CULTIVATION OF THE MUSHROOM "*Ganoderma lucidum*" ON AGRO-INDUSTRIAL WASTES AND IMPACT OF BIOCHAR APPLICATION ON GREENHOUSE GASES EMISSION

By

MAHMOUD ABDEL-MONIEM M. EL TOHAMY

B.Sc. Agric. Co-op. Sci., Ins. Agric., Ain Shams Univ., 2007

THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

In

Agricultural Sciences
(Agricultural Microbiology)

Department of Agricultural Microbiology
Faculty of Agriculture
Cairo University
EGYPT

2019

Format Reviewer **Vice Dean of Graduate Studies**

Name of Candidate: Mahmoud AbdEl-Moniem M. El Tohamy **Degree:** M. Sc.
Title of Thesis: Cultivation of the Mushroom "*Ganoderma lucidum"* on Agro-Industrial Wastes and Impact of Biochar Application on Greenhouse Gases Emission
Supervisors: Dr. Ferial Mohamed Rashad
Dr. Hayam Mohamed Fathy
Dr. Mostafa Hamdy El Kattan
Department: Agricultural Microbiology **Approval:** 30 / 4 / 2019

ABSTRACT

The present study was conducted in three phases, the first was *in vitro* using race tubes to assess the effects of different agro-wastes [broad bean stalks (BBS), cotton stalks (CS), maize stalks (MS), rice straw (RS), sugarcane bagasse (SCB) and wheat straw (WS)], supplements [wheat bran (WB) and corn gluten (CG)] and biochar incorporation at different levels on growth criteria of *Ganoderma lucidum* and the greenhouse gases emissions (GHGs). The selected agro-wastes were prepared either alone or in combinations with fixed levels of supplements, $CaCO_3$ and biochar for developing suitable formula for *G. lucidum* cultivation. Different types of spawns, sorghum and agro-wastes based spawns, were prepared. In the second phase, a comparative study was conducted using elephant grass (EG) formula versus selected agro-wastes formulae, different biochar levels and spawn types in mushroom house, a proximate analysis of mature fruiting bodies were also carried out. A greenhouse experiment, in the third phase, was executed to assess the effect of *Ganoderma* post mushroom substrate (GPMS) on the growth performance of cowpea infested with *Rotylenchulus reniformis*.

Among the substrates used, CS appeared best followed by SCB and RS. WB showed best supplementation for mycelial growth. CO_2 emission values exhibited accurate measurements to decide the suitability of such agro-waste for growth rather than visual observations. CO_2 was the major GHG released, but methane and nitrous oxide were not detected. Addition of biochar at rates of 10, 20, 30 and 40%, resulted in improvements of the mycelial growth with lower levels of CO_2 comparing to control faster growth rates were observed in formulae received 40% biochar and inoculated with agro-waste-based spawns. CS+RS+SCB+WB formula proven its superiority for *in vitro* growth and active spawn development substrate. Increasing spawning doses was parallel with speeding up the mycelium colonization rate. Higher longevity was obtained with sorghum spawn until 6 months. In mushroom house, this particular formula proved its superiority and was on par with recommended EG formula; it gave the highest yield (195.16 g Kg^{-1}), biological efficiency (19.52 %), protein (16.69 %), polysaccharides (3.61%) and minerals (3433 mg/100g). Spawn running period was the shortest in treatments inoculated with agro-waste-based spawns. With 40% biochar, days required to the complete mycelium colonization and fructification were 10.60 and 23.00, respectively. At 10% biochar, highest yields (238.40 g Kg^{-1}), biological efficiencies (23.84%), protein (19.58%) and minerals (4092 mg/100g) were obtained. The higher the biochar level, the higher the reduction in emitted CO_2, the loss in C and the increase in N of *Ganoderma* post mushroom substrates (GPMSs). Under greenhouse conditions, almost all the tested GPMSs, at 0.125 or 0.25%, encouraged the reproduction of reniform nematodes and improved plant growth criteria.

Key words: *G. lucidum*; agro-wastes; biochar, spawn; nutritional profile; GPMS; *R. reniformis*

LIST OF TABLES

No.	Title	Page
1.	Physicochemical properties of used materials…….	34
2.	Treatments for determination of suitable substrate formula for *G. lucidum* cultivation……	39
3.	Substrate formulae for *Ganoderma lucidum* cultivation……	42
4.	Growth measurements of *G. lucidum* on different agro wastes supplemented with 1.0% $CaCO_3$……..	51
5.	Effect of wheat bran doses on mycelium growth measurements and CO_2 emission of *G. lucidum*……	54
6.	Effect of different level of corn gluten on mycelium growth rate and CO_2 emission of *G. lucidum*…….	56
7.	Effect of different levels of biochar on mycelium growth rate and CO_2 emission of *G. lucidum*……	60
8.	Effect of different levels of biochar on mycelium growth rate CO_2 emission of *G. lucidum*……	61
9.	Mycelium growth measurements of *Ganoderma lucidum* on different formulated substrates incorporated with 40% biochar…….	65
10.	Mycelium growth measurements of *Ganoderma lucidum* as affected by pawn type……..	68
11.	Time required for complete colonization of different substrate formulae for spawn production in polyethylene bags and its	71

	life span…….	
12.	Chemical constituents of substrate formulae for in-door *G. lucidum* cultivation……	72
13.	*Ganoderma lucidum* production cycle, yield and biological efficiency (BE)……..	73
14.	Effect of different levels of biochar on crop cycle duration, productivity and biological efficiency (BE) of *G. lucidum*…..	76
15.	Effect of biochar on CO_2 emissions during the life cycle phases of *G. lucidum* mushroom……...	78
16.	Effect of different spawn types on crop cycle duration, productivity and biological efficiency (BE) of *G. lucidum*…..	81
17.	Effect of spawn type on CO_2 emissions during the life cycle phases of *G. lucidum* mushroom……..	83
18.	Effect of different substrate formulae, biochar levels and spawn sources on crude protein, polysaccharide, total sugars and mineral contents of fruiting body…….	84
19.	Physicochemical characteristics of Ganoderma post mushroom substrates (GPMSs)……...	89
20.	Influence of different post mushroom substrate treatments on the reproduction of reniform nematode, *R. reniformis* on cowpea plants……..	90
21.	Influence of different post mushroom substrate treatments on cowpea growth infected with the reniform nematode, *R. reniformis*……	91

LIST OF FIGURES

No.	Title	Page
1.	Specific types of medicinal mushrooms can boost human overall health…….	22
2.	Effect of agro-wastes on mycelial extension rates and CO_2 production. BBS: Broad bean stalk; CS: Cotton stalk; MS: Maize stalk; RS: Rice straw; SCB: sugarcane bagasse; WS: Wheat Straw……...	52
3.	Effect of different levels of wheat bran and corn gluten on mycelium growth measurements and CO_2 emission of *G. lucidum*. LGR, Linear growth rate; CR, Colonization rate = % {height of the colonized Substrate (GR after 21 days) ÷ 140 mm (the height of the substrate per tube)}; Average of 4 replicates…….	57
4.	Effect of different levels of incorporated biochar on mycelium growth measurements and CO_2 emission of *G. lucidum*. Substrate formulation was the same for each agro waste (80% agro waste + 18% wheat bran or corn gluten + 1% sucrose + 1% $CaCO_3$). LGR, Linear growth rate; CR ،Colonization rate = % {height of the colonized Substrate (GR after 21 days) ÷ 140 (the height of the substrate per tube .{(Average of 4 replicates……	62
5.	Mycelium growth measurements of *G. lucidum* as affected by different treatments of substrate formulae incorporated with 40 % biochar. T1: CS +CG; T2: CS + WB; T3: RS +CG; T4: RS+ WB; T5: CS + RS + CG; T6: CS + RS + WB; T7: CS + SCB + CG; T8: CS + SCB+ WB; T9: RS + SCB +CG; T10: RS + SCB + WB; T11:CS + RS +SCB +WB. LGR, Linear growth rate; CR, Colonizationrate = % {height of the	

colonized substrate (GR after 21 days) ÷ 140 mm (the height of the substrate per tube)}; GR, Growth rate; CS: Cotton stalk; RS: Rice straw; SCB: Sugarcane bagasse; CG: Corn gluten; WB: Wheat bran…… 66

6. Mycelium growth measurements of *G. lucidum* as affected Bydifferent inoculum sources on selected formulae I Incorporatedwith 40 % biochar. LGR, Linear growth rate ؛CR, Colonization rate = % {height of the colonized substrate (GR after 21 days)÷ 140 mm (the height of the substrate per tube)}; GR, Growth rate; PDA, Potato dextrose agar; SS, Sorghum spawn; AWS, Agro wastes-based spawn; CS: Cotton stalk; RS: Rice straw; SCB :Sugarcane bagasse; CG: Corn gluten; WB: Wheat bran ؛PDA: Potato dextrose agar; Average of 4 replicates. * The spawn growth medium has the same omposition as used in the growth substrate without biochar formulation without biochar…….. 69

7. Effect of cultivation substrate formulae on full colonization, cropcycle duration, yield and biological efficiency (BE) of G. lucidum.Formula 1: 49.80 % CS + 31.20 % SCB + 18% WB + 1% $CaCO_3$;Formula 2: 27.00 % CS + 27% RS + 27.00 % SCB + 18%WB+ 1% $CaCO_3$; Formula 3: 76.00 % EG + 22 %WB + 2 % $CaSO_4$.CS: Cotton stalk; RS: Rice straw; SCB: Sugarcane bagasse; WB:Wheat bran; EG: elephant grass…….. 74

8. Effect of different levels of biochar on crop cycle duration, productivity and biological efficiency (BE) of *G. lucidum*. Formula 1: 49.80 % CS + 31.20% SCB + 18% WB + 1% CaCO3; Formula 2: 27.00 % CS + 27% RS + 27.00 % SCB + 18% WB + 1% $CaCO_3$. S: Cotton stalk; RS: Rice straw; SCB: Sugarcane bagasse; WB: Wheat bran……. 77

9. Effect of biochar on CO_2 emissions during the life cycle phases of *G. lucidum* mushroom. Formula 1: 49.80 % CS + 31.20% SCB + 18% WB + 1% $CaCO_3$; Formula 2: 27.00 % CS + 27% RS + 27.00 % SCB + 18% WB + 1% $CaCO_3$. CS: Cotton stalk; RS: Rice straw; SCB: Sugarcane bagasse; WB: Wheat bran….. 79

10. Effect of spawn types on *G. lucidum* productivity, Biological efficiency (BE) and crop cycle duration. Formula 1: 49.80 % CS + 31.20% SCB + 18% WB + 1% CaCO3 incorporated with 10 % biochar; Formula 2: 27.00 % CS + 27% RS + 27.00 % SCB + 18% WB + 1% $CaCO_3$ incorporated with 10 % biochar. CS: Cotton stalk; RS: Rice straw; SCB: Sugarcane bagasse; WB: Wheat bran……. 82

11. Effect of spawn type on CO_2 emissions during the life cycle phases of *G. lucidum* mushroom. SS: Sorghum spawn; AWS: Agro-waste-based spawn; Formula 1: 49.80 % CS + 31.20% SCB + 18% WB + 1% CaCO3; Formula 2: 27.00 % CS + 27%RS + 27.00 % SCB +18% WB + 1% $CaCO_3$. CS: Cotton stalk; RS: Rice straw; SCB: Sugarcane bagasse; WB: Wheat bran….. 83

12. Effect of cultivation substrate formulae, biochar and spawn types on organic matter loss (OML) as an index of the biodegradation of cultivated substrate during mushroom crop cycle. SS: sorghum spawn; AWS: Agro-waste-based spawn. Formula 1: 49.80 % CS + 31.20% SCB + 18% WB + 1% $CaCO_3$; Formula 2: 27.00 % CS + 27% RS + 27.00 % SCB + 18% WB + 1% $CaCO_3$; Formula 3: 76.00 % EG + 22 % WB + 2 %$CaSO_4$; CS: Cotton stalk; RS: Rice straw; SCB: Sugarcane bagasse; WB: Wheat bran; EG: Elephant grass; *Formula incorporated with 10% biochar……. 87

13. Influence of *Ganoderma* post mushroom substrates on a, b) the reproduction; c) the buildup of the reniform nematode, *Rotylenchulusreniformis* infested cowpea.

Control 1, un-amended infested cowpea. Nematode buildup = final population/ initial population. Formula 1:49.80 % CS + 31.20% SCB + 18% WB + 1% $CaCO_3$; Formula 2: 27.00 % CS + 27% RS + 27.00 % SCB + 18% WB + 1% $CaCO_3$. CS: Cotton stalk; RS: Rice straw; SCB: Sugarcane bagasse; WB:Wheat bran 92

14. Influence of different post mushroom substrate treatments on cowpeagrowth infested with the reniform nematode, *Rotylenchulus reniformis*. Control 1, un-amended infested cowpea; control 2, un-amended cowpea plant without nematode infestation. Formula 1: 49.80 % CS + 31.20%SCB + 18% WB + 1% $CaCO_3$; Formula 2: 27.00 % CS + 27% RS + 27% SCB + 18% WB + 1% $CaCO_3$. CS: Cotton stalk; RS: Rice straw; SCB: Sugarcane bagasse; WB: Wheat bran…… 93

CONTENTS

	Page
INTRODUCTION.....	1
REVIEW OF LITERATURE.....	4
1. Occurrence and distribution of *Ganoderma* (Habitat) ..	5
2. Taxonomy and Characteristics of *Ganoderma*.....	7
3. Nutrition and life cycle of *Ganoderma lucidum*.....	8
4. Artificial cultivation of *Ganoderma lucidum* ……..	11
a. Methods of *Ganoderma* cultivation.....	12
b. Spawn production.....	13
c. Substrates and supplements……	14
d. Effect of environmental factors on the production…	17
e. Effect of biochar.....	19
5.Medicinal uses of *G. lucidum* mushroom.....	21
6. Potential uses of post mushroom substrate (PMS)…	30
MATERIALS AND METHODS…….....	33
1. Materials……..	33
a. Substrates……..	33
b. Supplements……..	33
1. Organic additives……..	33
2. Chemicals additives…….	33
c. Biochar……	35
d. Mushroom culture……	35
2. Methods……	35
a. Maintenance of the culture……	35
b. Preparation of standard inoculum…….	35
c. *In vitro* measurement and monitoring mycelial growth rate of *G. lucidum* and greenhouse gases (GHGs) …….	35
1.Effect of agro wastes and wheat bran on mycelial extension ratesof *G. lucidum* and GHGs production……	37
2. Effect of corn gluten on mycelial extension rates of *G. lucidum* and GHGs production……..	37
3.Effect of biochar on mycelial extension rates of *G. lucidum* and GHGs production……..	38
4. Effect of different combinations of agro wastes and supplementations on mycelial growth measurements of	

*G. lucidum*in the presence of 40% biochar……. 38
d. Evaluation of different substrates for spawn production…. 38
1. Sorghum spawn…… 39
2. Agro-wastes spawns……. 40
3.Effect of inoculum source on the mycelial growth measurements of *G. lucidum*…….. 40
4.Determination of suitable inoculum size for production of agro-waste-based spawn…… 41
5. Effect of storage on the viability of produced spawns…. 41
e. In-door cultivation of *G. lucidum* mushroom…… 42
1. Substrate preparation and spawning……. 42
a. Effect of different substrate formulae on yield and biological efficiency…… 42
b.Effect of biochar concentration on the growth, productivity and GHGs emissions ……..... 43
c. Effect of spawn type on yield and biological efficiency. 43
2. Spawn running……..... 43
3. Fruiting running…….. 43
4. Harvesting…… 44
5. Biological efficiency…….. 44
6. Proximate composition of fruiting bodies of *G. lucidum*…….. 44
7. Organic matter loss (OML)…….. 45
f. Effect of *Ganoderma* post mushroom substrates (PMSs) on *Rotylenchulus reniformis* infesting cowpea and plant growth response……. 45
g. Physical and chemical analyses…… 46
1. Moisture……. 46
2. pH values…… 46
3. Electrical conductivity (EC)…… 47
4. Estimation of total carbon and nitrogen…… 47
5. Determination of reducing and soluble sugars…… 47
6. Determination of cellulose, hemicellulose and lignin… 47
7. Determination of protein…… 47
8. Determination of crude polysaccharide and total sugar. 47
9.Determination ofcalcium, magnesium,phosphorus and Potassium……. 48
10. Determination of GHGs emissions…… 48
h. Statistical analysis…….. 48

i. Potato dextrose agar medium (Difco, 1985)…….. 48
RESULTS…….. 50
1. In vitro growth measurements of *Ganoderma lucidum* in race tubes…… 50
a. Effect of agro-wastes on mycelial extension rates and greenhouse gases (GHGs) production…….. 50
b. Effect of nutritional additives on mycelial extension rates and GHGs production…… 53
c. Effect of biochar on mycelial extension rates and GHGs production…… 59
d. Effect of different combinations of agro-wastes and supplementations on mycelial growth measurements of *Ganoderma* in the presence of 40% biochar…… 64
2. Evaluation of different substrates for spawn production…….. 67
a. Effect of inoculum source on the mycelial growth measurements of *G. lucidum*…… 67
b. Determination of suitable inoculum size for production of agro-waste based spawn…… 67
c. Effect of storage on the viability of produced spawns…. 70
3. In-door cultivation of *G. lucidum* mushroom…….. 70
a. Evaluation of different substrates formulae for supporting growth and productivity of *G. lucidum*…… 70
b.Effect of biochar concentration on the growth and productivity of *G. lucidum* and GHGs emissions…….. 75
c.Effect of different spawn types on growth and productivity of *G. lucidum*…… 79
d. Proximate composition of fruiting bodies of *G. lucidum*. 80
e. Organic matter loss…… 86
4.Effect of *Ganoderma* post mushroom substrates (GPMSs) on growth of cowpea plants infested with *Rotylenchulus reniformis*…….. 88
DISCUSSION..... 95
CONCLUSION…… 107
SUMMARY…….……..…….... 108
REFERENCES …….……..… 116
ARABIC SUMMARY …….……