (Simon et al., 2019). Others may have beneficial effects by improving the general fitness of the plant and eliminating diseases. (Vos et al., 2015). However, biological control provides a safe alternative for the monitoring of pathogens, due to its capacity as biocontrol which is helpful for the ecosystem, and they are considered potential agents in fungal diseases, because of they can interact directly with the roots, increasing plant growth, resistance to disease and tolerance to stress. (Silva et al., 2019).
The growth of rice is given by the combination of the genotype and the environment, which allow to improve the soil fertility and its yield; however, the indiscriminate use of fertilizers and agrochemicals decrease production and deterioration of soil health; likewise, indiscriminate use of fertilizers and agrochemicals cause a decrease in production and a degradation of soil health; Therefore, new alternatives must be sought to improve fertility and stimulate its growth. It was researched that microbes are an essential element to maintain the quality of the soil, increasing its production and growth. (Doni et al., 2014, Darmawan et al., 2018, Sabki et al., 2019).

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The Trichoderma spp species are faulty filamentous fungus belonging to the Hypocreales order in the Ascomycete division, it is one of the most widely researched genera with several applications in agriculture, industry and in the environment, several species have the ability for decrease diseases, stimulate growth and productivity in plants. (Lee et al., 2016, Hyder et al., 2017).

They release protein compounds which are similar to auxins thus stimulating the growth and development plants (Garnica et al., 2015; Contreras et al., 2016; Sachdev., Singh and Singh, 2018), as well as the quality of grape production (Pascale et al., 2017) wheat (Allen et al., 2017), soil improvement (Md et al., 2017, Shuangchen et al., 2020), and absorption of chemical agents from the soil (Zhenshuo et al., 2019).

Some researches about the use of *Trichoderma* spp strains show it increases the speed of germination, allows the decrease of spots on seeds which produce incidences to control weeds, likewise the results allow to have a clearer idea about its importance and the effect of strains on growth rice plants, therefore it is considered applicable in agricultural production (Núñez and Pavone., 2014). Another study shows the need to identify the potential of the fungus for synthesizing metabolites, being a barrier strain for filtered agents which harm the plant. The results of the study concluded it improves each process from planting to growth and development, therefore it is being considered a sustainable procedure (Ortuño et al., 2013). Thus, they play an important role in ecology by participating in the decomposition of organic waste, as well as in biodegradation of artificial chemicals and the bioaccumulation of high amounts of several metals from wastewater and soil. (Nongmaithem and Bhattacharya, 2016); therefore, it is important to study this genus in order to provide alternative solutions for good rice production and absorption of agrochemicals. Finally, the objective was to evaluate the decrease of imidacloprid and tebuconazole in soil plantations of *Oryza sativa* applying strains of *Trichoderma* spp.

2. Methods

2.1 Preparation of growing medium and growth of *Trichoderma* spp strains

The strains were provided by the Microbiology Laboratory of the Peruvian Amazon Research Institute, they were stored in vials at a temperature below 12 ° C for 24 hours; strain 1 (TS1), strain 2 (TS1 + T163) and strain 3 (T163). CMA growing medium (Merck) was prepared by adding 2 g of penicillin to the medium to sterilize its content, 50 ml of medium was poured into 3 petri plates of 35 x 10 mm, and it was dried at 37 ° C for 30 minutes, then a fraction of each strain was extracted from the vials, placing them in the center of the petri plate, it was sealed with parafilm to prevent its contamination and growth for 5 days (3 without light and 2 with light) at 35 ° C. The entire procedure was carried out in a laminar flow chamber (PB-48-115V) which meets the biosafety requirements.

2.2 Preparation of enriched medium for *Trichoderma* spp strains

The Peruvian Amazon Research Institute provided 1500 ml enriched medium of sugar cane molasses which was prepared by 45g of molasses and 7.5g of yeast. To sterilize the medium, we proceeded to selfclaved (BRAND) for 25 minutes, 121 ° C and 15atm, then it was allowed to cool for 3 hours, dividing the medium in 3 glass containers with 500 ml each of them for adding a spore suspension of strain 1, strain 2 and strain 3 of *Trichoderma* spp with 3 ml (1x106 spores ml-1), to homogenize and allow their multiplication the digital stirrer (MIU-GS-30) was used for 4 days during 24 hours and 35 ° C, leaving it ready for being inoculated on the soil. (Sachdev et al., 2018)

2.3 Soil and seed experimental conditions of *Oryza sativa*

The experiments were performed in an unheated polyethylene greenhouse at the César Vallejo University in the Cacatachi district, San Martín Region, at 295 meters above sea level and 12 km to the north of Tarapoto (6°29'40 " south latitude and 76°27'57" west longitude), 19 pots of 15 x 15 cm with 3.5 kg of contaminated soil with 19.28mg / kg Imidacloprid and 26.81mg / kg Tebuconazole were used, one pot was used for control group (without treatment) and 18 for experimental groups (6 pots for each strain), the soil was clay loam (74% clay, 18% silt and 8% sand), with 6.7 pH, electric conductivity 0.6 mS cm-1 and matter organic 1.35%.

The seeds of *Oryza sativa* Esperanza variety (MQR78) were superficially sterilized with 75% ethanol, followed by 20% sodium hypochlorite, then they were washed with sterilized distilled water. (Doni et al., 2014, Lee et al., 2016). 5 seeds of *Oryza sativa* were planted in each pot on 2 cm to the soil surface being autoclaved in greenhouse conditions at a temperature of 31 ± 4 ° C, 318 ± 3 µmol of light intensity, 80 ± 3% humidity and 11 h 15 m 17 s ± 9 s photoperiod, irrigation was carried out using drench technique with 50 ml of water every 24 hours for 90 days.

2.4 *Trichoderma* spp Inoculation and *Oryza sativa* growth

40 ml of strain 1, strain 2 and strain 3 of *Trichoderma* spp were added in 18 pots with *Oryza sativa* seeds, identifying that root measured 2 cm (6 pots for each strain), likewise, the fungus strains were inoculated after 45 days to nourish the soil. After 90 days a soil sample was taken to identify the absorption of tebuconazole (fungicide) and imidacloprid (insecticide) through the test analysis method of multi-residues of pesticides by QUECHERS in fruits and vegetables, juices, wines and food with high fat content using GC - MS and LC-MS / MS. (Stachniuk., 2019). Finally, the height of the plant (cm) was measured in each experimental strain, measuring from the ground level to the tip of the longest leaf.

2.5 Determination adsorption efficiency of the experimental group

The percentage difference formula was used to determine the percentage of efficiency, considering the control group and the experimental group as it is detailed below:

$$\text{Adsorption efficiency (\%)} = \left[\frac{CGC-CGE}{CGC}\right] \times 100 \qquad (1)$$

Where:
CGC: Control group concentration
CGE: Experimental group concentration

3. Results and discussion

3.1 Analysis of results in *Trichoderma* spp strain in soil

Figure 1a shows the data obtained from the control group by the analysis performed on the soil of the *Oryza sativa* plantations with 19.28 mg / kg of imidacloprid and 26.81mg / kg of tebuconazole before the treatment; in the experimental group the *Trichoderma* spp strains were applied, finding in strain 1 (TS1) 6.89 mg / kg of imidacloprid and 9.5 mg / kg of tebuconazole, in strain 2 (T163 + TSI) 9.67 mg / kg of imidacloprid and 13.78 mg / kg of tebuconazole and finally in strain 3 (T163) 10.34 mg / kg of imidacloprid and 18.6 mg / kg of tebuconazole; it is important to mention that *Trichoderma* spp strains play a very important role to achieve the production efficiency, therefore people must know their function in decomposing organic matter, capacity of the circulation of nutrients, decrease of chemical load by agro-fertilizers in the soil, therefore this organism would be beneficial for making the agriculture more sustainable. (Colla et al., 2017).

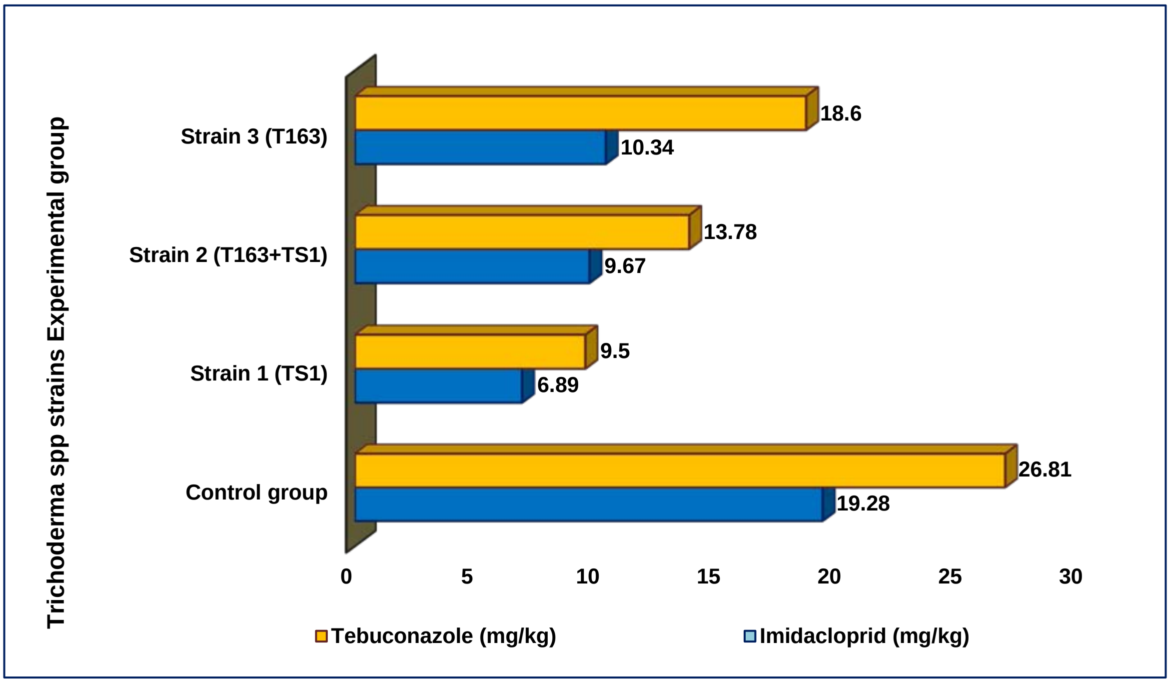

Figure 1.a: Tricoderma spp strains versus Imidacloprid and Tebuconazole control group (mg/kg)

Figure 1b shows the percentage of decrease imidacloprid and tebuconazole in *Oryza sativa* plantations, finding in strain 1 (TS1) 64.2% of imidacloprid and 64.5% in tebuconazole, strain 2 (T163 + TS1) 48.6% of tebuconazole and 64.5% in imidacloprid and finally in strain 3 (T163) 30.6% of tebuconazole and 46.3% of imidacloprid. It is important to improve the sowing processes by applying new biological control techniques that show their growth and better harvest, considering a sustainable procedure will not only help in production, but also in the recovery of contaminated soils. (Fiorentio et al., 2018).

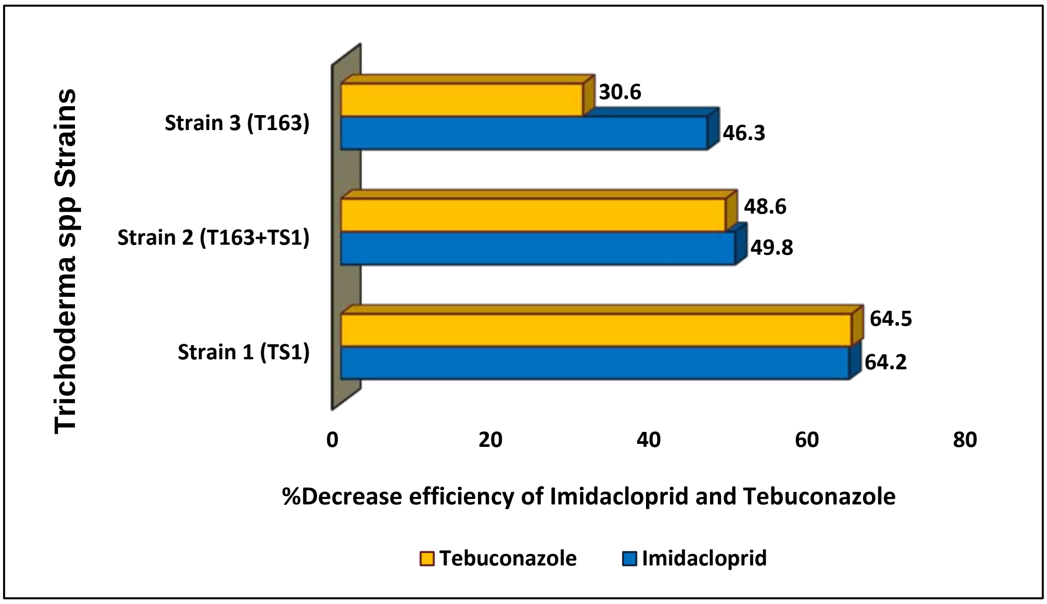

Figure 1.b: Percentage of decrease efficiency of Imidacloprid and Tebuconazole with Trichoderma spp. Strains.

3.2 Analysis of results in *Oryza sativa* plant

Figure 2 shows the control group (without treatment) where the height of *Oryza sativa* plant was 51.3 cm, in strain 1 (TS1) 61.8 cm, strain 2 (T162 + TS1) 69.5 cm and in the strain 3 (T163) 79.8 cm. It is necessary to look for alternatives which improve the growth of the plant, the microbes are resistant to the pathogens therefore they are an ecological way to maintain a clean ecosystem without pollutants; likewise, it is shown that fungi are growth promoters due to their ability to produce siderophores and phytohormones. (Doni et al., 2014)

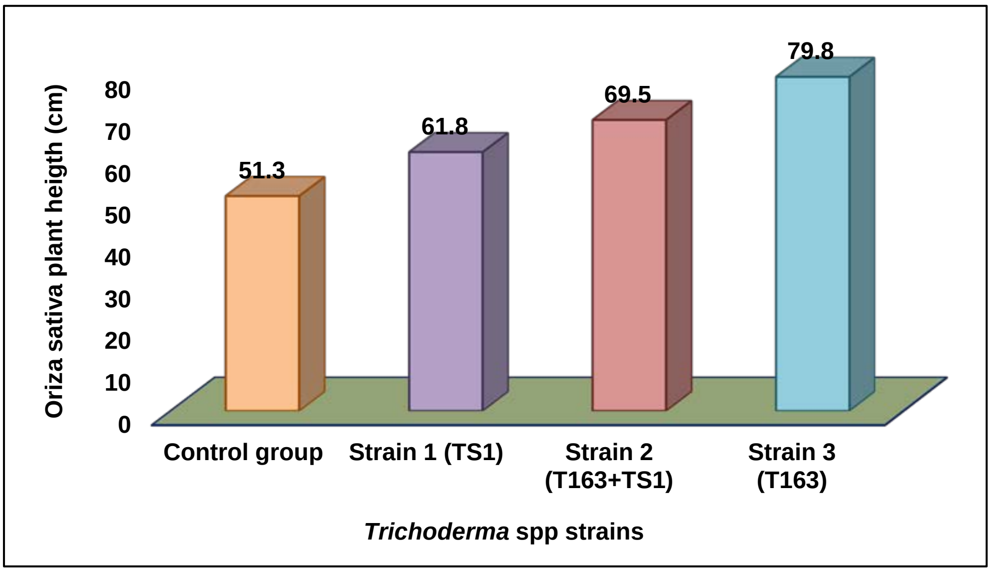

Figure 2: Oryza sativa plant height before and after applying Trichoderma sp strains

a) Increase in strains of Trichoderma spp. b) Nursery construction and implementation. c) Contamination with agrochemicals to the substrate. d) Soil sample for corresponding analysis. e) Cane molasses culture medium with inoculated fungi. f) Incorporating microorganisms into the pots. g) Presence of treatment. h) Evenly watering the pots with rice.

Figure 3: Photographic summary

4. Conclusions

It is evidenced that strain 1 (TS1) had a higher percentage of decrease efficiency of imidacloprid and tebuconazole in *Oryza sativa* plantations, strain 3 (T163) provides plant growth compared to the control group, reaching an approximately height of 79.8 cm. Therefore, *Trichoderma* spp strains are an alternative to be used as a bioremediation technique of pesticide-laden soils, thus the productivity of crops would be improved in a sustainable way and the contamination decrease in some way.

References

Allen G.X., Wei G.,Yuanhong C., Iffat S., Genevieve M., Jinghui L., Changzhong R., 2017, Effect of seed treatment with novel strains of Trichoderma spp. on establishment and yield of spring wheat, Crop protection, 96, 97-102

Colla G., Hoagland L., Ruzzi M., Cardarelli M., Bonini P., Canaguier R., (2017), Biostimulant action of protein hydrolysates: unraveling their effects on plant physiology and microbiome. Front. Plant Sci, 8, 2202.

Contreras H.A., Macias L., Val E., Larsen J., 2016, Ecological functions of Trichoderma spp. and their secondary metabolites in the rhizosphere: interactions with plants. FEMS Microbiolgy Ecology.

Darmawan A., Biddinika M.K., Huda M., Tokimatsu K., Aziz M., 2018, Toward sustainable agricultural: integrated system of rice processing and electricity generation, Chemical Engineering Transactions, 70, 1669-1674

Doni F., Isahak A., Che Mohd C.R., Wan W.M., 2014, Physiological and growth response of rice plants (Oryza sativa L.) to Trichoderma spp. inoculants. AMB Express, 4, 45.

Fiorentino N., Ventorio V., Woo S., Pepe O., De Rosa A., 2018, Trichoderma-Based Biostimulants Modulate Rhizosphere Microbial Populations and Improve N Uptake Efficiency, Yield, and Nutritional Quality of Leafy Vegetables, Front plant Eci.

Garnica V.A., Barrera S., Munoz E., Raya J., Mendez A., Macias L., Ruiz L.F, López J., 2015, The volatile 6-pentyl-2H-pyran-2-one from Trichoderma atroviride regulates Arabidopsis thaliana root morphogenesis via auxin signaling and ethylene insensitive 2 functioning. N Phytol, 209, 1496–1512.

Hyder S., Inam M.D., Bibi S., Malik A.H., Ghuffar S., Iqbal S., 2017, Novel potential of Trichoderma spp. as biocontrol agent. J Entomol Zool Stud, 5, 214–222.

Lee S., Yap M., Behringer G., Hung R., Bennett J., 2016, Volatile organic compounds emitted by Trichoderma species mediate plant growth, Fungal Biology and biotechnology, 29, 3 – 7

Md Y.K., Md M.H., Abul H.M., Mizanur R., Mohammad Z.A., 2017, Antioxidant compounds and minerals in tomatoes by Trichoderma-enriched biofertilizer and their relationship with the soil environments, Journal of Integrative Agriculture, 16, 691-703

Núñez M.L., Pavone M.D., 2014, Biological treatment of rice cultivation under nursery conditions using the fungus Trichoderma spp. Interscience, 39 (3), 185-190. redalyc.org/pdf/339/33930206008.pdf

Nongmaithem N., Roy A., Bhattacharya P.M., 2016, Screening of Trichoderma isolates for their potential of biosorption of nickel and cadmium, Braziliam Journal of Microbiology, 47, 305.

Ortuno N., Miranda C, Claros M., 2013, Selection of strains of Trichoderma spp. generators of secondary metabolites of interest for use as a growth promoter in cultivated plants. Journal of the Selva Andina Biosphere, 1, (1), 16-32 scielo.org.bo/scielo.php?pid=S2308-38592013000100003&script=sci_abstract

Pascale A., Vinale F., Manganiello G., Nigro M., Lanzuise S., Ruocco M., Marra R., Lombardi N., S.L.Woo S.L., Lorito M., 2017, Trichoderma and its secondary metabolites improve yield and quality of grapes, Crop protection, 92, 176-181.

Sabki M.H., Lee C.T., Bong C.P.C., Zhang Z., Li C., Klemeš J.J., 2019, Sustainable organic waste management framework: a case study in Minhang district, Shanghai, China, Chemical Engineering Transactions, 72, 7-12.

Sachdev S., Singh A., Singh R.P., 2018, Optimization of culture conditions for mass production and bio-formulation of Trichoderma using response surface methodology, 3 biotech, 8, 326.

Shobhit R.V., Jay S.S., Naveen K.A, Surendra S., 2017, Soil-Plant-Microbe Interactions in Stressed Agriculture Management: A Review, Pedosphere, 27, 177 – 192.

Shuangchen C., Yaru Y., Yaqi W., Meijuan W., Qi M., Yifei C., Jingj R., Airong L., Xiaomin L., Golam J.A., 2020, Trichoderma asperellum reduces phoxim residue in roots by promoting plant detoxification potential in Solanum lycopersicum L, Environmental Pollution, 259.

Silva N.R., Monteiro V.N., Stecca Steindorff S.A., Gomes V.E., Ferreira N.E., Ulhoa J.C., 2019, Trichoderma/pathogen/plant interaction in pre-harvest food security, Fungal Biology, 123, 565 – 583. Simon V.A., Ian S., Naresh M., Gustavo H.G., 2019, The fungal threat to global food security, Fungal Biology, 123, 555 - 557

Stachniuk A., 2019, LC-MS / MS Determination of pesticide residues in fruits and vegetables. On: Mérillon JM., Ramawat K. (eds) Bioactive Molecules in Food. springer.com/referenceworkentry/10.1007%2F978-3-319-78030-6_82

Vos C.M, De Cremer K., Cammue B.P., De Coninck B., 2015, The toolbox of Trichoderma spp. in the biocontrol of Botrytis cinerea disease, Molecular plant pathologyc, 16, 4

Zhenshuo W., Yan L., Lubo Z., Yue Y., Jia L., Lixia Z., Zhenjiang G., Yufeng W., Wa G., Guo D., Qi W., 2019, A Rhizosphere-Derived Consortium of Bacillus subtilis and Trichoderma harzianum Suppresses Common Scab of Potato and Increases Yield, Computational and Structural Biotechnology Journal,17, 645-653