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The potential use of *Lentinus edodes* to manage and control water hyacinth in Zimbabwe

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The rapid expansion and reproduction of certain plant species represents one of the biggest problems in aquatic environments, ranging from eutrophication to the limited availability of water for human consumption. Among these plants is water hyacinth (Eichhornia crassipes), a herbaceous hydrophyte often branded the world's worst aquatic weed due to its invasive aggression, negative impact on aquatic environments, and the cost usually associated with its management. Water hyacinth is a biomass, typically rich in lignocellulosic material and making it a potential raw material for the synthesis of products of industrial and domestic interest; e.g. edible fungi. Among the commonly known edible fungi is Lentinus edodes, a commercial mushroom whose versatile nature as a white rot fungus provides basis for the continued exploration of its biochemical processes during solid state fermentation on various lignocellulosic biomass as potential substrates. The fungus naturally feeds on lignocellulose by secreting various extracellular enzymes responsible for breaking down this organic polymer into simple soluble molecules that the hyphae can absorb and develop into mycelia. In this study, L. edodes was assessed for its ability to grow on water hyacinth and possibly utilizing it as a substrate. When cultured onto this noxious biomass followed by assessment by agar plate-based clearing assay and spectrophotometry, the fungus demonstrated its ability to secrete cellulases, xylanases, pectinases, peroxidases and laccases, thus showing its capabilities to physiologically utilize this hydrophyte as a substrate. If properly optimized, this approach can be remarkably used as a sustainable way to control water hyacinth in Zimbabwe.

Key words: *Lentinus edodes*, water hyacinth, lignocellulosic biomass, lignocellulolytic enzymes, cellulases, xylanases, pectinases, lignin peroxidases, laccases.

INTRODUCTION

Water hyacinth or *Eichhornia crassipes* (Mart.) Solms-Laubach. is a tropical perennial aquatic plant belonging to the family Pontederiaceae (Crow et al., 2000; Penfound and Earle, 1948). It is a free-floating aquatic organism, originating from the Amazon River Basin in South America (Sornvoraweat and Kongkiattikajorn, 2010). The plant tolerates extremes in seasonal variations particularly in terms of flow velocity, nutrient availability, pH, temperature, water level fluctuations and toxic substances (Penfound and Earle, 1948). The hydrophyte also has an extensive dispersal capacity and an extremely fast growth rate (Gutierrez et al., 2001; Villamagna and Murphy, 2010) and duplication time of 7.4 days on average, by which a total of 144 ton/ha of dry matter can be accumulated in a year (Mwangi, 2013; Nigam and Singh, 2002).

Water hyacinth has emerged as a major weed, polluting water bodies in more than 50 countries in the tropical and sub-tropical regions, with profuse and permanent impacts (Mwangi, 2013). In Zimbabwe, this invasive exotic plant was first reported in watersheds as early as the 1940s, but had not yet posed any management problem (Magadza, 2003). The plant was introduced in the country initially, as an ornamental flower and then eventually spreading out to other water bodies uncontrollably (Chikwenhere, 1994). By the mid-1960s, most of the major aquatic bodies in that country, including lake Chibero (the largest water body in Harare - the capital city) and Hunyani River (the main tributary of lake Chibero) were invaded (Magadza, 2003).

Besides natural factors, human activities in most cases, also promote the spread of this weed by providing conducive conditions and environments for its proliferation and establishment (Gutierrez et al., 2001; Villamagna and Murphy, 2010). Run-offs from agricultural and industrial developments, pollution from septic and sewer systems and the other human-related practices, continuously increase the influx of organic and inorganic substances into water bodies (Chikwenhere, 1994). The fast growth rate of this weed and robustness of its seeds lead to various problems, which among others, include coverage of water ways, destruction of ecosystems through death of the aquatic life and eventually, the uncontrollable speeding up of eutrophication (Nigam and Singh, 2002). The usual humankind livelihood activities such as fishing and tourism have, to date, been severely constrained by the explosive infestations of this aquatic plant in various local and regional water bodies (Cilliers et al., 2003).

By this day, water hyacinth has been marked the world's worst aquatic weed and has garnered increasingly a lot of international attention as an invasive species (Mwangi, 2013). The plant has been classified by the International Union for Conservation of Nature (IUCN) as one of the 100 top-most aggressive invasive species and one of the 10 top-most worst weeds in the world (Saha et al., 2014). Attempts to control the weed have resulted in very high marginal costs, which at times, were rather futile as they could only manage to temporarily reduce the weed but not completely eradicating it (Cilliers et al., 2003; Gutierrez et al., 2001). Some of the methods used e.g. chemical treatment, had very detrimental effects on aquatic life, further with the water being deemed unsafe for domestic and agricultural uses (Mwangi, 2013). According to Brown (2006), the economic impacts of this weed in several African countries, including Zimbabwe have been estimated to be between 20 and 50 million US dollars every year while across the whole continent of African, it is as much as US$100 million annually.

As a problematic weed but with an attractively high content of the lignocellulosic biomass, water hyacinth's possible use in industries and commercial set-ups could potentially have significant benefits not only to the industries themselves but also to either or both the natural aquatic environments and/or local communities situated around such water-infested bodies. However, and like any other flowering land plant, water hyacinth is composed mainly of the biologically stable polymer - lignocellulose that is very resistant to either the physical, chemical, or enzymatic attacks (Dorado et al., 2001). Notably, white-rot fungi, which are a specialized group of microorganisms belonging to the unique class of Basidiomycetes, have been reported to be capable of attacking the lignocellulose fibre (Dorado et al., 2001; Jurado et al., 2011; Wang et al., 2019). These fungi secrete various extracellular enzymes and organic acids that breakdown fiber (Dorado et al., 2001; Jurado et al., 2011; Pandya and Albert, 2014). Among the enzymes are oxidases (laccases and peroxidases) that breakdown lignin (Wesenberg et al., 2003; Zirbes and Waldvogel, 2018), glucanases (exo- and endo-) that degrade cellulose (Kuhad et al., 2011; Legodi et al., 2019), xylanases that breakdown hemicelluloses (Punniavan, 2012), and pectinases that degrade pectins (Baldrian and Valášková, 2008; Collins et al., 2005).

A number of white rot fungi produce a whole cocktail of these enzymes while others produce only one or a few of them (Baldrian and Valášková, 2008; Maganhotto de Souza Silva et al., 2005). *Lentinus edodes* (Berk. &

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Mont.) Pegler., (shiitake) and *Pleurotus* spp. (Jacq.: Fr.) Kumm., (oyster) comprise a group of white rot fungi that are edible (mushrooms) with important medicinal properties and biotechnological and environmental benefits (Cohen et al., 2002; Jia et al., 2019; Leatham, 1985; Reddy and D'Angelo, 1990; Thakur, 2018). If successfully grown on water hyacinth, these edible white rot fungi would yield mushrooms (Thakur, 2018), a whole cocktail of enzymes (Wang et al., 2019) and fine chemicals (Zirbes and Waldvogel, 2018), whose properties would be very essential and useful for the food and feed industries (Kiiskinen et al., 2004; Mikiashvili et al., 2006).

In this reported work, *L*. *edodes* was tested for its practical capability to grow on water hyacinth growing locally in Zimbabwe and its potentials to utilize it as a substrate. The study was designed on the backdrop that if this edible white rot fungus could successfully utilize water hyacinth as a substrate, then this whole approach could then provide a viable strategy for sustainable management (Jia et al., 2019; Thakur, 2018) of this problematic weed in local aquatic environments of the country. On the other hand, the same approach would also provide prospects for the possible conversion of a low-value indigenous lignocellulosic rich waste into products of high commercial value such as mushrooms, enzymes and fine chemicals (Buswell et al., 1993; Thakur, 2018; Villamagna and Murphy, 2010; Wang et al., 2019; Zirbes and Waldvogel, 2018).

MATERIALS AND METHODS

Source of the test fungus

The *L*. *edodes* strain used in this study as the test fungus was purchased from Sylvan Africa (PTY) Ltd., RSA, in form of a partially-dried spawn, maintained at 4°C.

Viability assessment of the test fungus

Growth viability of the purchased test fungus was tested and ascertained as already detailed elsewhere (Sibanda et al., 2019).

Source of substrate and substrate preparation

The water hyacinth and liver seed grass biomasses used in this study as the test and control substrates were obtained from Zimbabwe (Permit number: P0079761; Appendix; Figure A1) and South Africa respectively. The biomasses were dried and prepared for experimental work as outlined before (Sibanda et al., 2019).

Cultivation of fungi and production of enzymes

The culture cultivation of *L*. *edodes* on the two prepared biomasses and the subsequent preparation of crude enzyme extracts were undertaken as previously detailed (Sibanda et al., 2019).

Assaying for lignocellulolytic activities

L. *edodes'* probable ability to secrete various lignocellulolytic enzymes when cultured on water hyacinth as a potential substrate (and liverseed grass as a control substrate) was assessed via the agar plate-based clearing assay and spectrophotometric methods (Miller, 1959; Pointing, 1999; Sibanda et al., 2019a; Téllez-téllez et al., 2013).

Resolution and analysis of the secreted total protein content in the crude enzyme extract

Total protein content secreted by *L. edodes* in the crude enzyme extract during its growth on water hyacinth was resolved by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), according to a previously established method (Laemmli, 1970). In addition, the subsequent zymographic analyses of the resolved protein bands for cellulolytic and xylanolytic activities were carried out in accordance with the method of Téllez-téllez et al. (2013) and Pointing (1999). For more detail, refer to Sibanda et al. (2019a).

Reaction kinetics of the cellulolytic and xylanolytic protein fractions in the crude enzyme extract

Reaction kinetics of the SDS-PAGE resolved cellulolytic and xylanolytic protein fractions in the crude enzyme extract were assessed and determined via the Hanes-Woolf plot and in accordance with the established method of Kwezi et al. (2011) and Meier et al. (2010). For further detail, refer to Sibanda et al. (2019a).

Statistical analysis

All data from enzyme assaying in this work are means of triplicate assays (n = 3) subjected to analysis of variance (ANOVA) (Super-Anova, Statsgraphics Version 7; Statsgraphics Corp., The Plains, VI, USA). Wherever ANOVA revealed significant differences between treatments, means were separated by *post hoc* Student–Newman–Keuls (SNK) multiple range test ($p < 0.05$).

RESULTS

L. edodes has a good viability status to grow on water hyacinth as a potential substrate

When the viability status of *L*. *edodes*, as a test fungus for the study, was tested through spawn culturing, it became apparent that this white rot fungus could both viably and significantly grow on either the two provided commercial grade substrates (carboxymethylcellulose and birchwood xylan) (Figure 1a) or the test experimental substrate (water hyacinth) (Figure 1b). On water hyacinth, *L*. *edodes* produced hyphal growths that could be visually

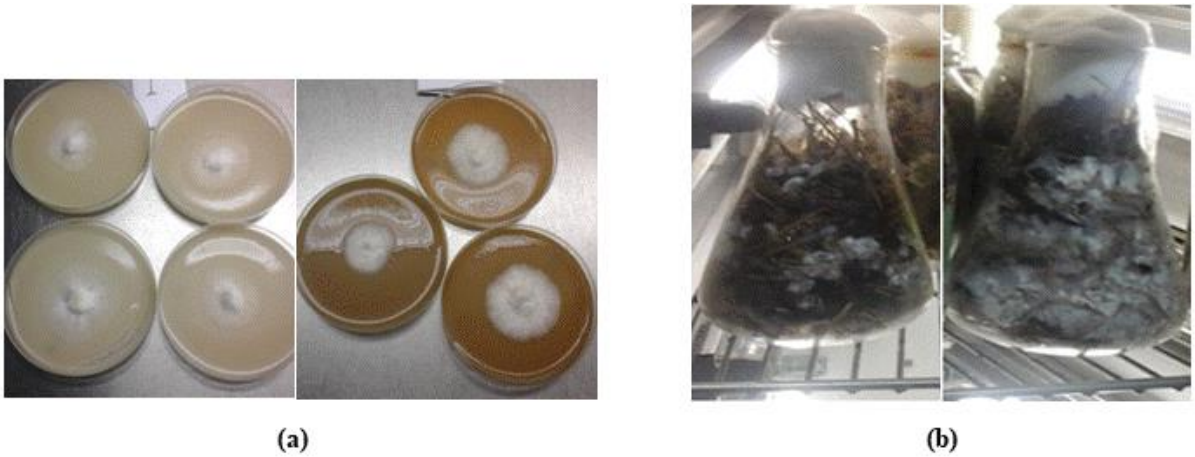

Figure 1. Assessing the viability status of *L*. *edodes* and testing for its ability to grow on water hyacinth as a possible substrate. (**a**) *L*. *edodes* cultured on commercial grade carboxymethylcellulose (left plane) and birchwood xylan (right plane). **(b**) *L*. *edodes* growing on water hyacinth as a potential substrate (left plane) and liverseed grass as a control substrate (right plane).

observed on the substrate biomass, thus demonstrating its probable capabilities to physiologically utilize the tested experimental biomass as a substrate.

L. edodes has the ability to secrete lignocellulolytic enzymes when cultured on water hyacinth

Using the agar plate-based clearing assay method, it was noticed that the *L*. *edodes* could viably secrete a whole cocktail of the lignocellulolytic enzymes during its growth on water hyacinth as a potential substrate (Figure 2). In this particular assaying method, lignocellulolytic enzymes secreted by microbes, breakdown and solubilize complex lignocellulosic polysaccharides in the media to generate zones of clearance on the solidified agar plates that can be easily visualized as clear halos after addition of particular dyes and clearing with specific de-staining solutions (Sibanda et al., 2019a; Téllez-téllez et al., 2013) (Figure 2a-e).

Validating the lignocellulolytic capacity of *L. edodes* when cultured on water hyacinth

Using spectrophotometry, it was validated that the *L*. *edodes* could actually produce a whole cocktail of the lignocellulolytic enzymes when grown on water hyacinth as a substrate (Figure 3). In this advanced scientific technique, a 3,5-dinitrosalicylic acid detection system for reducing sugars is employed. It results in the generation of coloured compounds that are measurable by various analytical equipment and easily convertible into rates of enzyme activity (Miller, 1959; Pointing, 1999); for instance, and in this case, ~ 1.2650, 2.0625, 2.4375, 0.9375, 2.0225 µmole/sec/ml for lignin peroxidases, laccases, cellulases, pectinases and xylanases, respectively (Figure 3).

Resolution and activity assaying of the various protein fractions in the crude enzyme extract

When SDS-PAGE was used to resolve the various enzymatic protein fractions secreted by *L. edodes* during its growth on water hyacinth for probable further analysis, it emerged that only fractions of the molecular weight sizes of around 50-70 kDa and 20-25 kDa could be visually observed (Figure 4a). This kind of resolution thus suggested cellulases and xylanases respectively. Notably, a further analysis of the same SDS-PAGE gel by zymography then firmly confirmed that such ~50-70 kDa and ~20-25 kDa protein fractions were indeed carboxymethylcellulose-degrading (Figure 4b) and birchwood xylan-degrading proteins (Figure 4c) respectively.

Kinetic assaying of the cellulolytic and xylanolytic activities in the crude enzyme extract

After determining that, when cultured on water hyacinth, *L. edodes* mostly produces cellulolytic and xylanolytic proteins as its major components, the kinetic rates of these two most secreted protein components were then assessed and ascertained via the Hanes-Woolf plot

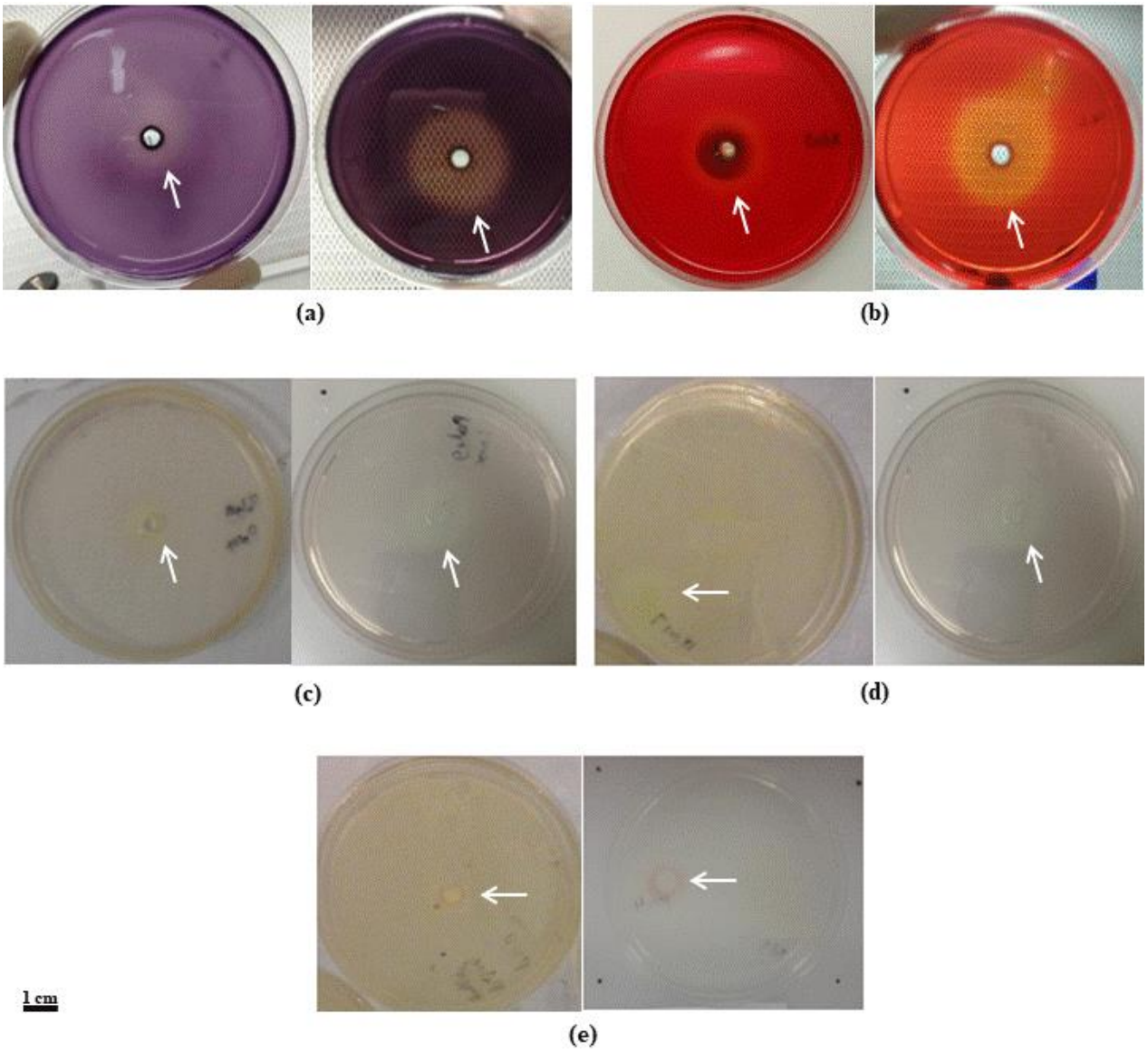

Figure 2. Testing the ability of *L*. *edodes* to secrete various lignocellulolytic enzymes when cultured on water hyacinth. (**a**) Cellulase, (**b**) xylanase, (**c**) pectinase, (**d**) lignin peroxidase and (**e**) laccase activities of the extracellular enzyme extract secreted by *L*. *edodes* when cultured on water hyacinth as a potential substrate (left planes) and liverseed grass as a control substrate (right planes), as is determined by the agar plate-based clearing assay method. Arrows mark ends of the zones of clearance and the scale relates to sizes of diameters of the observed zones of clearance.

method (Figure 5). This was undertaken in order to relate activities of these two major enzymatic protein components to their counterparts in other known organisms and/or related microbial systems. Based on this approach and as is shown in Figure 5, a K_m value of 0.247 mM and a V_{max} value of 2 177.88 µmol/sec for the cellulolytic proteins were obtained (Figure 5a) while a K_m value of 0.147 mM and a V_{max} value of 1 208.33 µmol/sec for the xylanolytic proteins were obtained (Figure 5b).

DISCUSSION

Water hyacinth or *E. crassipes* (Mart.) Solms-Laubach. originates from Brazil (Crow et al., 2000; Penfound and Earle, 1948) and has by this day spread to almost all tropical and sub-tropical nations such as Zimbabwe (Parsons et al., 2001), where it is considered as one of the world's most deadliest invasive aquatic plants (Mwangi, 2013). The plant is perennial and mostly

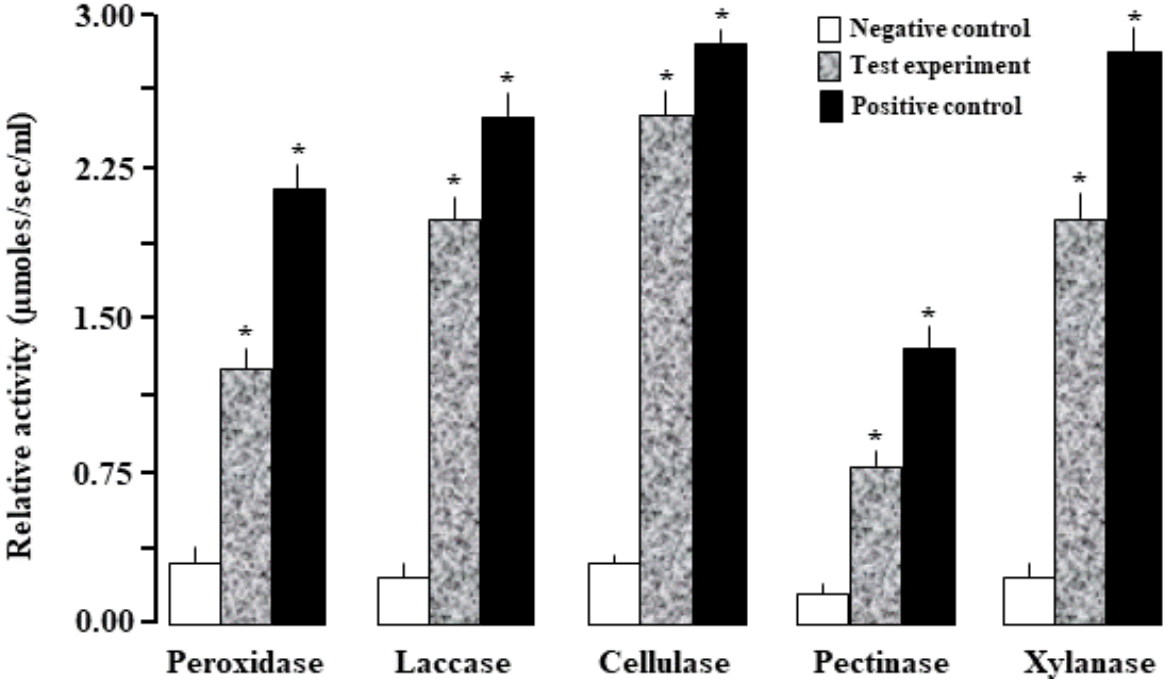

Figure 3. Spectrophotometric determination of the ability of *L. edodes* to secrete lignocellulolytic enzymes when cultured on water hyacinth. Activity was measured in the absence of enzymes (clear bars; negative control) and in the presence of enzymes secreted by the *L. edodes* when cultured on water hyacinth (grey texture; test experiment) and liverseed grass (black bars; positive control). Data are mean values (n = 3) and error bars show standard errors (SE) of the mean. Asterisks denote significant differences from the negative controls ($P < 0.05$) as determined by the ANOVA and *post hoc* Student-Newman-Keuls multiple range tests.

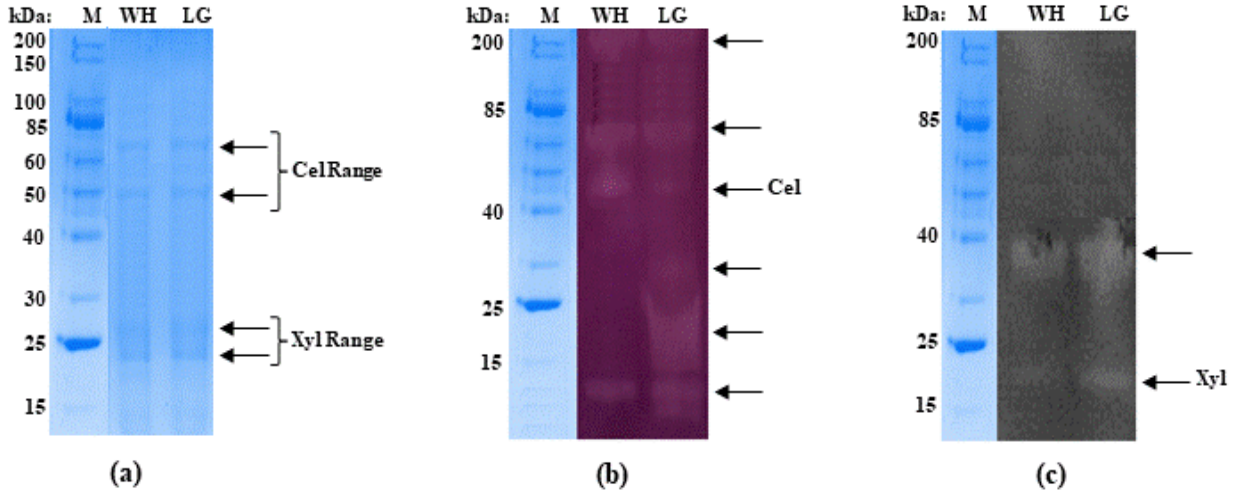

Figure 4. Resolution and activity assaying of the various protein fractions secreted by *L. edodes* when cultured on water hyacinth (WH) as a test substrate or liverseed grass (LG) as a control substrate. (**a**) an SDS-PAGE gel showing the resolution of secreted prominent protein fractions in the crude extract, (**b**) a zymogram showing the decomposition of carboxymethylcellulose in the gel by cellulases in the crude extract and (**c**) a zymogram showing the degradation of birchwood xylan in the gel by xylanases in the crude extract. In all the three gels, M represents the standard molecular weight marker (Fermenters Int., Burlington, Canada) while arrows mark the various ranges of cellulases and xylanases in the crude extract resolved by the gels.

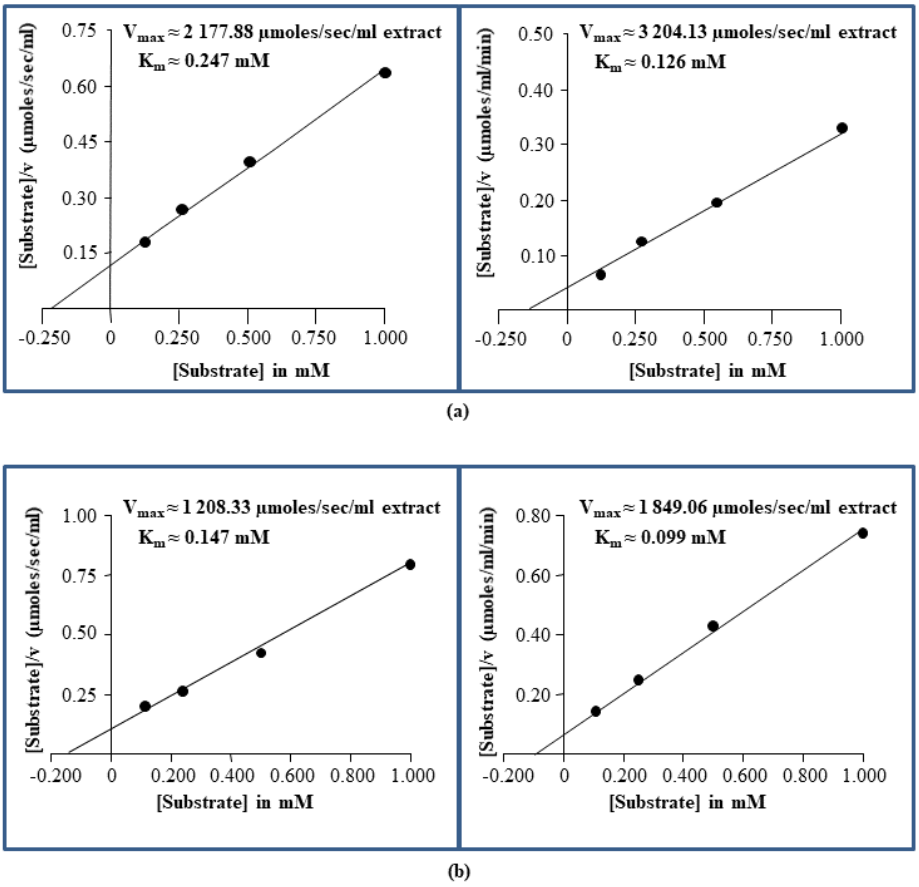

Figure 5. Reaction kinetics. Kinetic rates of the (**a**) cellulolytic and (**b**) xylanolytic protein fractions in the crude extracellular extract secreted by *L. edodes* when cultured on water hyacinth as a test substrate (left planes) or liverseed grass (right planes) as a control substrate. K_m was determined as the negative value of the x-intercept ($x = -K_m$, when $y = 0$) of the linear fit of the data set while V_{max} was calculated from the y-intercept ($y = K_m/V_{max}$, when $x = 0$) of the same linear fit. All values obtained indicate means of three independent and fully representative experiments (n = 3).

wide-spread on freshwater wetlands of most tropical and sub-tropical areas, particularly in stagnant water (MWBP/RSCP, 2006). The hydrophyte multiplies very rapidly, forming dense mats (Gopal and Goel, 1993) that normally interfere with existing waterways, ruin aquatic life and create suitable conditions for breeding of parasitic vectors and the outbreak of their related diseases (Kushwaha, 2012). Water hyacinth is also known to have various notable ecological and socio-economic issues, which among others, include suppression of the local aquatic biodiversity, obstruction of river flows which may aggravate flooding and promote siltation, interference with water utilization for activities such as recreation, tourism and/or irrigation, and increased rates of evapotranspiration from water storages (Chikwenhere, 1994; Cilliers et al., 2003). Its infestation also poses a potential health risk in that the plant has been implicated in the creation of breeding habitats for mosquitos and/or their larvae that can cause malaria as well as other water disease-carrying vectors like bilharzia snails (Villamagna and Murphy, 2010).

However, and despite its various negative impacts as a notorious weed, water hyacinth has several other potential benefits to humankind, which include its use as a protein supplement in animal feeds (Mako et al., 2011; Yagi et al., 2019), for water purification, as fibreboard or fertilizer and in paper and biogas production (Lindsey and Hirt, 1999). The fact that this plant has very high levels of protein content (especially in its leaves) (Virabalin et al., 1993) accompanied by its rapid growth (Gopal, 1987), has essentially made it very suitable for this hydrophyte to be commonly used as fodder (Yagi et al., 2019) for the

various kinds of livestock such as cows (Rashid and Iqbal, 2012), sheep (Abdalla et al., 1987), goats (Dada et al., 2002) and pigs (Men et al., 2006), and domesticated birds such as ducks (Jianbo et al., 2008) as well as the aquatic fishes like tilapia fingerlings (El-Sayed, 2003). Moreover, the harvesting process of this hydrophyte for use as fodder is quite simple and straightforward as it can be done manually on a small scale level and without requiring any new harvesting techniques to be introduced (Gunnarsson and Petersen, 2007). In the middle-income earning countries such as Vietnam and Thailand, where poor quality rice straw is mainly the major source of fibre, water hyacinth has proved as an excellent alternative. Furthermore, in tropical African countries like Tanzania, this aquatic plant has since been proven to be very good substrate for large-scale production of either the exotic mushroom, *Pleurotus ostreatus* (Çağlarırmak, 2007) or its indigenous counterpart, *P*. *flabellatus* (Kivaisi et al., 2003). The use of this plant (for example as livestock feed or for production of mushrooms) for the general benefit of mankind is typically considered as an effective method of its mechanical control in most nations (Murugesan et al., 2006).

Apparently, in an African tropical country like Zimbabwe, none of these efforts have ever been reported and/or made. As a result, this present study was therefore, undertaken to assess if the edible fungus *L*. *edodes* could perhaps grow successfully on water hyacinth native to the waters of that country and utilizing it as a substrate. A successful use of this problematic plant as a substrate for *L*. *edodes* could probably serve as a sustainable and cost-effective way of controlling it in the Zimbabwean local aquatic ecosystems while at the same time, generating protein-rich foods for the surrounding communities and perhaps, also production of commercial enzymes and/or fine chemicals for the local and/or national industries.

The *L*. *edodes* strain used in this study is an exotic mushroom that was commercially acquired from a local supplier Sylvan Africa (PTY) Ltd., (Pretoria, South Africa) in form of a partially-dried spawn. However, before this fungal strain could be used in the planned study, its growth viability was first checked and ascertained through culturing on two different substrates of commercial grade. As is shown in Figure 1a, the test fungus could both viably and vigorously grow on either carboxymethylcellulose or birchwood xylan. When the *L*. *edodes* was then cultured on water hyacinth, followed by assessment of its ability to grow on this test substrate, biomass colonization was relatively good with hyphal almost completely covering the whole substrate (Figure 1b). This could be as a result of the *L*. *edodes* secreting the various lignocellulolytic enzymes that then enabled it to grow and colonize the provided substrate. Generally, white rot fungi like *L*. *edodes* are known to be capable of secreting oxidases (laccases and peroxidases) that degrade lignin (Wesenberg et al., 2003; Zirbes and Waldvogel, 2018), glucanases (exo- and endo-) that breakdown cellulose (Kuhad et al., 2011; Legodi et al., 2019), xylanases that degrade hemicelluloses (Punniavan, 2012), and pectinases that breakdown pectins (Collins et al., 2005).

Naturally, some white rot fungi produce the whole cocktail of lignocellulolytic enzymes while others produce only one or a few of them (Baldrian and Valášková, 2008; Maganhotto de Souza Silva et al., 2005; Wang et al., 2019). Therefore, in order to ascertain if the *L*. *edodes* was capable of secreting the whole cocktail of the white rot fungal enzymes or part of it when growing on water hyacinth, its crude extracellular extract was tested for the various lignocellulolytic enzyme activities via the agar plate-based clearing assay method (Figure 2) and spectrophotometry (Figure 3). Under the agar plate-based clearing assay method, the Congo red assay showed zones of clearance in diameters of over 2.74 cm for cellulases (Figure 2a) and 2.53 cm for xylanases (Figure 2b), demonstrating ability of the excreted enzyme extract to breakdown carboxymethylcellulose and birchwood xylan respectively. These revealed zones of clearance were not that much different from the ones generated by plant endophytes, *P*. *ostreatus* and some filamentous fungi from termite mounds on the same commercial substrates, which were >2 cm (Eichlerová et al., 2012; Sibanda et al., 2019a). Such capabilities may be gained due to the adaptation abilities of fungi to their habitats, which are a whole set of lignocellulosic materials (Yopi et al., 2014). A related trend of clearance was also observed for the pectinases (Figure 2c), lignin peroxidases (Figure 2d) and laccases (Figure 2e), signifying ability of the excreted enzyme extract to hydrolyze polygalacturonic acid, veratryl alcohol, and guaiacol respectively.

The same results as is reported above were also revealed by spectrophotometry (Figure 3), a method that is alternative to the agar plate-based clearing assay but being rather more sensitive. Collectively, these findings therefore, showed that the *L*. *edodes* is capable of secreting the whole cocktail of the white rot fungal enzymes when grown on water hyacinth, and thus able to utilize this notorious weed as an alternative substrate. *Lentinus* spp. have previously been reported to have a unique ability to produce xylanases (Bhagobaty et al., 2007), carboxymethylcellulases, β-glucosidases, β–xylosidases, and extracellular lignocellulolytic enzymes, including laccases, pectinases and lignin peroxidases (Elisashvili et al., 2015; Jia et al., 2019; Wang et al., 2019).

When the various fractions of the total protein content secreted by the *L*. *edodes* during its growth on water hyacinth were resolved by SDS-PAGE for further

analysis, it emerged that the dominant protein fractions produced were most likely cellulases (~50-70 kDa) and xylanases (~20-25 kDa) (Figure 4a). Cellulases are multi-enzyme complexes composed of various protein components with endoglucanase, exoglucanase and β-glucosidase activities that normally operate synergistically (Legodi et al., 2019; Liming and Xueliang, 2004; Stajić et al., 2006). Of these protein components, cellobiohydrolase I (52.2 kDa) and cellobiohydrolase II (47.2 kDa) are the predominant ones (>90%) while endoglucanases and hemicellulases represent less than 10% (Da Vinha et al., 2011). On the other hand, xylanases are single polypeptide chain proteins with a molecular weight size of around 21 kDa (as judged by SDS-PAGE) and a pI value of 4.5 (Bray and Klarke, 1995; Zirbes and Waldvogel, 2018). Unlike cellulases, xylanases are not glycosylated (Bray and Klarke, 1995).

Notably, when the same SDS-PAGE gel described above (Figure 4a) was further subjected to a zymogram analysis, results obtained then showed that the resolved ~50-70 kDa proteins were indeed responsible for the decomposition of carboxymethylcellulose (Figure 4b) while the ~20-25 kDa proteins were responsible for the degradation of birchwood xylan (Figure 4c), thus firmly affirming our initial claim in the SDS-PAGE analysis (Figure 4a) that the ~50-70 kDa proteins were cellulases while the ~20-25 kDa proteins were xylanases. Incidentally, our work also managed to reveal a number of carboxymethylcellulose-decomposing proteins, ranging from ~10-200 kDa (Figure 4b), concurring with the fact that cellulases are multi-enzyme complexes composed of various protein components such as endoglucanase I (46.0 kDa), II (42.2 kDa), IV (33.4 kDa), V (22.8 kDa) and VII (25.1 kDa); cellobiohydrolase I (52.2 kDa) and II (47.2 kDa); β-glucosidase I (75.3 kDa) and II (52.1 kDa); and β-glucosidase-1,4-glucanase (23.5 kDa) (Da Vinha et al., 2011; Legodi et al., 2019). This outcome is closely related to that of Elisashvili et al. (2015), who recorded carboxymethylcellulose-decomposing proteins of around 25, 50 and 100 kDa from three unnamed Indonesian endophytic fungi, isolated from medicinal plants (Yopi et al., 2014); and to that of Ncube et al. (2012), who reported molecular masses of 20-45 kDa for cellulases isolated from *Aspergillus niger* when *Jatropha curcas* seed cake was substrate (Ncube et al., 2012). Furthermore, Nayebyazdi et al. (2012) reported a range of cellulolytic proteins of the molecular weight size 25-50 kDa in *Trichoderma reesei* and *Phanerochaete* spp. (Nayebyazdi et al*.,* 2012). Overall, other studies that have been undertaken and reviewed independently, also have reported the molecular masses of fungal cellulases to be as low as 12 kDa and up to 250 kDa (Kuhad et al., 2011; Li et al., 2011; Liming and Xueliang, 2004; Ritter et al*.,* 2013; Vivekanandan et al., 2014; Zhang and Zhang, 2013). In addition, our work also revealed numerous birchwood xylan-hydrolyzing proteins of the molecular size range of ~20-40 kDa (Zirbes and Waldvogel, 2018) (Figure 4c), relating closely with findings of the other previously undertaken studies. For instance, some xylanolytic proteins of the molecular weight size ranges of 20-50 kDa, 18-52 kDa, 29 kDa, 19 kDa, and 45-70 kDa were reported in endophytes (Polizeli et al*.,* 2005), *A*. *aculeatus* (Fujimoto et al*.,* 1995), *Hypocrea lixii* (Sakthiselvan et al., 2014), *A*. *fumigatus* (Silva et al*.,* 1999), and *Neocallimastix frontalis* (de Segura and Fevre, 1993) respectively.

After determining that, when grown on water hyacinth, *L*. *edodes* mostly secretes cellulases and xylanases as its main protein components, the kinetic rates of these two highly produced lignocellulolytic protein components were then assessed and ascertained via the Hanes-Woolf plot method (Figure 5) (Irving et al., 2011; Meier et al., 2010; Sibanda et al., 2019a). This was done in order to relate activities of these two major *L*. *edodes* enzymes to their counterparts in other known organisms and/or related microbial systems. For the cellulases, the K_m value of 0.247 mM and V_{max} of 2 177.880 µmol/sec were obtained (Figure 5a). These obtained kinetic values are in close agreement with those previously shown by other closely related cellulases (Sibanda et al., 2019a) and the other various recombinant cellulases isolated from other different organisms such as termites, filamentous fungi and protists, whose K_m values ranged from 2.0 to 14.7 mM and V_{max} values ranging from 0.84 to 1 667.00 µmol/sec (Table 1).

For the xylanases, the K_m value of 0.147 mM and V_{max} of 1 208.330 µmol/sec were obtained (Figure 5b). Once more, these values do concur with the reaction kinetic rates of the other closely related xylanases (Sibanda et al., 2019a) and the other numerous recombinant and non-recombinant xylanases isolated from other different microorganisms such as termite fungal symbionts and bacteria, whose K_m values ranged from 3.920 to 6.960 mM and V_{max} values ranging from 256.000 to 7 407.000 µmol/sec (Table 2).

Apparently, when comparing the kinetic ratios (i.e., V_{max}/K_m) of cellulases to those of xylanases within as a single organism, it emerged from our work that the *L*. *edodes* cellulases had a relatively higher ratio than that of the xylanases (Figure 5). Arguably, this scenario is not unusual because previously, a recombinant protein from *Clostridium thermocellum*, CtCel5E, that had a dual function as a cellulase and xylanase, displayed a K_m value of 2.1 mM and a V_{max} of 1 564 µmol/sec for the cellulase and a K_m value of 4.6 mM and a V_{max} of 883.5 µmol/sec for the xylanase (Yuan et al., 2015). Notably, all the kinetic values of the CtCel5E together with most of the proteins in Tables 1 and 2 were generally lower than those of our own crude enzyme extract in this study, probably due to three possible technical reasons. Firstly,

Table 1. Reaction kinetics of the various recombinant cellulases isolated from different organisms.

Name of protein	Origin of the protein	Name of the expression host	K_m Value (mM)	V_{max} value (µmol/sec)	Reference
PA68	Chimeric termite	*Escherichia coli*	12.7	889.00	Ni et al. (2007)
RsEG	*Reticulitermes speratus*	*Aspergillus orizae*	2.0	1 429.00	Hirayama et al. (2010)
NtEG	*Nasutitermes takasagoensis*	*Aspergillus orizae*	4.7	1 667.00	Hirayama et al. (2010)
Cell-1	*Reticulitermes flavipes*	*Escherichia coli*	14.7	0.84	Zhou et al. (2010)
CfEG5	*Coptotermes formosanus*	*Escherichia coli*	5.6	548.00	Zhang et al. (2011)
RsSymEG1	Protist from *Reticulitermes flavipes*	*Aspergillus orizae*	2.0	769.60	Todaka et al. (2010)

CMC being the sole substrate of the reactions (adapted from Ni and Tokuda (2013)).

Table 2. Reaction kinetics of the various recombinant and non-recombinant xylanases isolated from different microorganisms.

Name of protein	Origin of the Protein	Name of the expression host	K_m value (mM)	V_{max} value (µmol/sec)	Reference
Xyl6E7	Fungal symbiont isolated from the gut of the termite *Macrotermes annandalei*	*Escherichia coli*	6.96	1 057.8	Liu et al. (2011)
Extracellular xylanase	*Saccharopolyspora pathumthaniensis* S582	Non-recombinant	3.92	256.0	Sinma et al. (2011)
GHF10 xylanase	*Paenibacillus macerans* IIPSP3	Non-recombinant	6.00	7 407.0	Dheeran et al. (2012)

Birchwood xylan being the sole substrate of the reactions (adapted from Ni and Tokuda (2013).

most of the proteins in Tables 1 and 2, including CtCel5E were recombinant while proteins in our own extract were not. Secondly, the source of some of the proteins, including CtCel5E was bacterial or prokaryotic whilst that of our own was fungal or eukaryotic, of which fungi are naturally known to be superior producers of lignocellulolytic enzymes (Favaro et al., 2013; Ramanjaneyulu et al., 2015). Lastly and in the event that substrate concentration was a limiting factor in the study, the cellulose content of most lignocellulosic substrates is always higher than that of hemicellulose, e.g., wheat straw, rice straw, switch grass and sugarcane bagasse - all have around 35% cellulose and at most 25% hemicellulose content (Chen, 2014; Koshy and Nambisan, 2012; Shawky et al., 2011).

Overall, comparing water hyacinth to its control substrate (liverseed grass), it is apparent that the control substrate was always performing better throughout the study. However, it is rather worth to note that the control substrate naturally has a higher biomass composition compared to the experimental substrate; that is, 30% cellulose, 50% hemicellulose, 20% lignin and 1% pectin for liverseed grass (Howard et al., 2013; Saito et al., 2003) and 20% cellulose, 33% hemicelluloses, 10% lignin, and 1% pectin for water hyacinth (Avci et al., 2013). In addition, water hyacinth has always been reported to possess a very high adsorption capacity that makes it capable of taking up numerous nutrients, toxic chemicals and metal substances, which perhaps may inhibit enzyme activity on its biomass (Idrees et al., 2013; Moyo and Mapira, 2012; Reddy and D'Angelo, 1990; Saha et al., 2014; Tham, 2012; Usha et al., 2014). However, even though the general production of mushrooms has always been undertaken using liverseed grass, rice or wheat straw, the overall performance of water hyacinth in this study as a substrate (0.75-0.98 folds) strongly proposes it as a probable alternate.

Finally, by collectively summing up all findings of this study, it is conceivable that the water hyacinth native to Zimbabwe can be viably utilized as a substrate of *L*. *edodes*, which if properly optimized, the approach can then be used as a sustainable and cost-effective way (Jia et al., 2019; Thakur, 2018) of managing this problematic and noxious weed in the country. More so, the possible effective utilization of this aquatic weed as a substrate of *L*. *edodes* can also be tailor-made towards the production of protein-rich mushrooms (Thakur, 2018) for local communities and a whole cocktail of the white rot lignocellulolytic enzymes (Wang et al., 2019) as well as specific fine chemicals (Zirbes and Waldvogel, 2018) for various applications in the industry and/or commercial systems. In addition, the degradation of a highly lignocellulosic biomass like water hyacinth by the white rot fungus *L*. *edodes* (Wang et al., 2019) produces various

fermentable carbohydrates with numerous potential industrial applications such as bio-fuel, food, brewery and winery, animal feed, textile and laundry, pulp and paper and agriculture, which when properly optimized, may encourage communities to harvest this noxious aquatic weed and ameliorate its unabated growth and expansion. In this regard therefore, our study hereby strongly recommends for a further optimization of its findings so that *L*. *edodes* can be viably utilized for the sustainable and cost-effective management of water hyacinth in Zimbabwe (and even in other tropical and sub-tropical countries, where the weed is endemic).

CONFLICT OF INTERESTS

The authors declare no competing interests.

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REFERENCES

Abdalla A, Ambrosano EJ, Vitti SS, Silva FJC (1987). Water-hyacinth (Eichhornia crassipes) in ruminant nutrition. Water Science Technology 19(10):109-112.

Avci A, Saha BC, Dien BS, Kennedy GJ, Cotta MA (2013). Response surface optimization of corn stover pretreatment using dilute phosphoric acid for enzymatic hydrolysis and ethanol production. Bioresources Technolology 130:603-612.

Baldrian P, Valášková V (2008). Degradation of cellulose by basidiomycetous fungi. FEMS Microbiology Reviews 32:501-521.

Bhagobaty RK, Joshi SR, Malik A (2007). Microbial degradation of organophosphorous pesticide: Chlorpyrifos (Mini-Review). International Journal of Microbiology 4:1-5.

Bray MR, Clarke AJ (1995). Identification of an essential tyrosyl residue in the binding site of *Schizophyllum commune* xylanase A. Biochemistry 34:2006-2014.

Brown C (2006). Marine and coastal ecosystems and human well-being: Synthesis. United Nations Publications.

Buswell J, Cai Y, Chang S (1993). Fungal- and substrate-associated factors affecting the ability of individual mushroom species to utilize different lignocellulosic growth substrates. Conference Proceedings of The First International Conference on Mushroom Biology and Mushroom Products pp. 111-140.

Çağlarırmak N (2007). The nutrients of exotic mushrooms (*Lentinula edodes* and *Pleurotus species*) and an estimated approach to the volatile compounds. Food Chemistry 105:1188-1194.

Chen H (2014). Chemical composition and structure of natural lignocellulose. In Biotechnology of lignocellulose. Springer pp. 25-71.

Chikwenhere GP (1994). Biological control of water hyacinth (*Eichhornia crassipes*) in Zimbabwe: Results of a pilot study. FAO Plant Protection Bulletin 42:185-190.

Cilliers CJ, Hill MP, Ogwang JA, Ajuonu O (2003). Aquatic weeds in Africa and their control. CABI Publishing. Technology and Engineering 1991:161-178.

Cohen R, Persky L, Hadar Y (2002). Biotechnological applications and potential of wood-degrading mushrooms of the genus *Pleurotus*. Applied Microbiology and Biotechnology 58:582-594.

Collins T, Gerday C, Feller G (2005). Xylanases, xylanase families and extremophilic xylanases. FEMS Microbiology Review 29:3-23.

Crow GE, Hellquist CB, Fassett NC (2000). Aquatic and wetland plants of northeastern North America pp. 121-150.

Da Vinha FNM, Gravina-Oliveira MP, Franco MN, Macrae A, da Silva Bon EP, Nascimento RP, Coelho RRR (2011). Cellulase production by *Streptomyces viridobrunneus* SCPE-09 using lignocellulosic biomass as inducer substrate. Applied Biochememistry and Biotechnology 164:256-267.

Dada SAO, Akinsoyinu AO, Smith JW, Dashiell KE (2002). The effect of leaf-pruning on nutrient intake and *in vivo* digestibility of soybean stovers by sheep. Journal of Sustainable Agriculture 19:5-14.

Dheeran P, Nandhagopal N, Kumar S, Jaiswal YK, Adhikari DK (2012). A novel thermostable xylanase of *Paenibacillus macerans* IIPSP3 isolated from the termite gut. Journal of Industrial Microbiology and Biotechnology 39:851-860.

de Segura BG, Fevre M (1993). Purification and characterization of two 1, 4-beta-xylan endohydrolases from the rumen fungus *Neocallimastix frontalis*. Applied Environmental Microbiology 59(11):3654-3660.

Dorado J, Field JA, Almendros G, Sierra-Alvarez R (2001). Nitrogen-removal with protease as a method to improve the selective delignification of hemp stemwood by the white-rot fungus Bjerkandera sp. strain BOS55. Applied Microbiology and Biotechnology 57:205-211.

Eichlerová I, Šnajdr J, Baldrian P (2012). Laccase activity in soils: Considerations for the measurement of enzyme activity. Chemosphere 88:1154-1160.

Elisashvili V, Kachlishvili E, Asatiani MD (2015). Shiitake medicinal mushroom, *Lentinus edodes* (higher basidiomycetes) productivity and lignocellulolytic enzyme profiles during wheat straw and tree leaf bio-conversion. International Journal of Medicinal Mushrooms 17:77-86.

El-Sayed AFM (2003). Effects of fermentation methods on the nutritive value of water hyacinth for Nile tilapia *Oreochromis niloticus* (L.) fingerlings. Aquaculture 218:471-478.

Favaro L, Jooste T, Basaglia M, Rose SH, Saayman M, Görgens JF, van Zyl WH (2013). Designing industrial yeasts for the consolidated bioprocessing of starchy biomass to ethanol. Bioengineered 4(2):97-102.

Fujimoto H, Ooi T, Wang SL, Takizawa T, Hidaka H, Murao S, Arai M (1995). Purification and properties of three xylanases from *Aspergillus aculeatus*. Bioscience, Biotechnology and Biochemistry 59(3):538-540.

Gopal B (1987). Water Hyacinth. Aquatic Plant Studies 1:471.

Gopal B, Goel U (1993). Competition and allelopathy in aquatic plant communities. The Botanical Review 59:155-210.

Gunnarsson CC, Petersen CM (2007). Water hyacinths as a resource in agriculture and energy production: A literature review. Waste Management 27:117-129.

Gutierrez EL, Ruiz EF, Uribe EG, Martinez JM (2001). Biomass and productivity of water hyacinth and their application in control programs. Biological and integrated control of water hyacinth, *Eichhornia crassipes.* ACIAR Proceedings 102:109-199.

Hirayama K, Watanabe H, Tokuda G, Kitamoto K, Arioka M (2010). Purification and characterization of termite endogenous β-1,4-endoglucanases produced in *Aspergillus oryzae*. Bioscience,

Biotechnology and Biochemistry 74:1680-1686.
Howard RL, Abotsi E, Jansen Van Rensberg EL (2013). Development of a fungal cellulolytic enzyme combination for use in bioethanol production using Hyparrhenia spp as a source of fermentable sugars. PhD Thesis. University of Limpopo P 239.
Idrees M, Adnan A, Sheikh S, Qureshi FA (2013). Optimization of dilute acid pre-treatment of water hyacinth biomass for enzymatic hydrolysis and ethanol production. EXCLI Journal 12:30-40.
Irving HR, Wheeler JI, Iacuone S, Kwezi L, Gehring C, Thompson PE, Ruzvidzo O, Govender K (2011). The phytosulfokine (PSK) receptor is capable of guanylate cyclase activity and enabling cyclic GMP-dependent signaling in plants. Journal of Biological Chemistry 286:22580-22588.
Jia Z, Chen N, Shi W, Tang X, Xu H (2019). Bioremediation of cadmium-dichlorophen co-contaminated soil by spent *Lentinus edodes* substrate and its effects on microbial activity and biochemical properties of soil. Journal of Soils and Sediments 17(2):315-325.
Jianbo LU, Zhihui FU, Zhaozheng YIN (2008). Performance of a water hyacinth (Eichhornia crassipes) system in the treatment of wastewater from a duck farm and the effects of using water hyacinth as duck feed. Journal of Environmental Sciences 20:513-519.
Jurado M, Martinèz ÀT, Martinez MJ, Saparrat MCN (2011). Application of white-rot fungi in transformation, detoxification, or revalorization of agriculture wastes. Second Edition. Comprehensive Biotechnology. pp. 75-104.
Kiiskinen L-L, Kruus K, Bailey M, Ylo¨sma¨ki E, Siika-aho M, Saloheimo M (2004). Expression of Melanocarpus albomyces laccase in Trichoderma reesei and characterization of the purified enzyme. Microbiology 150:3065-3074.
Kivaisi A, Magingo F, Mamiro B (2003). Performance of *Pleurotus flabellatus* on water hyacinth (*Eicchornia crassipes*) shoots at two different temperature and relative humidity regimes. Tanzanian Journal of Science 5:11-25.
Koshy J, Nambisan P (2012). Pre-treatment of agricultural waste with pleurotus sp. for ethanol production. International Journal of Plant, Animal and Environmental Sciences 2(2):244-249.
Kuhad RC, Gupta R, Singh A (2011). Microbial cellulases and their industrial applications. Enzyme Research 20:11-24.
Kushwaha SPS (2012). Remote sensing of invasive alien plant species. In: Bhatt JR, Singh JS, Singh SP, Tripathi RS, Kohli RK, editors. Invasive alien plants: An ecological appraisal for the Indian sub-continent. CABI International; United Kingdom pp. 131-138.
Kwezi L, Ruzvidzo O, Wheeler JI, Govender K, Iacuone S, Thompson PE, Irving HR (2011). The phytosulfokine (PSK) receptor is capable of guanylate cyclase activity and enabling cyclic GMP-dependent signaling in plants. Journal of Biological Chemistry 286(25):22580-22588.
Laemmli UK (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227(5259):680-685.
Leatham GF (1985). Extracellular enzymes produced by the cultivated mushroom *Lentinus edodes* during degradation of a lignocellulosic medium extracellular enzymes produced by the cultivated mushroom lentinus edodes during degradation of a lignocellulosic medium.Applied and Environmental Microbiology 4:859-867.
Legodi LM, Grange DL, Van Rensburg EL, Ncube I (2019). Isolation of cellulose degrading fungi from decaying banana pseudo stem and *Strelitzia alba.* Enzyme Research 2019:1-10.
Liming X, Xueliang S (2004). High-yield cellulase production by *Trichoderma reesei* ZU-02 on corn cob residue. Bioresource Technology 91:259-262.
Lindsey K, Hirt HM (1999). Use water hyacinth! A practical hanbook of uses for the water hyacinth from across the world. Anamed: Winnenden P 114.
Liu S, Liu L, Uzuner U, Zhou X, Gu M, Shi W, Zhang Y, Dai SY, Yuan JS (2011). HDX-analyzer: A novel package for statistical analysis of protein structure dynamics. BMC Bioinformatics 12(1):S43.
Magadza CHD (2003). Lake Chivero: A management case study. Lakes and Reserviors Research and Management 8:69-81.
Maganhotto de Souza Silva CM, Soares de Melo I, Roberto de Oliveira P (2005). Ligninolytic enzyme production by *Ganoderma spp*. Enzyme and Microbial Technology 37:324-329.
Mako AA, Babayemi OJ and Akinsoyinu AO (2011). An evaluation of nutritive value of water hyacinth (*Eichhornia crassipes* Mart. Solms-Laubach) harvested from different water sources as animal feed. Livestock Research for Rural Development 23(103) http://www.lrrd.org/lrrd23/5/mako23106.htm
Meier S, Ruzvidzo O, Morse M, Donaldson L, Kwezi L, Gehring C (2010). The Arabidopsis wall associated kinase-like 10 gene encodes a functional guanylyl cyclase and is co-expressed with pathogen defense related genes. PLoS One 5.
Men LT, Yamasaki S, Caldwell JS, Yamada R, Takada R, Taniguchi T (2006). Effect of farm household income levels and rice-based diet or water hyacinth (*Eichhornia crassipes*) supplementation on growth/cost performances and meat indexes of growing and finishing pigs in the Mekong Delta of Vietnam. Animal Science Journal 77:320-329.
Mikiashvili N, Wasser SP, Nevo E, Elisashvili V (2006). Effects of carbon and nitrogen sources on *Pleurotus ostreatus* ligninolytic enzyme activity. World Journal of Microbiology and Biotechnology 22(9):999-1002.
Miller GL (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugars. Analytical Chemistry 31:426-428.
Moyo P, Mapira J (2012). Bio-remediation with water hyacinth (*Eichhornia crassipes*): A panacea for river pollution in the city of Masvingo (Zimbabwe). Journal of Sustainable Development in Africa 14:115-131.
Murugesan K, Arulmani M, Nam IH, Kim YM, Chang YS, Kalaichelvan PT (2006). Purification and characterization of laccase produced by a white rot fungus *Pleurotus sajor-caju* under submerged culture condition and its potential in decolorization of azo dyes. Applied Microbiology and Biotechnology 72:939-946.
Mwangi T (2013). Water hyacinth - Can its aggressiveness be controlled? Ecosystem Management-UNEP/DEWA, Nairobi, Kenya.
MWBP/RSCP (2006). Invasive alien species in the lower Mekong basin: Current state of play. Mekong Wetland Biodiversity Programme and Regional Species Conservation Programme, The World Conservation Union (IUCN), Asia, Sri Lanka.
Nayebyazdi N, Salary M, Ghanbary MAT, Ghorbany M, Bahmanyar MA (2012). Investigation of cellulase activity in some soil borne fungi isolated from agricultural soils. Annals of Biological Research 3(12):5705-5713.
Ncube T, Howard RL, Abotsi EK, van Rensburg ELJ, Ncube I (2012). *Jatropha curcas* seed cake as substrate for production of xylanase and cellulase by *Aspergillus niger* FGSCA733 in solid-state fermentation. Industrial Crops and Products 37:118-123.
Ni J, Tokuda G, Takehara M, Watanabe H (2007). Heterologous expression and enzymatic characterization of beta-glucosidase from the drywood-eating termite, Neotermes koshunensis. Applied Entomology and Zoology 42:457-463.
Nigam P, Singh D (2002). Enzyme and microbial systems involved in starch processing. Enzyme and Microbial Technology 22:375-407
Parsons WT, Parsons WT, Cuthbertson EG, Cuthbertson EG (2001). Noxious weeds of Australia (CSIRO Publishing) Available at: https://books.google.co.ke/books?id=sRCrNAQQrpwC
Pandya B, Albert S (2014). Evaluation of *Trichoderma reesei* as a compatible partner with some white rot fungi for potential bio-bleaching in paper industry. Annals of Biological Research 5:43-51.
Penfound WT, Earle TT (1948). The biology of water hyacinth. Ecological Monographs 18:447-472.
Pointing SB (1999). Qualitative methods for the determination of lignocellulolytic enzyme production by tropical fungi. Fungal Diverssity 2:17-33.
Polizeli M, Rizzatti A, Monti R, Terenzi H, Jorge J, Amorim D (2005). Xylanases from fungi: Properties and industrial applications. Applied Microbiology and Biotechnology 67(5):577-591.

Punniavan S (2012). Effect of medium composition and ultrasonication on xylanase production by *Trichoderma harzianum* MTCC 4358 on novel substrate. African Journal of Biotechnolology 11:12067-12077.
Ramanjaneyulu G, Reddy GPK, Kumar KD, Reddy BR (2015). Isolation and screening of xylanase producing fungi from forest soils. International Journal of Current Microbiology and Applied Sciences 4:586-591.
Rashid M, Iqbal M (2012). Effect of phosphorus fertilizer on the yield and quality of maize (*Zea mays* L) fodder on clay loam soil. Journal of Animal and Plant Sciences 22(1):199-203.
Reddy KR, D'Angelo EM (1990). Biomass yield and nutrient removal by water hyacinth (*Eichhornia crassipes*) as influenced by harvesting frequency. Biomass 21:27-42.
Ritter CET, Camassola M, Zampieri D, Silveira MM, Dillon AJP (2013). Cellulase and xylanase production by *Penicillium echinulatum* in sub-merged media containing cellulose amended with sorbitol. Enzyme Research 2013:1-9.
Saha P, Alam MF, Baishnab AC, Khan MR, Islam MA (2014). Fermentable sugar production and separation from water hyacinth using enzymatic hydrolysis. Sustainable Energy 2:20-24.
Saito T, Hong P, Kato K, Okazaki M, Inagaki H, Maeda S, Yokogawa Y (2003). Purification and characterization of an extracellular laccase of a fungus (family Chaetomiaceae) isolated from soil. Enzyme and Microbial Technology 33:520-526.
Sakthiselvan P, Naveena B, Partha N (2014). Molecular characterization of a xylanase-producing fungus isolated from fouled soil. Brazilian Journal of Microbiology 45(4):1293-1302.
Shawky BT, Mahmoud MG, Ghazy EA, Asker MM, Ibrahim GS (2011). Enzymatic hydrolysis of rice straw and corn stalks for monosugars production. Journal of Genetic Engineering and Biotechnology 9(1):59-63.
Sibanda A, Ruzvidzo O, Ncube I, Ncube T (2019a). Diversity of cellulase- and xylanase-producing filamentous fungi from termite mounds. Journal of Yeast and Fungal Research 10(2):15-29.
Sibanda N, Ruzvidzo O, Zvidzai CJ, Mashingaidze AB, Murungweni C (2019). Exploring for the possibility of utilizing *Pleurotus ostreatus* to manage *Eichhornia crassipes* in Zimbabwe. Journal of Yeast and Fungal Research 10(1):1-14.
Sinma K, Khucharoenphaisan K, Kitpreechavanich V, Tokuyama S (2011). Purification and characterization of a thermostable xylanase from *Saccharopolyspora pathumthaniensis* S582 isolated from the gut of a termite. Bioscience, Biotechnology and Biochemistry 75:1957-1963.
Silva CHC, Puls J, Sousa MV, Ferreira-Filho EX (1999). Purification and characterization of a low molecular weight xylanase from solid-state cultures of *Aspergillus fumigatus* Fresenius. Revista de Microbiologia 30:114-119.
Sornvoraweat B, Kongkiattikajorn J (2010). Separated hydrolysis and fermentation of water hyacinth leaves for ethanol production. KKU Research Journal 15:794-802.
Stajić M, Persky L, Friesem D, Hadar Y, Wasser SP, Nevo E, Vukojević J (2006). Effect of different carbon and nitrogen sources on laccase and peroxidases production by selected Pleurotus species. Enzyme and Microbial Technology 38:65-73.
Téllez-téllez M, Díaz R, Sánchez C, Díaz-godínez G (2013). Hydrolytic enzymes produced by Pleurotus species. African Journal of Microbial Research 7:276-281.
Thakur M (2018). Mushrooms as a biological tool in myco-remediation of polluted soils. Emerging Issues in Ecology and Environmental Science. Briefs in Environmental Science 15:27-42.
Tham HT (2012). Water hyacinth (*Eichhornia crassipes*) - Biomass production, esilability and feeding value to growing cattle pp. 115-132.
Todaka N, Lopez CM, Inoue T, Saita K, Maruyama J, Arioka M (2010). Heterologous expression and characterization of an endoglucanase from a symbiotic protist of the lower termite, *Reticulitermes speratus*. Applied Biochemistry and Biotechnology 160:1168-1178.
Usha KY, Praveen K, Reddy BR (2014). Enhanced production of ligninolytic enzymes by a mushroom *Stereum ostrea*. Biotechnology Research International 2014:815-849.
Villamagna AM, Murphy BR (2010). Ecological and socio-economic impacts of invasive water hyacinth (*Eichhornia crassipes*): A review. Freshwater Biology 55:282-298.
Virabalin R, Kositsup B, Punnapayak H (1993). Leaf protein concentrate from water hyacinth. Journal of Aquatic Plant Management 31:207-209.
Vivekanandan KE, Sivaraj S, Kumaresan S (2014). Characterization and purification of laccase enzyme from *Aspergillus nidulans* CASVK3 from vellar estuary South East Coast of India. International Journal of Current Microbiology and Applied Sciences 3(10):213-227.
Wang X, Ding Y, Gao X, Liu H, Zhao K, Gao Y, Qui L (2019). Promotion of the growth and plant biomass degrading enzymes production in solid-state cultures of *Lentinula edodes* expressing *Vitreoscilla hemoglobin* gene. Journal of Biotechnology 302:42-47.
Wesenberg D, Kyriakides I, Agathos SN (2003). White-rot fungi and their enzymes for the treatment of industrial dye effluents. Biotechnology Advances 22:161-187.
Yagi F, Minami Y, Yamada M, Kuroda K, Yamauchi M (2019). Development of animal feeding additives from mushroom waste media of *Shochu lees*. International Journal of Recycling of Organic Waste in Agriculture 8(2):215-220.
Yopi, Tasia W, Melliawati R (2014). Cellulase and xylanase production from three isolates of indigenous endophytic fungi. International Journal for Qualitative Research 8:73-86.
Yuan SF, Wu TH, Lee HL, Hsieh HY, Lin WL, Yang B, Huang CH (2015). Biochemical characterization and structural analysis of a bifunctional cellulase/xylanase from *Clostridium thermocellum*. Journal of Biological Chemistry 290(9):5739-5748.
Zhang DH, Lax AR, Bland JM, Allen AB (2011). Characterization of a new endogenous endo-β-1,4-glucanase of Formosan sub-terranean termite (*Coptotermes formosanus*). Insect Biochemistry and Molecular Biology 41:211-218.
Zhang XZ, Zhang YHP (2013). Cellulases: Characteristics, sources, production, and applications. Bioprocessing Technology in Biorefinery for Sustainable Production of Fuels and Chemical Polymers 8:131-146.
Zhou X, Kovaleva ES,Wu-Scharf D, Campbell JH, Buchman GW, Boucias DG (2010). Production and characterization of a recombinant β-1,4-endoglucanase (glycohydrolase family 9) from the termite *Reticulitermes flavipes*. Archives of Insect Biochemistry and Physiology 74:147-162.
Zirbes M, Waldvogel SR (2018). Electro-conversion as sustainable method for the fine chemical production from the biopolymer lignin. Current Opinion in Green and Sustainable Chemistry 14:19-25.

APPENDIX

Resource import permit.

PERMIT FOR THE IMPORTATION OF CONTROLLED GOODS

Permit No. P0079761

IMPORTANT : This permit does not exempt the holder from the provisions of any other Act, ordinance or

DEPARTMENT OF AGRICULTURE FORESTRY AND FISHERIES
DIRECTORATE PLANT HEALTH
2016 -11- 10
DIRECTORATE PLANT HEALTH
PO Box 6604, ARCADIA, 0007

Executive Officer

PHYTOSANITARY REQUIREMENTS FOR THE IMPORTATION OF REGULATED ORGANISM (S) FOR RESEARCH PURPOSE

1. The consignment must be inspected at the point of entry and found free from contaminants.
2. Quarantine label to be affixed to the parcel;
3. The imported **AQUATIC WEED[maximum 50 KG]** sample (s), shall only be handled at **PLANT BIOTECHNOLOGY RESEARCH LABORATORY** facility
4. Import sample(s) in a sealed container(s) shall be addressed to **Prof. OZINIEL RUZVIDZO / Ms. TSEGOFATSO DIKOBE**
   Name of institution / company: **UNIVERSITY OF NORTH WEST**
   Postal address: **CHINHOYI UNIVERSITY OF TECHNOLOGY**
   P. BAG 7724
   CHINHOYI
   ZIMBABWE
5. The importer shall be responsible for custom clearance;
6. The container(s) shall be opened and the material handled in the facility at **PLANT BIOTECHNOLOGY RESEARCH LABORATORY**
7. Name of Laboratory facility: **PLANT BIOTECHNOLOGY RESEARCH LABORATORY**
   Physical address: **PLANT BIOTECHNOLOGY RESEARCH LABORATORY**
   NORTH WEST UNIVERSITY
   MAFIKENG CAMPUS
   1 UNIVERSITY DRIVE
   MMABATHO
   2735
8. Responsible laboratory technician: **Prof. OZINIEL RUZVIDZO**
   TSEGOFATSO DIKOBE
   Tel no: **018 389 2289**
9. Destroy all packing material and wrapping by incineration or autoclaving.
10. Due to the foreign status of the sample(s) all experimental material shall be marked as potentially dangerous to the South African agricultural industry.

DEPARTMENT OF AGRICULTURE FORESTRY AND FISHERIES
2016 -11- 10
DIRECTORATE PLANT HEALTH
PO Box 6604, ARCADIA, 0007

11. Take precautions at all times to prevent the escape and introduction of any pest(s), which may be present in/on the samples into the RSA.
12. No cultures, sub-cultures or specimens of the imported sample(s) may be given to any other person or be used for any work outside the facility mentioned in 3. of this condition for any reason whatsoever, without the written consent of the Director
13. **All imported sample(s) must be destroyed by autoclaving/incineration after completion of the laboratory analysis and notify the Manager, (for attention: Ms Rorisang Mahlakoana: Fax 012 319 6101) immediately thereof in writing.**
14. If any of the above-mentioned conditions are not complied with or are violated, the material shall be destroyed, at the importer's expense.

THE FOLLOWING UNDERTAKING TO BE COMPLETED BY IMPORTER OR HIS AUTHORISED AGENT:

I the undersigned,

ID No:

am fully aware of the above-mentioned conditions and understand that should I contravene or fail to comply with any of the above conditions I shall be guilty of an offence and be liable for prosecution under the Agricultural Pests Act, 1983 (Act No. 36 of 1983).

SIGNED: DATE:

PERMIT NO.:

DEPARTMENT OF AGRICULTURE FORESTRY AND FISHERIES
2016 -11- 10
DIRECTORATE PLANT HEALTH
PO Box 6604, ARCADIA, 0007

DIRECTORATE: PLANT HEALTH

GENERAL INFORMATION TO IMPORTER/PERMIT HOLDER/AGENT

AGRICULTURAL PESTS ACT, 1983 (ACT NO. 36 OF 1983)

Subject to the provisions of section 3 of the agricultural pests Act, the importation of plants, plant products and other controlled goods is subject to a permit.

A copy of the permit and conditions should be communicated to the foreign supplier, by the importer/permit holder /agent.

The national plant protection (NPPO) of the exporting country must issue a phytosanitary certificate, complying with the conditions of the South African permit. Each consignment must be accompanied by an original phytosanitary certificate.

Should the NPPO of the exporting country not be able to comply with the conditions of the permit, export cannot proceed. For assistance in this regard, the importer/permit holder should contact this Directorate.

Controlled goods can only be imported through a prescribe port of entry, except where determined otherwise by the Executive Officer.

On arrival, each consignment with relevant documentation must be presented (by the importer/permit holder/agent) to the Executive Officer for inspection at the port of entry. Goods may not be removed from the port of entry without the written authorization of the Executive Officer.

Please note:

i. where any other place than the port of entry has been determined or when goods are imported via a courier service, the importer/permit holder/agent must on arrival, present the goods to the Executive Officer;
ii. if brought in per passenger, the material must first be declared at the customs control point before presenting it to the Executive Officer. The red line to be taken at the customs control point.

Please take note of the expiry date of the permit. A permit expires on the date indicated on the permit. Should you wish to proceed with a similar import, please apply at least 30 days prior to the expiry date of the permit, for a new permit.

Please accept the above as guideline, but take note that all stipulations of the Agricultural Pests Act should be complied with. Authorization in terms of this Act does not exempt the holder from the provisions of any other Act, ordinance or agreement.

Other Acts that may be relevant: Plant Improvement Act & GMO Act.

Permit Office:

Mr. Banks Kgomo Tel: (012) 319 6130
Mr. Emmanuel Liabara Tel: (012) 319 6207
Ms. Priscilla Baloyi Tel: (012) 319 6306

Email:

Figure A1. Resource import permit. The resource permit that was applied for and then granted by the Department of Agriculture, Forestry and Fisheries, South Africa with the permit number; P0079761, to allow the researchers to transport water hyacinth from Zimbabwe into South Africa and undertake research at the North-West University.