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Sustainable strategy to harness residual lignin from wheat straw to mitigate climate change

Abstract

Triticum aestivum (wheat) straw is one of the main rural wastes in the world that is incinerated, consequently air pollution with the release of greenhouse gases that will accelerate global warming. An option for biotechnological exploitation of wheat straw is the extraction of lignin with acetic acid and heat to obtain residual lignin from wheat straw (RELIWS). Therefore, the objectives of this work were: i) depolymerization of RELIWS by *Aspergillus fumigatus* into aromatics ii) Conversion of these aromatics from RELIWS into potential gibberellins (GIT) by *Micromonospora echinospora*, iii) evaluate the effect of potential GIT of *M. echinospora* on the phenology and biomass of *Zea mays*. To this end, RELIWS was extracted from wheat straw, depolymerized with *A. fumigatus* into aromatics, then transformed into potential GITs with *M. echinospora*, and the effect of the potential GIT on *Z. mays* was analyzed using the response variables: phenology: plant height (PH) and root length (RL); biomass: aerial and radical fresh weight (AFW/RFW); aerial and radical dry weight (ADW/RDW). The experimental data were analyzed using the ANOVA-Tukey program.

The results showed the depolymerization of RELIWS by *A. fumigatus* laccase, as well as the conversion of aromatics from RELIWS by *M. echinospora* into potential GITs. While the effect of GITs by *M. echinospora* caused *Z. mays* (maize) to register a PH of 11.95cm, a LR of 18.06cm while in the biomass; an AFW value of 0.7g and 0.9g of RFW was registered with a dose of 50µL/seed of *Z. mays*. Compared the values of the pure std GI with a µL PH of 12.61cm, a RL of 17.09cm, these numerical values were statistically different from the seed treated with the std GI, with an AFW of 0.6g, an RFW of 0.4g. The above supports that RELIWS is a source of aromatics for conversion into a possible GITs. These numerical values were statistically different from those of *Z. mays* seed treated with the 100% mineral solution. Intelligent use of RELIWS prevents greenhouse gas generation and air pollution, and supports sustainable agriculture.

Keywords: Lignin, agricultural waste, plant growth promoters, phycomycetes, actinomycetes, mitigating climate change

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Introduction

Among the variety of commercially valuable agricultural crops, *Triticum aestivum* (wheat) is one of the most important due to its high straw production;[1-3] with a global wheat straw production of 675x109kg year-1.[1,4-6] This agricultural waste is calcined and generates high concentrations of ash and greenhouse gases such as CO, CO_2, NO_2, SO_2, and O_3, which pollute the air.[3,6-9] One alternative for its intelligent disposal[9-12] is its use as a vegetal carbon source in agricultural biotechnological products. Wheat straw contains plant cell wall compounds: cellulose 39%, hemicelluloses 38% and lignin 17%.[3,4,13-15] In wheat straw, to remove hemicelluloses and cellulose; acetic acid (CH_3-COOH) is used and it is autoclaved at 121°C for 60 minutes; therefore, residual lignin from wheat straw (RELIWS) is usable if degraded, but not by chemical methods that are expensive due to their byproducts that pollute the environment;[2,5,9,14,16] a less explored alternative is ligninolytic mitosporic fungi such as *Aspergillus fumigatus* laccase that degrade with laccase this complex polymer[4,13,17-19] in the breakdown of RELIWS.[12,13,20,21] Some bacterial genera such as *Azotobacter beijerinckii* a genus and species of plant growth promoting bacteria (PGPB) have the ability to transform them into or into possible gibberellins (GIT) by *A. beijerinckii*.[22,23] But non reports are about *Micromonospora echinospora* an actinomycetes endophytic from nodules of wild legumes able to produce GIT from RELIWS. Based on the above, the objectives were: a) depolymerization of RELIWS by *A. fumigatus* into aromatics b) conversion of aromatics from RELIWS into potential gibberellins (GIT) by *M. echinospora*, c) evaluate the effect of potential GIT of *M. echinospora* on the phenology and biomass of *Zea mays* (maize).

Materials and methods

Origen and activation of *A. fumigatus*

A. fumigatus isolated from roots of *Zea mays var mexicana* (teocinte) belongs to the Environmental Microbiology Laboratory collection of the Chemical-Biological Research Institute of the UMSNH. RELIWS was extracted from wheat straw, ground, and subsequently sieved through a 0.0841mm mesh. To partially remove cellulose, hemicelluloses, and pectin, 10% (v/v) acetic acid (CH_3-COOH) was used for 30min in a 1:2 (w/v) ratio. The mixture was then neutralized with 10% (w/v) NaOH, washed with distilled water, autoclaved at 120°C for 60min, and oven-dried at 70°C for 24h[13] *A. fumigatus* was activated on RELIWS agar with the following chemical composition (g/L): RELIWS 10.0; casein peptone 5.0; yeast extract 1.3; K_2HPO_4 0.17; KH_2PO_4 2.61; $MgSO_4$ 1.5; NaCl 0.9; $CuSO_4$ 0.05; bromothymol blue 10 ppm; 2.5mL of 10% (w/v) Roma [MR] detergent, and 1 mL/L of trace element solution with the following chemical composition (g/L): H_3BO_3 2.86; $ZnSO_4$-$7H_2O$ 0.22; $MnCl_2$-$7H_2O$ 1.81; $KMnO_4$ 0.09; agar 18, the pH was adjusted to 5.5; it was sterilized at 121°C/20min.[12,14,17,21]

Kinetics of depolymerization of residual lignin from wheat straw by *A. fumigatus*

The *A. fumigatus* mycelium was removed from the Petri dish with 15.0mL of sterile saline detergent, 12mL of 0.85% (w/v) NaCl, and 3.0 mL of 0.01% (w/v) detergent (Roma®) using a 5mL pipette; then, 12.5mL of *A. fumigatus* was inoculated into 500 mL Erlenmeyer flasks with 250mL of RELIWS broth with the following composition (g/L): RELIWS 10.0; casein peptone 5.0; yeast extract 1.3; K_2HPO_4 0.17; KH_2PO_4 2.61; $MgSO_4$ 1.5; NaCl 0.9; $CuSO_4$ 0.05; 10 ppm bromothymol blue; 2.5mL of 10% (w/v) detergent, and 1 mL L-1 of trace element solution with the following composition (g/L): H_3BO_3 2.86; $ZnSO_4$-$7H_2O$ 0.22; $MnCl_2$-$7H_2O$ 1.81; $KMnO_4$ 0.09, adjusted to pH 5.5 and sterilized at 121°C/20 min (Bonilla et al., 2013); the flasks were incubated on a rotary shaker (Thermo Scientific MaxQ 4000) for 2 weeks at 30°C at 150rpm; samples were taken from the RELIWS broth to measure laccase as evidence of depolymerization on days 3, 6, 9, 12 and 15; that were frozen.[12,18,19]

Measurement of *A. fumigatus* laccase

Measurement of laccase for aromatics generation by *A. fumigatus* was carried out on days 3, 6, 9, 12, and 15. The samples were centrifuged at 3500rpm at 4°C for 15min to eliminate the *A. fumigatus* mycelium. A mixture was then prepared in a test tube covered with aluminum foil, to which 2.4mL of 25mM sodium acetate trihydrate buffer, pH 3.0, 300µL of 10 mM 2,2'-azino-bis-3-ethylbenzothiazolin-6-sulfonic acid (ABTS), and 300µL of RELIWS broth with laccase were added. The mixture was vortexed (Genie II G560). From there, 1 mL was taken and transferred to a spectrophotometer cell (Benchtop-Educational Spectrophotometer 7305) by oxidation of ABTS; with a molar extinction coefficient of ε420 = 3600 M-1 cm-1. For the measurement of laccase, the spectrophotometer was calibrated with a blank adjustment with 3.0mL (buffer 2.4mL, sodium acetate trihydrate and acetic acid at pH 3.0); distilled water 300µL and ABTS 300µL), adjusted the problem sample was measured at 0, 3 and 5 minutes.[11,17,19,24,26] To determine the laccase units, the following equation was used:[20,21,24]

$$UL^{-1} = \frac{A(1x106)(Vt)(C)}{t(\varepsilon)(Vm)}$$

C = Cell size (1.0cm)

Ɛ = Molar extinction coefficient of ABTS

Vm = Sample volume (mL.)

Vt = Total volume of the reaction (mL.)

t = Reaction time (min.)

A= Δ Abs 420 nm = Final Abs - Initial Abs

Micromonospora echinospora

This actynomycete belongs to the collection of the Environmental Microbiology Laboratory of UMSNH. *M. echinospora* was isolated from nodules of *Melilotus indicus* (yellow clover). To prepare *M. echinospora* for transforming aromatics of RELIWS GIT was activated on avocado agar with the following composition (g/L): avocado pit 10.0; casein peptone 5.0; yeast extract 1.3; K_2HPO_4 0.17; KH_2PO_4 2.61; $MgSO_4$ 1.5; NaCl 0.9; $CuSO_4$ 0.05; bromothymol blue 10 ppm; 2.5 mL of 10% (w/v) detergent, and 1 mL/L of trace element solution with the following composition (g/L): H_3BO_3 2.86; $ZnSO_4$-$7H_2O$ 0.22; $MnCl_2$-$7H_2O$ 1.81; $KMnO_4$ 0.09; agar 18, adjusted to pH 7.0, was sterilized at 121°C/ 20min. The RELIWS broth, transformed by *A. fumigatus* was filtered to remove it and enriched with casein peptone 4g/L (Bioxon), yeast extract (Bioxon) 2.0, glucose 2.0 and $CuSO_4$ $5H_2O$ 160 ppm the pH was adjusted to 6.8-7.0, sterilized at 121°C/20min. Then *M. echinospora* with 15mL of sterile detergent saline solution 12mL NaCl 0.85% (w/v) and 3.0mL detergent (Roma®) 0.01% (w/v), was extracted with a 5mL pipette; A 12.5mL M. echinospora was inoculated into depolymerized and enriched RELIWS broth for 16 days at 30°C and 150rpm, samples were taken on days 9, 12, and 16.[22-25] Once the potential *M. echinospora* GITs were synthesized, the supernatant was recovered in sterile test tubes and frozen to eliminate both genera and species of *M. echinospora*.

Bioassay with the RELIWS transformation of *M. echinospora* GIT on the phenology and biomass of *Z. mays*.

Effect of *Z. mays* seed was disinfected with 70% ethanol, then washed six times with sterile distilled water; disinfected with 6% sodium hypochlorite (NaClO) and then washed six times with sterile distilled water. The seed was treated with the possible GIT of M. echinospora at dose of 50µL *Z. mays* seed, since previous experiments showed that doses: 100 and 150µL caused an negative effect on germination phenology and biomass of *Z. mays* (data not shown) and was placed in sterile Petri dishes on a cotton bed with sterile distilled water as a support, covered with sterile filter paper; According to the experimental design, the seed used as absolute control (AC) was irrigated only with sterile distilled water, the seed used as relative control (RC) was fed with the 50% mineral solution[5,26,27] with the following composition (g/L): NH_4Cl or NH_4NO_3 10.0; K_2HPO_4 2.5; KH_2PO_4 2.0; $MgSO_4$ 1.0; NaCl 0.1; $CaCl_2$ 0.1; $FeSO_4$ traces, trace element solution 10 ml/L with the following composition (g/L): H_3BO_3 2.86; $ZnSO_4$-$7H_2O$ 0.22; $MnCl_2$-$7H_2O$ 1.81; $KMnO_4$ 0.09 and the pH was adjusted to 6.5-6.8). The Petri dishes with the *Z. mays* seeds were placed in a solarium in the dark for 3 days; 15 days later, phenology was measured: PH/RL, biomass: AFW/RFW, and the aerial and radical parts were dried at 80 °C/24 h in an oven for ADW/RDW.[27-29] The experimental data were analyzed by according to ANOVA, p > 0,05, Tukey HSD using the Statgraphics Centurion 16.103 ® program,[24,30] on Table 1 shown the experimental design to evaluate the effect of conversion of RELIWS aromatics by *A. fumigatus* into potential gibberellins (GIT) by *M. echinospora.*

Table 1 Experimental design to analyze the conversion of RELIWS aromatics by *Aspergillus fumigatus* into possible gibberellins (GIT) by *Micromonospora echinospora*

Biosaay	Absolute control (AC)	Relative control (RC)	Std Giberellin	Potential Giberellin (GIT) of Micromonospora echinospora from RELIWS
	Water only	mineral solution at 100%	mineral solution at 50%	mineral solution at 50%
Dose (µL)	-	50	50	50
[1]Zea mays	+	+	+	+

(+) added (-) no added

Results and discussion

In Figure 1, the low activity of laccase of *A. fumigatus* is shown during the depolymerization of RELIWS that started on the third

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and sixth day, after using the sugars of cellulose, hemicelluloses and pectin of RELIWS,[20,21,26] then the laccase increased the velocity of depolymerization of RELIWS until day 12,[4,11,17,31] which reached maximum activity with 10.8 U/L with which generated the highest amount of aromatics of RELIWS to decrease on day 15, it was evident that this mitosporic fungy has a depolymerization capacity of RELIWS[12,17,20,21] equal to or greater than any basidiomycete,[18,19] in a relatively shorter time, so it has a potential value to avoid the burning of agricultural wheat straw responsible for the release of greenhouse gases and mitigate global warming.[15,31-33]

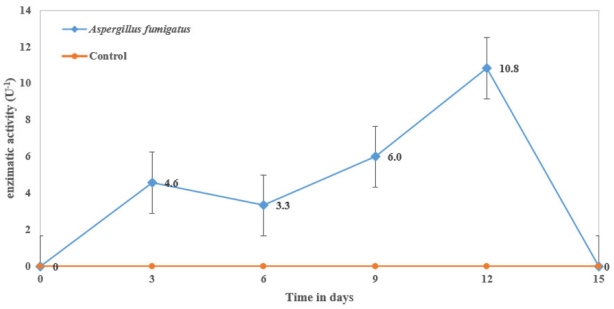

Figure 1 Kinetics of the laccase activity of *Aspergillus fumigatus* in the depolymerization of RELIWS into aromatics.

Effect of RELIWS transformation of aromatics by *A. fumigatus* on gibberellins (GIT) by *M. echinospora* on the phenology and biomass of *Zea mays.*

Table 2 shows the effect of the GIT and the 50% mineral solution on Z. mays at a dose of 50 µl/seed on plant height (PH) (18.06cm) and root length (RL) (11.95cm); compared to *Z. mays* with standard GIT and the 50% mineral solution with 12.61cm of pH and 17.09cm of RL; these numerical values were statistically different compared to *Z. mays* fed with the 100% mineral solution without any GIT, with 4.96cm of pH and 6.62cm of RL. In relation to the biomass of *Z. mays* with the possible GIT of *M. echinospora,* the 50% mineral solution, which registered an aerial fresh weight (AFW) of 0.7g, 0.9g RFW, an aerial dry weight (ADW) of *Z. mays* treated with std GIT registered a AFW of 0.6g and RFW of 0.15g, a radical dry weight (DRW) with 0.07 g; compared to *Z. mays* treated with the std GIT and the 50% mineral solution with an ADW of 0.06 g, an RDW of 0.06g; numerical values with statistical difference compared to *Z. mays* used as RC fed with the 100% mineral solution without any type of GIT, with 0.03g of ADW and 0.03g of RDW; while *Z. mays* fed with the 50% mineral solution reached an AFW of 0.6g and an RFW of 0.4g, in Table 2 shows the results obtained in this research, the positive effect of possible GITs on *Z. mays* was evident at a dose of 50 µL/seed. It is demonstrated that what is synthesized by *M. echinospora* from the aromatics of RELIWS generated by *A. fumigatus* are possible GITs because these substances are biologically active in stimulating cell division or cell elongation or both actions.[22,23,32] Since the exogenous application of GITs in seeds causes favorable growth stimulation.[14,27,29] Based on these results, it should be noted that GITs induce the elongation of the aerial part; likewise, also GITs are involved in promoting root growth and in the abundance of root hairs,[23] which promotes an increase in the biomass of *Z. mays* both in the aerial part and in the radical part. As evidence on Table 2, that *Z. mays r*esponse is due to the applied dose of the possible GITs since at high concentrations in this case equivalen to 100 and 150µL/seed GITs inhibit the growth of *Z. mays* roots; as it has been reported.[22,31,32] This research supports an alternative ecological solution in intensive agricultural areas of wheat production to avoid, as until today,[14] a problem of loss of soil fertility due to the non-return of plant carbon produced by wheat growth, as well as to prevent the generation of greenhouse gases by burning wheat straw residue.[7,8,15] In this sense, RELIWS can be fermented to serve as compost, inoculated with *A. fumigatus* to become a feed for livestock or animal feed, as well as double fermentation so that the aromatics of RELIWS are converted into potential gibberellins through the action of *M. echinospora*.[22,23] This research in progress indicates that aromatics from RELIWS are an uncommon type of gibberellins analyzed by gas chromatography and nuclear magnetic resonance (data not shown), a type of intelligent disposal of RELIWS to be part of the strategies required to reduce the carbon footprint and mitigate global warming.[1,6,9,10]

Table 2 Effect of the conversion of aromatics derived from RELIWS generated by *A. fumigatus* into potential gibberellins (GIT) by *M. echinospora* on the phenology and biomass of *Zea mays*

Dosis 50 µL	Phenology		Biomass		Biomass	
*Treatment	Plant height (cm)	Radical length (cm)	Aerial fresh weight (g)	Radical fresh weight (g)	Aerial dry weight (g)	Radical dry weight (g)
Absolute control (water)	3.79^{d**}	6.69^{c}	0.2d	0.1^{d}	0.02^{d}	0.02^{d}
Relative control Mineral solution at 100%	4.96^{c}	6.62^{c}	0.2^{c}	0.2^{c}	0.03^{c}	0.03^{c}
Std Giberellin	12.61^{a}	17.09^{a}	0.6^{b}	0.4^{b}	0.06^{b}	0.06^{b}
Potential Gibberellins (GIT) (GIT)*Micromonospora echinospora*	11.95^{a}	18.06^{a}	0.7^{a}	0.9^{a}	0.15^{a}	0.07^{a}

*n = 20, **different letters had a statistical difference according to ANOVA, p >0,05, Tukey HSD.

Conclusion

The conversion of RELIWS into aromatics by *A. fumigatus,* a mitosporic fungus that has the genetic and biochemical potential to depolymerize it, with the generation of aromatics that *M. echinospora* converts into potential gibberellins, ensures added value of economic and environmental value to the enormous agricultural waste whose highest content is lignin, an option to avoid the burning of agricultural waste with the release of greenhouse gases, therefore this double biological action *A. fumigatus / M. echinospora* helps mitigate global warming that affects planet Earth from productive agricultural areas of the world.

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Conflicts of interest

The author declare no conflicts of interest.

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