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Journal of Yeast and Fungal Research

Full Length Research Paper

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).

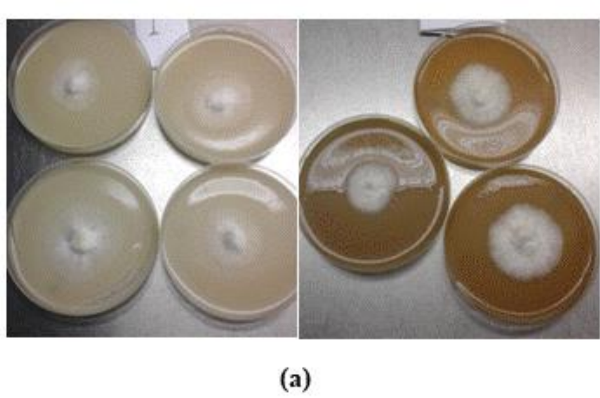
Statistical analysis

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



(a)

(b)

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

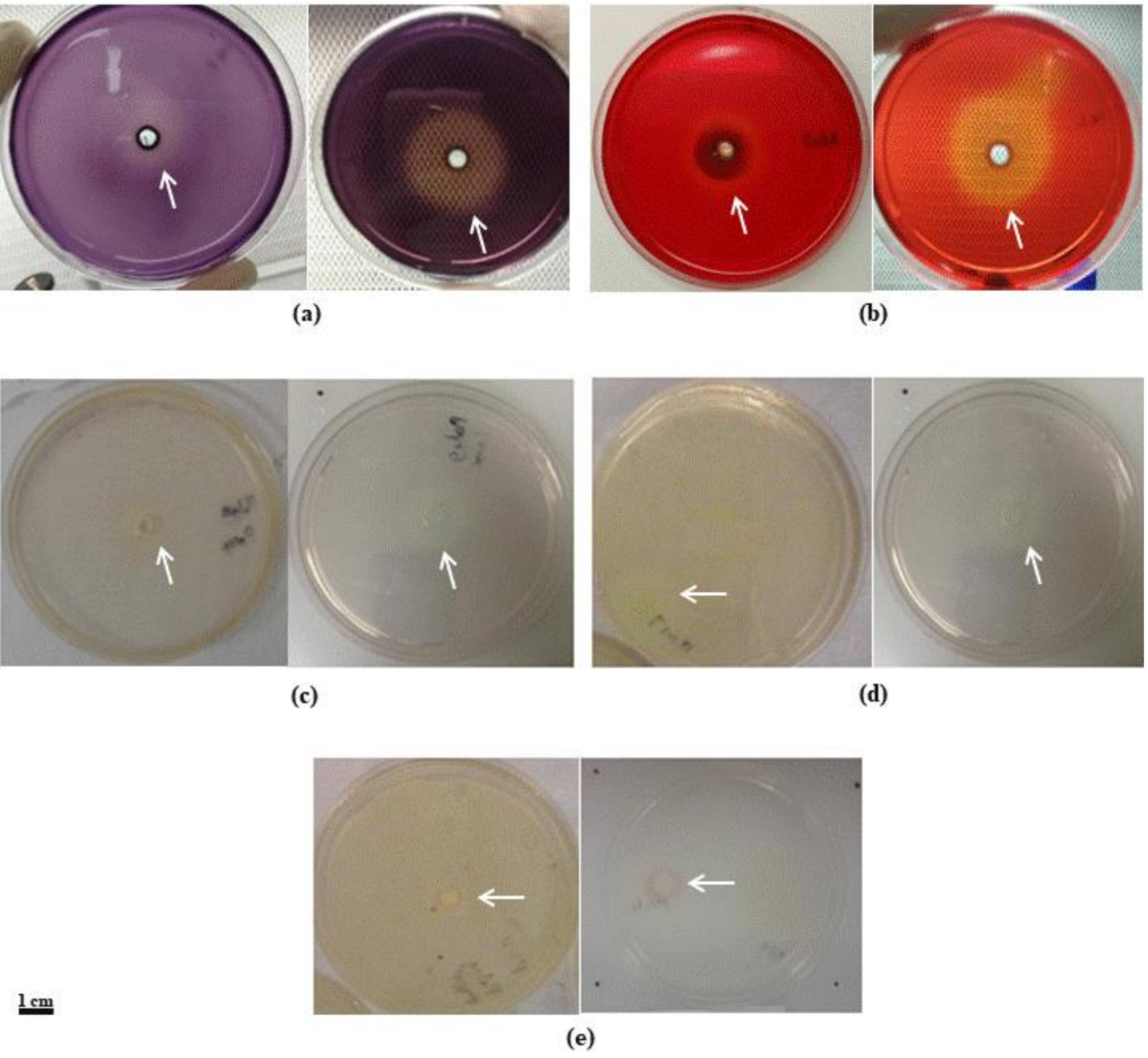


Figure 2. Testing the ability of *L. edodes* to secrete various lignocellulolytic enzymes when cultured on water hyacinth. (**a**) Cellulase, (**b**) xylanase, (**c**) pectinase, (**d**) lignin peroxidase and (**e**) laccase activities of the extracellular enzyme extract secreted by *L. edodes* when 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

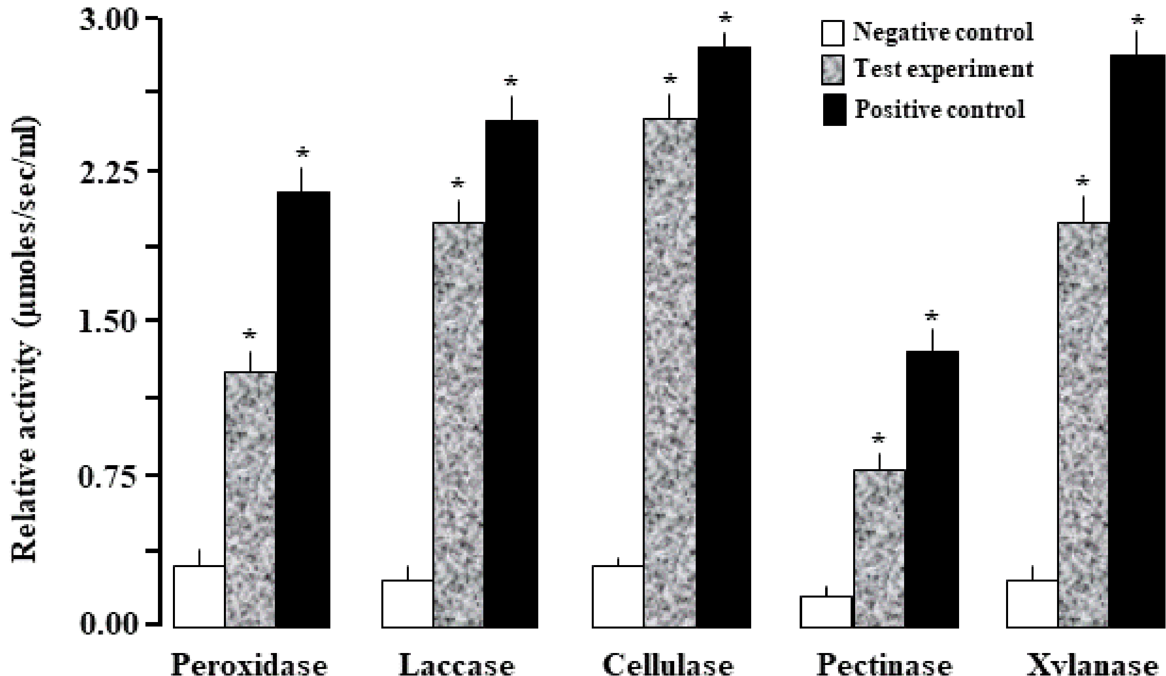


Figure 3. Spectrophotometric determination of the ability of *L. edodes* to secrete lignocellulolytic enzymes when cultured on water hyacinth. Activity was measured in the absence of enzymes (clear bars; negative control) and in the presence of enzymes secreted by the *L. edodes* when cultured on water 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.

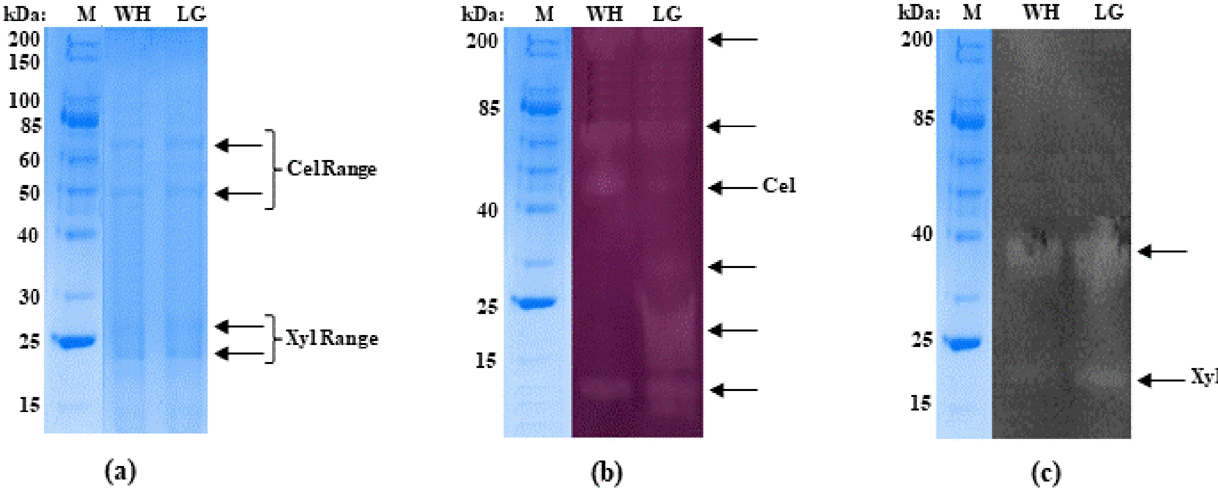


Figure 4. Resolution and activity assaying of the various protein fractions secreted by *L. edodes* when cultured on water hyacinth (WH) as a test substrate or liverseed grass (LG) as a control substrate. (**a**) an SDS-PAGE gel showing the resolution of secreted prominent protein fractions in the crude 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.

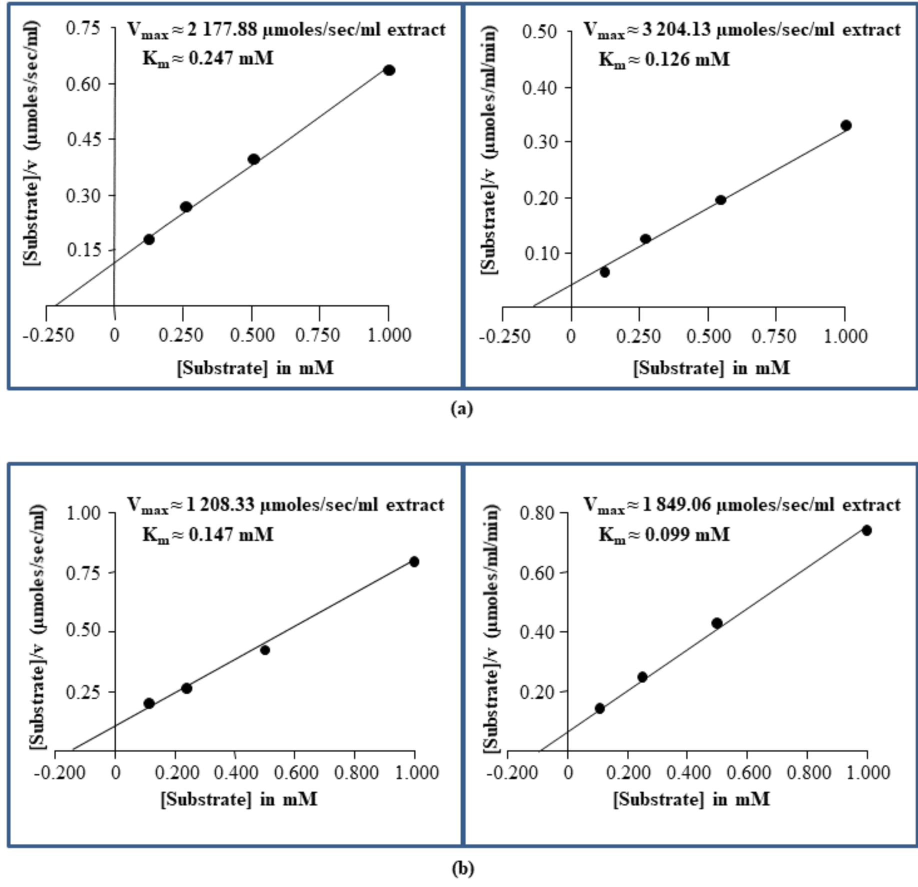


Figure 5. Reaction kinetics. Kinetic rates of the (**a**) cellulolytic and (**b**) xylanolytic protein fractions in the crude extracellular extract secreted by *L. edodes* when cultured on water hyacinth as a test substrate (left planes) or liverseed grass (right planes) as a control substrate. K_m was 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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APPENDIX

Resource import permit.

agriculture, forestry & fisheries

Department: Agriculture, Forestry and Fisheries REPUBLIC OF SOUTH AFRICA

Directorate Plant Health

Permit No. P0079761

PERMIT FOR THE IMPORTATION OF CONTROLLED GOODS

NOMFUNDELELO SIBANDA

PRIVATE BAG 7724

CHINHOYI, ZIMBABWE

to import into the Republic the following controlled goods: AQUATIC PLANTS

AQUATIC PLANTS 50 KG

Conditions: 1. AS ATTACHED

Port of Entry: BEIT BRIDGE / O R TAMBO INTERNATIONAL AIRPORT

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

DEPARTMENT OF AGRICULTURE, FORESTRY AND FISHERIES, DIRECTORATE PLANT HEALTH, 2016 -11- 10, DIRECTORATE PLANT HEALTH, PO Box 40024, ARCADIA, 0007

Executive Officer

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, ________________

I D 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, DIRECTORATE PLANT HEALTH, 2016 -11- 10, DIRECTORATE PLANT HEALTH, PO Box 40024, ARCADIA, 0007

agriculture, forestry & fisheries

Department: Agriculture, Forestry and Fisheries REPUBLIC OF SOUTH AFRICA

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 / Ms. 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.

agriculture, forestry & fisheries

Department: Agriculture, Forestry and Fisheries REPUBLIC OF SOUTH AFRICA

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.

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

Permit Office:

Mr. Boris Kgomo Tel: (012) 319 6130

Mr. Emmanuel Liabara Tel: (012) 319 6207

Ms. Priscilla Baloyi Tel: (012) 319 6396

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.

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Journal of Yeast and Fungal Research

Full Length Research Paper

Selection of appropriate substrate for production of oyster mushroom (*Pleurotus ostreatus*)

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Mushroom production is an economically viable biotechnology process for conversion of various agro-industrial wastes into food. Mushroom, a fruiting body of macrofungi has been valued throughout the world as either food or medicine for more than three thousand years ago. The mushroom grows on a vast number of substrate and environment. Substrate compromises different agro-industrial residues that possess varied property for supporting the growth of mushroom. Though, the most appropriate composition of the substrate should be selected to obtain a better result. Hence, the study was conducted to select appropriate substrate for production of oyster mushroom and to identify the suitable combination from a selected substrate to get a high yield of oyster mushroom. The effects of different selected agro industrial residues on growth and bioconversion efficiency of oyster mushroom was determined. For this study, Oyster mushroom (*Pleurotus ostreatus*) were grown on different substrates namely cotton seed, enset waste, sawdust, and teff straw with different composition. The spawn was produced using three grains to know the performance of oyster mushroom. The main step used for oyster mushroom production includes preparation of culture media, spawn production, preparation of the substrate, fruiting, and harvesting. The highest bioconversion efficiency and yield were obtained from the combination of sawdust and teff straw. While the lowest yield and bioconversion efficacy was obtained from combination teff straw and enset waste.

Key words: Mushroom, oyster, spawn, substrate, waste.

INTRODUCTION

Mushroom production could be a possible option to alleviate poverty and improve the lifestyle of vulnerable people (Imtiaj and Rahman, 2008). The production is important for food shortage (Beetz and Kustudia, 2004; Tibuhwa, 2013), especially for low-income countries like Ethiopia. Food insecurity remains one of the world's biggest challenges; particularly, the problem is very severe in low and middle-income countries. Therefore, finding ways of improving food production in increasing population is paramount important. More than 2,000 species composed of 31 genera are identified to be edible over the world (Moore, 2005). Twelve species are commonly grown for food and/or medicinal purposes, across tropical and temperate zones. *P.* spp, commonly

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known as oyster mushrooms, are edible fungi cultivated worldwide especially in South East Asia, India, Europe and Africa. China produces 64% edible mushrooms in the world and 85% of all oyster mushrooms all over the world (*P.* spp.) is also produced in China (Chang, 1999). The three most commonly cultivated mushrooms are *Agaricus bisporus* (button mushroom), *P. ostreatus* (oyster mushroom) and *Lentinula edodes* (shiitake mushroom). Oyster mushrooms are the second largest commercially produced mushroom in the world (Sánchez, 2010; Mohamed et al., 2011) next to *A. bisporus*. Shorter growth time is required to oyster mushrooms in comparison to other edible mushrooms. It converts a high percentage of the substrates to fruiting bodies and hence increases profitability and low-cost cultivation technology (Baysal et al., 2003).

Mushrooms are highly valued for their rich characteristic flavor, potent nutritional properties and possess various types of dietary supplements. Mushrooms are low in calorific value but rank very high for their vitamins, minerals and protein contents (Beetz and Greer, 1999). The consumption of oyster mushrooms has an advantage of preventing as well as reducing diseases such as diabetes, heart disease, high blood cholesterol level, gastric cancer, hepatitis B, liver illness, kidney problems, hypertension, microbial infection, chronic fatigue syndrome and impaired immune response (Ooi, 2000).

Oyster mushrooms are known to contain therapeutic ingredients such as dietary fibers and phenolic compounds various bioactive compounds. Mushroom production is an appropriate technology for the management of agricultural and agro-industrial residues. Oyster mushrooms are saprophytes that decompose agricultural plant by-product as they have the ability to use cellulose, hemicelluloses, and lignin materials as a source of their nourishment. Mushroom cultivation provides an environmentally friendly and economical way of converting agricultural and forest wastes into nutritious food (Ragunathan et al., 1996). On the surface of the earth, around 200 billion tons per year of organic matter is produced through the photosynthetic process (Zhang, 2008). However, the majority of this organic matter is not directly edible by humans and animals in many cases, becomes a contaminated source of an environment.

Though, mushroom cultivation is a useful method to produce alternative food sources using different environmental wastes. Oyster mushroom is known for its ability to degrade lignocelluloses residues from agricultural fields and forests and convert them into protein-rich biomass (Rowel et al., 2000). Species of oyster-mushroom show good adaptability to a wide range of temperature, making it possible to grow this mushroom almost all year round without controlled climatic conditions (Chadha, 2001; Baysal et al., 2003). Many agricultural and industrial by-products are important in mushroom production including teff straw, coffee pulp, wood chips, and cotton waste has high cellulose, hemicellulose and lignin contents. Therefore, there is a limited study conducted on utilization efficiency of agricultural waste by the mushroom. Hence, this study was conducted to evaluate the growth, the economic feasibility of small scale production and yield (bioconversion efficiency) of oyster mushroom using locally available agro-industrial by-products.

Oyster mushroom production is a useful method of environmental waste management and waste disposal. The cultivation of oyster-mushroom adds value to the economy, environmental restoration and food security (provision) worldwide. Mushroom production is one of the strategies that can be used for poverty intervention and also for combating malnutrition. Therefore, this study provided to identify the best and appropriate composition of a substrate for production of oyster mushroom.

Providing food for rapidly growing world population and waste management belongs to major problems found in the world. The cultivation of oyster mushroom on agro-industrial residues is a prime factor for the conversion of low-value inedible wastes into a higher value commodity which can serve as food. Among bioconversion processes, mushroom cultivation is an appropriate technology for the management of agricultural and agro-industrial residues. The oyster mushrooms have gained popularity, because of their simplicity and low-cost cultivation technology. The cultivation of mushroom in a natural environment is limited by season and space. Hence, it necessitates the cultivation of mushroom in the controlled environment through maintaining appropriate growing condition as it is in the natural environment. One method of cultivation needed for selecting appropriate growing substrate composition. Hence, this study conducted to evaluate different types of a substrate for production of oyster mushroom.

MATERIALS AND METHODS

Study area

This study was conducted in Wolkite University, Department of Biotechnology laboratory. Wolkite is located in Southern Nation, Nationalities and Peoples Region (SNNPR) regional zone and the administrative center of the Gurage Zone. It found at 175 km distant from Addis Ababa, Ethiopia.

Study material

Oyster mushroom (*Pleurotus ostreatus*) was collected from small enterprise working on spawn production in Addis Ababa. A pure culture of oyster mushroom was maintained on potato dextrose agar and malt extract agar plates. Agro-industrial waste namely cottonseed, enset waste, bagasse, teff straw, and sawdust was collected from Gurage zone. In this study, we took five (5) different agro-industrial wastes as a substrate to know the combination appropriate for oyster mushroom production. The material used in this study includes the hood, plastic bag, autoclave, Petri dish, jar, aluminum foil, sprayer, bunsen burner, hot plate, incubator, metric ruler, measuring cylinder, electronics balance, glove, and cotton.

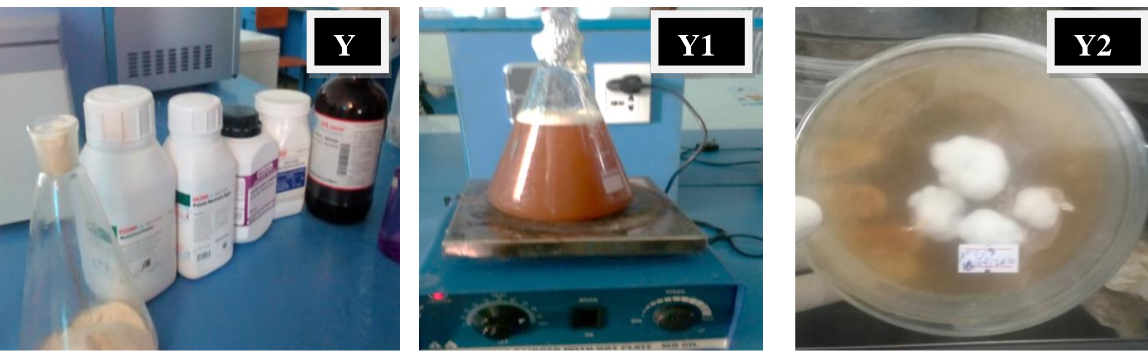

Figure 1. Preparation of culture media (Y. Chemical, Y1. Prepared media, Y2. Mycelia grow on prepared media).

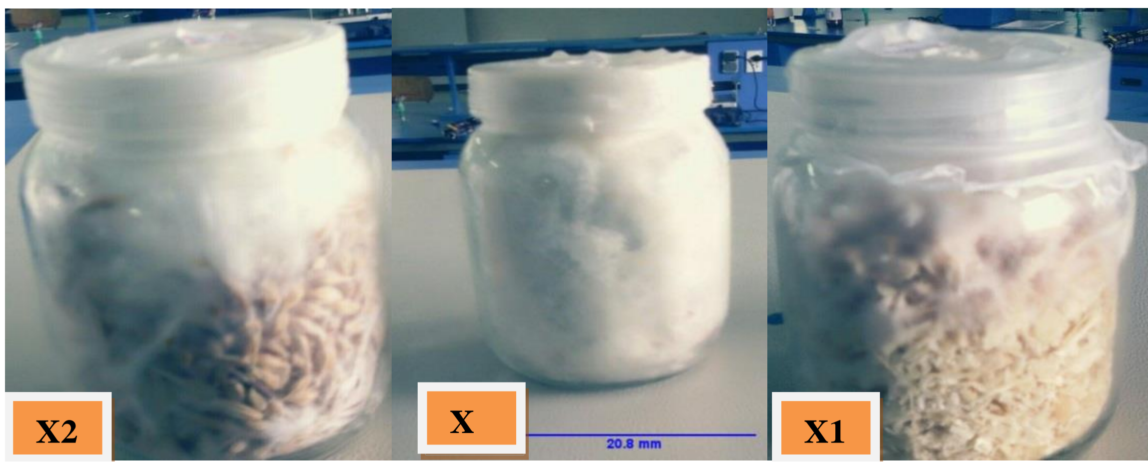

Figure 2. Spawn produced using three grains (X. Sorghum, X1. Bagasse, X2. Barley); Scale bar: 20.8 mm.

Experimental design and procedure

The experiment was laid down in CRD (completely randomized design) with four different substrate compositions in three different repeats. Then, collected data analyzed by SAS statistical analysis software.

Preparation of culture media

Pure cultures of oyster mushroom maintained on media prepared from potato dextrose agar (PDA), Malt extract agar (MEA), peptone and agar. 78 g of PDA, 60 g of malt extract agar, 30 g of Agar and 10 g of peptone was added to into 2 dm^3 of distilled water into a flask (Figure 1). Then it was placed on Bunsen burner to mix. The prepared media was placed on a hot plate to dissolve agar and then autoclaved at 121°C for 15 min. Fifteen milliliters (15 ml) of the medium dispensed into 9 cm diameter petri dishes. These were inoculated with the oyster mushroom cultures by using a spatula and incubated at 25°C.

Spawn production

Mushroom spawn is a medium that serves as the inoculum of the mushroom growth medium. Spawn was prepared by using barley, sorghum, and bagasse for comparison of the performance of oyster mushroom. Those two grains and bagasse washed and soaked in water overnight. The water was changed often to prevent fermentation. Once the grains have been prepared, they were boiled till it becomes soft but remain firm, then the water was drained and spread on a cheesecloth. Calcium carbonate (2%) was mixed with the grains. These grains were filled in half litter-size-empty bottles to three-fourths their capacity. These grains were sterilized in an autoclave for 15 minutes at 121°C temperature and 15 Pa (Pascal). Inoculation was carefully done in total aseptic conditions using pure culture or previously prepared grain spawn. After the inoculation, the bottles were incubated at 25°C temperature until mycelia fully cover the grains (Figure 2).

Substrate preparation and inoculation

The following substrates including sawdust, teff straw, and cottonseed waste, bagasse, enset waste were used for the study after soaked separately in water for moisture absorption. For the best results, substrates were mixed with wheat bran (10%) and gypsum (3%) supplement at a similar concentration (Figure 3).

Mixed substrates were placed in heat resistant polypropylene bags and sterilized in an autoclave at 121°C for 15 mins and allowed to cool at room temperature. After sterilization, each of experimental polypropylene bags was inoculated at the center of

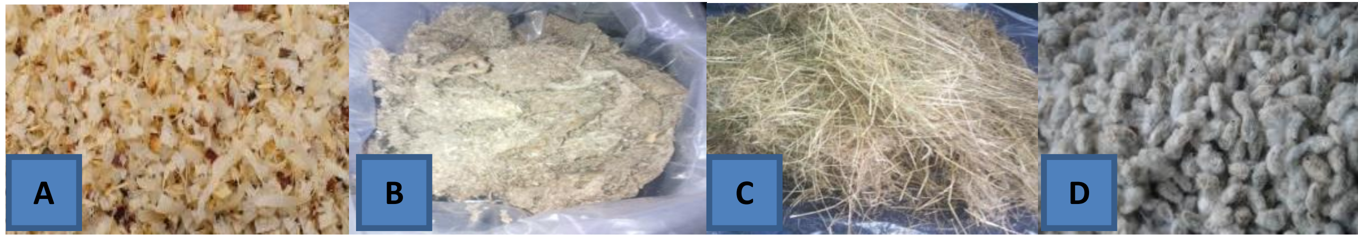

Figure 3. Different substrate used for Oyster mushroom cultivation (A. sawdust, B. enset waste, C. teff straw and D. cotton seed).

Figure 4. Fruiting bodies on combination of different substrate (T. sawdust+teff straw, T1. cotton seed+teff straw, T2. sawdust+enset waste, T3. Teff staw+enset waste); Scale bar: 6 mm.

the substrate with of pure culture of *P. ostreatus* under aseptic condition and bags tiled with wrap. They kept in dark room at 25 to 30°C and 90% relative humidity for 21 days.

Fruiting

When the mycelium has fully colonized the substrate, it can be able to produce fruiting bodies in response to a sudden change to the external physical environment, which first promoted the formation of initial fruiting bodies that later develop into fruiting bodies. The environmental changes that may trigger fruiting include changes in temperature, gas exchange, relative humidity, light and water availability within the compost. These induction factors also have an impact on the quality of the mushroom.

For *P.* Spp., decreasing the temperature from the spawn run temperature (25 to 27°C) to between 12°C and 18°C is the most common induction method used to stimulate fruiting. The primordial formed at the top of the bags and developed within 3 to 4 days and was ready to be collected (Figure 4). Under proper fruiting conditions, the additional flushes have occurred without any new inductions, but the flushes can be controlled by heating the blocks/bags followed by reducing the temperature.

Harvesting

The procedure for oyster mushroom harvesting involves grasping each mushroom stalk individually and twisting the mushroom until it is pulled out of the substrate. As the mushrooms begin fruiting, it is important to keep the humidity high (85 to 90% is recommended). As before, allow air to flush through the growing area prior to spraying (oyster mushrooms require a consistent source of fresh air). Temperatures can now be higher than for the initial pinning stage. The mushroom harvesting periods vary between different mushroom strains and usually range from 6 to 12 weeks and they can be harvested on a number of flushes. The determining factor in the number of flushes to be harvested and production time is substrate formulation together with the environmental conditions during cultivation. However, a period between the flushing of mushrooms named the resting stage, whereby the mushroom mycelia have to accumulate nutrients.

During this stage, contamination must be prevented to allow rapid mycelia growth. Harvesting of mushrooms can be carried out at different maturation stages depending on the mushroom species, market value and consumer preferences (Figure 5). Harvesting was carefully carried out before gills open in order to avoid a decreased market value and quality of mushroom.

Product evaluation

The bags were regularly disinfected using alcohol and hypochlorite to avoid contamination of substrates by unwanted microorganisms. When mycelia had fully covered the substrates bags, the bags were moved to another room for fructification. Bags were opened and regularly watered for fructification. After 27 days of inoculation and weights of the harvested mushroom was taken and recorded. The cap and stipe of the mushrooms were measured in cm using a metric ruler. Weight fruiting bodies of the mushroom were harvested in three different flushes.

Data collection

The data was collected from the result obtained. The growth and development of mushroom were monitored daily. The time (number of days) required from inoculation to completion of mycelium

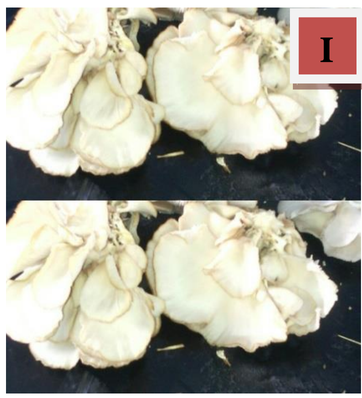

Figure 5. harvesting oyster mushroom produced on combination of selected substrate (I. harvested fresh oyster mushroom, J. Dry oyster mushroom, and K. packed oyster mushroom).

running, time elapsed between opening the plastic bags to pinhead formation and time required from opening the plastic bags to first-round harvesting were recorded. Growth parameters including stipe length (cm), cap diameter (cm), and ring diameter (cm) were recorded after each harvest. Yield parameters, such as a number of fruiting bodies per bunch, number of flushes and total fresh weight (g) of mushroom were also recorded at harvest time. Matured fruiting bodies were harvested by severing the base just above the surface of the substrate with a sharp blade. Two rounds of mushroom harvests were made across all substrate types in the course of the experiment. To evaluate the growth performance of mushroom on different substrates, yield and biological efficiency were calculated.

Data analysis

Analysis of variance

The analysis of variance (ANOVA) was carried out using statistical analysis system (SAS) version 9.2 software program using the Proc GLM procedure for completely randomized design (CRD) design (SAS Institute, 2008). The significant differences among substrate was presented by mean ± standard deviation (SD) at a level of p<0.05. The model used for CRD design was:

$$yij = \mu + \tau i + Eij$$

where, yij= the jth observation of ith treatment, μ=the overall mean, Ţi= i^{th} effect (μi- μ) and Eij= the effect of jth observation of ith treatment, j=1…r, I=1…t.

Estimation of Bio-conversion efficiency (BE)

Weights of all fruiting bodies harvested from polypropylene bags were recorded as total yield of mushroom based on (Chang et al., 1993).

$$\mathbf{BE} = \frac{\mathbf{FW}}{\mathbf{DW}} \times \mathbf{100}$$

where, BE = bio-conversion efficiency, FW = fresh weight of mushrooms harvested (g), DW= dry weight substrate (g). Yield performance and bio conversion efficiency of oyster mushroom on four kinds of substrates were calculated.

In this study, the collected data is analyzed by statistical analysis system (SAS) version 9.2 software program using the Proc GLM procedure for completely randomized design (CRD) design (SAS Institute, 2008).

RESULT

Production of spawn

After inoculation of spawn in prepared grains, the grains were fully covered by mycelia within 14 to 17 days. The mycelia coverage for sugarcane bagasse, sorghum and barley it takes 14, 16, 17 days, respectively as shown on Figure 6.

Analysis of variance for substrate

The analysis of variances for the studied growth parameters including stipe length (cm), cap diameter (cm), and ring diameter (cm) presented in Table 1. Also the yield parameters, such as a number of fruiting bodies per bunch, number of flushes and total fresh weight (g) and Dry weight (DW) of mushroom are indicated on Table 1.

The analysis of variance is highly significant for fresh weight, cape diameter and dry weight of mushroom at p<0.001 significance level. On the other hands, ring diameter and stipe length showed no significance level of (p<5%). The parameters that showed high significance indicate that the source of variation for the cultivation of mushroom is varying among the type of substrate used in the experiment. The non-significance value of ring diameter and stipe length data indicated that the model used to evaluate this parameter is limited to show the difference by using different substrates.

Figure 6. Spawn production using three grains (A. sorghum, B. sugarcane bagasse and C. barley).

Table 1. Analysis of variances (ANOVA) with four treatments and three repeats.

Sources of variation	Degree of freedom	Mean square					
		FW	CD	RD	SL	NF	DW
Substrate	3	15603.35***	68.37***	0.52^{ns}	13.24^{ns}	1059.85*	1079.68***
Error	8	1150.96	2.44	0.94	6.03	142.75	119.52
CV		15.17	14.11	23.26	35.86	38.69	28.09
R^2		0.83	0.91	0.17	0.45	0.73	0.77

Where: *, **, ***significant at 5%, 1% and 0.1%, respectively; ns=non-significant at 5 % probability level, FW=fresh weight, CD= cape diameter, RD= ring diameter, SL= stipe length, NF=number of flush, and DW=dry weight, CV=coefficient of variation, R^2= coefficient of determination.

Table 2. Mean and standard deviation comparison of studied parameter on different substrate.

Substrate	Mean ± S.D					
	FW (g)	CD (cm)	RD (cm)	SL (cm)	NF (n)	DW (g)
Cotton + teff straw	279.02±20.95	17.88±0.42	3.78±0.18	6.45±2.01	20.44±5.01	43.51±17.89
Sawdust+teff straw	291.90±41.06	10.95±2.44	4.64±0.19	9.88±4.27	58.89±23.11	62.55±8.80
Sawdust+enset waste	172.54±26.05	7.82±1.26	4.40±1.64	5.95±1.28	24.50±1.8	31.89±8.68
Teffstraw+enset waste	151.08±42.42	7.69±1.42	3.86±0.99	5.1±0.33	19.66±2.90	17.68±2.22

SD=standard deviation, FW=fresh weight, CD=cape diameter, RD= ring diameter, SL=stipe length, DW=dry weight, g=gram, cm=centimeter, n=number for counting flush at each harvest.

Mean performance of substrate

The mean value of the growth and yield parameter are presented in Table 2. Oyster mushroom cultivated on a mixture of sawdust + teff straw recorded the highest fruit body weight followed by a mixture of cottonseed + teff straw; teff straw + enset waste and sawdust + enset waste. A maximum value of fresh weight is attained when we have used sawdust + teff straw and cottonseed + teff straw with a yield of 279.02 and 291.90 g, respectively. The least yield obtained from the combination of teff straw+ enset waste with a yield of 151.08 g.

Mushrooms growing on sawdust + teff straw had the highest cap diameter. The least stipe and ring diameter of the cultivated oyster mushroom was recorded in the combination of teff straw + enset waste. Oyster mushroom that grows on combination of cotton seed + teff straw and sawdust + teff straw has ring diameter 3.78 and 4.64 cm, respectively.

The response of substrate on yield parameter

The yield parameters have been determined mainly from growth parameter hence become the main criteria for the selection of substrate.

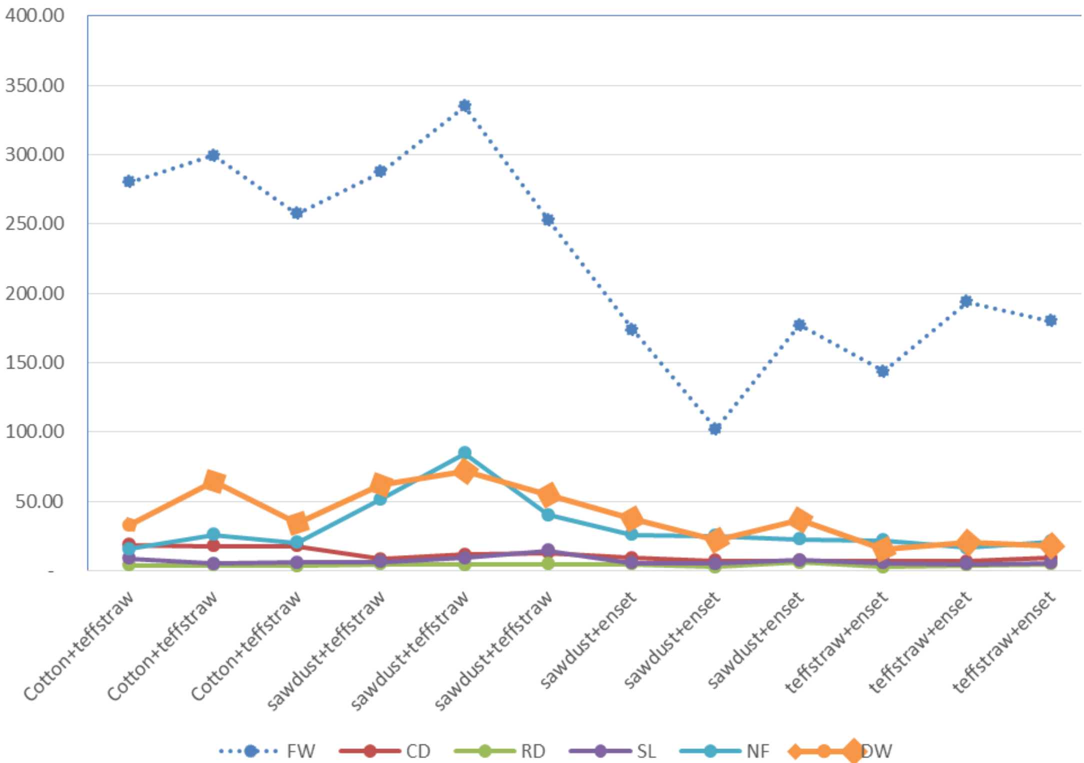

Figure 7. Response of different combination of substrate for production of Oyster mushroom.

The fresh yield of mushroom has declined as teff straw is substituted with enset waste as shown in Figure 7. In addition, a number of flush and dry weight has contributed to the yield performance of the substrate. Based on the result the highest fresh weight harvested when teff straw and sawdust used for a substrate.

Oyster mushroom grow on the combination of substrate

The growth of oyster mushroom on the combination of sawdust and teff straw is higher than the remaining three combinations of a substrate in these studies. Highest fresh weight of oyster mushroom was obtained from the combination of sawdust and teff straw. While the lowest of fresh weight recorded from the combination of teff straw and enset waste. Four different combinations of substrates are used for the production of oyster mushroom. This combination of substrate used for growth of oyster mushroom includes sawdust + teff straw, cotton seed + teff straw, sawdust + enset waste and teff straw + enset waste as shown on Figure 8.

Bioconversion efficiency of oyster mushroom

Mushroom bioconversion efficiency on different substrates mixtures having 45:45 and 90:10 WW main materials and additive wheat bran for three consecutive flushes are shown in Figure 8.

The mixtures of sawdust and teff straw reached their bioconversion efficiency of 73%, followed by 52% of teff straw + cottonseed, and 30% of sawdust + enset waste mixture. The lowest bioconversion efficiency is obtained 26.6% of teff straw + enset waste mixture (Figure 9).

Periods of oyster mushroom fruiting bodies maturation

The bags took 3 to 5 days from primordial formation to maturation of mushroom fruiting body. After 3 days, mushrooms became ready for picking. Duration for the maturation of fruiting bodies after primordial formation showed variations among different substrates and replicates (Figure 10).

DISCUSSION

Various grains such as sorghum, barley and bagasse are used for the production of spawn. Amongst these different grains used during the current investigation, oyster mushroom mycelia invasion took the minimum number of days (14) for spawn running on sugarcane

Figure 8. Oyster mushroom growing on different selected substrate (ST. Sawdust+teff straw, CT. cotton seed+teff straw,SE. sawdust+enset waste and TE. teff straw+enset waste).

bagasse followed by sorghum (16 days), barley (17 days). Similarity, Tsegaye and Tefera (2017) reported the production of spawn on sugarcane bagasse took the shortest time (14 days) compared to other grains sorghum and millet that took (16 to 17 days). The result further supported by (Rana et al., 2007) on oyster mushroom showed significantly rapid growth on different grains as compared to the rest of other mushroom species. Thus, sugarcane bagasse may be an appropriate source of carbon and energy for mycelia colonization and spawn production. The growth of Oyster mushroom (*P. ostreatus*) mycelia was relatively faster on a combination of sawdust + teff straw wastes as compared to the remaining three combinations.

The highest mycelium colonization, primordial initiation, fruiting bodies formation, and fresh weight were obtained from sawdust + teff straw with a yield of 730 g/kg. Previously, Tsegaye and Tefera (2017) reported highest fresh yield of mushroom (790 g/kg) harvested from a combination of cotton waste + coffee pulp which is closer to the current study. Adjapong et al. (2015) reported about 32.99 g of fruiting bodies of mushroom harvested per crop on maize husk.

Mushroom cultivation requires carbon, nitrogen and inorganic compounds as their nutritional sources, and main nutrients are carbon sources such as cellulose and lignin. Oyster mushrooms require less nitrogen and more carbon source. Thus, most organic matters containing cellulose, hemicellulose and lignin can be used as the mushroom substrate. The ability of oyster mushroom to grow successfully on the combination of sawdust and teff straw associated with the essential chemical composition of selected substrates is important for the growth of mushroom. Variations observed in the number of fruiting bodies produced may be associated with the physical nature of the substrates as well as the nature of the

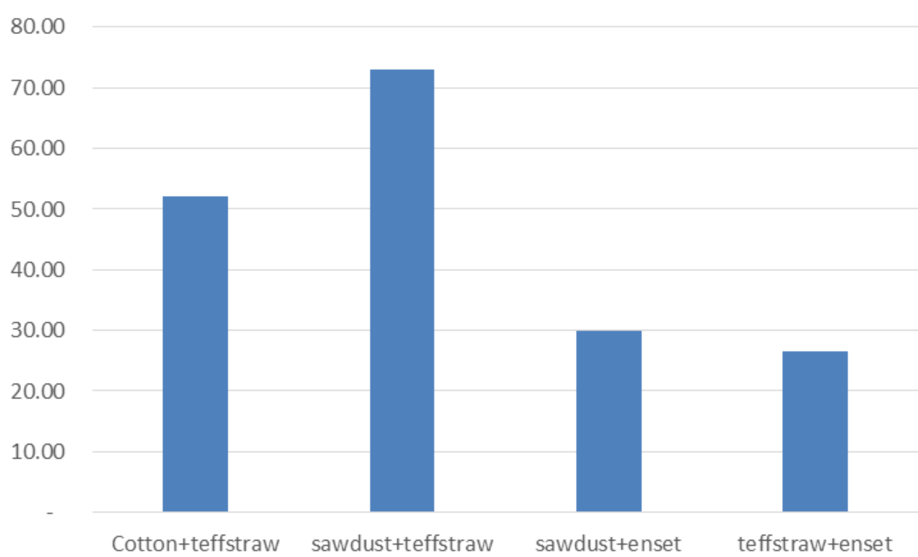

Figure 9. Bioconversion efficiency of oyster mushroom.

Figure 10. Progressive development of oyster mushroom on combination of different substrate (E. primordial formation, F. fruiting bodies, G. growth of fruiting bodies, H. matured oyster mushroom for harvest).

mushroom species. The number of fruit bodies recorded is related to their mycelia colonization.

In this study, data were collected on yield parameters and growth parameters. Yield parameter includes fresh weight (FW), number of flush and dry weight (DW). While the growth parameters are: cap diameter, stipe and ring diameter. The maximum value of fresh weight is obtained when we have used sawdust + teff straw and cotton seed + teff straw with a yield of 291.02 g and 279.90 g, respectively. While the least yield of fresh weight obtained from a combination of teff straw and enset waste with the yield of 151.08 g. The growth parameters such as ring diameter and stipe length showed the non-significance level at probability less than 5% ($p<5\%$). Parameters that showed non-significance do not indicate the source of variation for production of oyster mushroom. On the other hand, analysis of variance is highly significant for FW, CD and DW of oyster mushroom at $p<0.1\%$. The parameters that showed high significance indicates the source of variation for the cultivation of mushroom is vary among the type of substrate used in the experiment.

The highest mean value of growth and yield parameters are obtained from the combination of sawdust and teff straw. While the combination of teff straw and enset waste gives the lowest mean value of growth and yield parameters. In this study, four substrates such as teff straw, enset waste, sawdust, and cottonseed are used. The supplements used to enhance the growth of oyster mushroom are wheat bran and gypsum. Alcohol and bleach are chemicals used to control unwanted microorganism during oyster mushroom production. Continuous spraying of water is required during mycelia growth because it requires high humidity.

The bioconversion efficiency is calculated as fresh weight over the dry weight of substrate multiplied by one hundred. Highest bioconversion efficiency was obtained from the combination of sawdust and teff straw. This

indicates a high yield of oyster mushroom.

The mixtures of sawdust and teff straw reached their bioconversion efficiency of 73%, followed by 52% of teff straw + cottonseed, and 30% of sawdust + enset waste mixture. Previously, the biological efficiency of maize husk substrate was 39% (Adjapong et al., 2015). The lowest bioconversion efficiency is obtained 26.6% of teff straw + enset waste mixture.

Conclusion

Oyster mushroom production is the most important as a nutritional supplement and cash crop for the landless poor. Oyster mushrooms are high yielding, fast-growing crop. They are known to help lower cholesterol levels and are a great source of potassium, iron, and protein. Cultivation of edible oyster mushroom is a prime factor for the conversion of low-value inedible wastes into a higher value commodity which can serve as food material for humans as well as a source of the commercially important metabolites. Also, their spent can be used as cattle feed, fertilizer or landfills. Therefore, cultivation of oyster mushroom on agro-industrial residues provides multi-disciplinary advantages for the human being, animals as well as for the ecosystem. The highest yield (bioconversion efficiency) of oyster mushroom was obtained from the combination of sawdust and teff straw which are easily available substrates and large biomass exists in the country. The fresh mushroom biological efficiency was directly related to the nutritional composition of the substrate used for growing mushrooms. The observed differences in the substrates media may be due to the percentage composition of cellulose materials and essential chemicals and biomolecules that are important for the growth of oyster mushroom.

The highest values of yield parameters are obtained from the combination of sawdust and teff straw. While the lowest values of yield parameters are obtained from the combination of teff straw and enset waste. The analysis of variance is highly significant for fresh weight, cap diameter and dry weight of mushroom at probability less than 0.1%. This indicates the source of variation for the production of oyster mushroom is varying among the type of substrate used in the experiment. In this study, ring diameter and stipe length showed the non-significance level at probability less than 5%. Generally, in this study the combination of sawdust and teff straw is the best appropriate substrate for high yield production of oyster mushroom

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Assessment of yield loss due to northern leaf blight in five maize varieties grown in Tanzania

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Field experiments were conducted in the Agricultural Research Institute, Uyole, using five susceptible maize varieties during 2013 and 2014 growing seasons. The varieties were used to determine yield losses due to northern leaf blight disease in Mbeya Region of Tanzania. The trials were laid out in two blocks of *E. turcicum* inoculated and Mancozeb treatment arranged in randomized complete block design in three replicates. Five fungicide sprays were done at weekly interval, starting from 35 days after planting (DAP) while disease inoculation was done twice at 35 and 45 DAP, using whorl placement technique. Data on disease severity index were collected using visual scale of 0-5, and grain yield of each treatment was recorded after harvest and drying. Such data were subjected to analysis of variance, correlation coefficient and coefficient of determination (R^2). Means were separated using Turkey's-Kramer simultaneous test at P≤0.05. Results indicated that Mancozeb sprays enhanced maize grain yield by 30 to 46.6% and 1000-grain weight by 19 to 24%. Disease severity index range of 78.7 to 95.7% indicated that Bora, Kilima, Situka-1, Staha and TMV-1 varieties were more susceptible to Northern leaf blight (NLB) disease. Yield losses ranged from 46 to 62.8% in grain yield (tons/ha) and 31.9 to 38.9% (g/plot) in 1000-grain weight. Disease severity index assessed at silking dry stage had a strong relationship to yield in all the varieties, but varied from Kilima ($r = -0.7617$, $R^2 = 0.580$, $P \leq 0.078$) in 2014 to Staha ($r = -0.9901$, $R^2 = 0.9803$, $P \leq 0.001$) in 2013 in grain yield. The minimum relationship between1000-grain weight and severity index of NLB was recorded in Staha ($r = -0.9300$, $R^2 = 0.8649$, $P \leq 0.007$) in 2013. The grain yield was enhanced and crop loss models indicated good fitness with strong and reliable validity in all the varieties, and as such can be used to estimate potential losses of maize caused by NLB disease in Mbeya, Tanzania.

Key words: *Zea mays*, northern leaf blight, yield loss, Tanzania.

INTRODUCTION

Maize is an important food crop extensively grown in both developed and developing countries of the world (International Maize and Wheat Improvement Centre (CIMMYT) and IITA, 2011). About 100 million hectares are under cultivation in 125 developing countries (FAOSTAT, 2010), with an annual worldwide production of 822 and 817 million tons in 2008 and 2009, respectively (Food and Agriculture Organization (FAO),

2009, 2010). The demand for maize is estimated to double by 2050 (Rosegrant et al., 2008; Yan et al., 2011). Northern leaf blight of maize (*Zea mays* L.) caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs (syn. *Helminthosporium turcicum (*Pass.) is almost ubiquitous in all the countries where maize is grown and is a threat to maize production in many areas of the world (Pandurangegowda et al., 1993; Carlos, 1997; Harlapur et al., 2000; Muiru et al. 2010).

In East Africa, the disease has been reported as an important foliar fungal disease of maize, resulting to substantial yield losses (Nkonya et al., 1998; Muriithi and Mutinda, 2001; Pratt et al., 2003; Ramathani et al., 2011). The disease epidemics commonly occurs in cool humid regions characterized by heavy dew during the growing season (Jordan et al. 1983; Dorothea et al. 1998; Juliana et al., 2005), temperature range of 20-27°C, relative humidity from 90-100% and low luminosity and the presence of large amount of inocula (Ullstrup, 1970; Shurtleff, 1980; Hennessy et al., 1990; Bentolila et al., 1991; Khatri, 1993; Gregory, 2004; Levicet al., 2008).

Northern leaf blight causes premature death of blighted leaves and results in significant yield reductions due to loss of photosynthetic leaf areas to blighting (De Vries and Toenniessen, 2001; Veerabhadraswamy et al., 2014). Severity of 40-70% on susceptible variety and yield loss of 60% have been reported in Tanzania neighbouring countries of Zambia, Uganda, Kenya, South Africa and Ethiopia (Simelane, 2007). High infection of *E. turcicum* diverted sugar from the stalks for grain filling resulting to crop lodging (Ferguson and Carson, 2004).When the disease is established before silking and spreads to upper leaves during grain filling, severe yield losses can occur (Ullstrup and Miles, 1957; Raymundo and Hooker, 1981). Yield losses of as high as 98% have been reported (Chenulu and Hora, 1962; Kachapur and Hegde, 1988).On average, grain yield losses of maize due to NLB ranged from 15-50% (Perkins and Hooker, 1981; CIMMYT, 1985; Nwanosike et al., 2015a) however varies based on the plant stage when infection occurred, severity of disease and the resistance of the maize genotype (Jha, 1993).

Leaf position in maize and other cereals contributed significantly to yield. Reports have shown that, top, middle and bottom leaves contributed approximately 10:5:1%, respectively to grain yield(Hooker, 1979; Bowen et al., 1991). The first and second leaves above the ear contributed significantly to yield and their mechanical removal reduced yield by 32% (Levy and Leonard, 1990). Based on entire leaf canopy and leaf positions, several models have been reported (Raymundo and Hooker, 1981; Perkins and Pedersen, 1987; Campbell and Madden, 1990; Harlapur et al., 2005) for predicting and estimating NLB disease. However, Perkins and Pedersen (1987) reported that critical point (CP) and area under disease progress curve (AUDPC) models gave relatively good fit of $r^2 = 0.68$ and $r^2 = 0.66$, respectively.

Seed treatment and application of fungicides (Raid, 1990, 1991; Patilet al., 2000; Wise and Muelle, 2011; Reddy et al., 2013; Veerabhadraswamy et al., 2014; Wathaneeyawech et al., 2015), host plant resistance and tolerant genotypes (Degefu, 2003; Harlapur, 2005; Ramathani et al*.,* 2011), field sanitation and conventional tillage (de Nazareno et al., 1993), sowing date (Ngugi et al., 2000; Fininsa and Yuen, 2001; Rai et al., 2002) and crop rotation (Pataky and Ledencan, 2006; Lipps and Mills, 2011) have been recommended for management of NLB. Despite these control measures, NLB continues to be a major constraint in maize production worldwide. Previous reports have shown variations among genetic background of different maize varieties and cultural practices in different regions and countries. This spatial and temporal variation makes it difficult to develop common strategies to combat northern leaf blight disease.

In Tanzania, among the diseases adversely affecting productivity, ubiquitous incidence of maize leaf blight in the pre-harvest stage was prominent particularly in the highlands of Mbeya and Arusha regions (Nwanosike et al., 2015b). Mbeya region is a major maize producing area in the Southern High lands of Tanzania. The climate varies from tropical to temperate, with altitude ranging from 400 and 3,000 masl. Temperatures are warm in the lowlands and cool in the highlands with cumulative rainfall between 750 to 3,500 mm annually (Bisanda et al., 1998). Such environmental conditions according to Nwanosike et al. (2015b) favoured NLB development and may be responsible for relatively low grain yield of 1.3 to 1.5 tons/ha (Rowhaniet al., 2011). The study therefore aimed at determining yield losses associated with northern leaf blight in five commonly grown varieties of maize and to develop yield loss models for estimating potential losses caused by *E. turcicum*in maize.

MATERIALS AND METHODS

Study area and field management

Field experiments were conducted at Agricultural Research Institute, Uyole in Mbeya Region 2013 and 2014 growing seasons using five commonly grown maize varieties under artificial

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inoculation. The maize varieties were Bora, Kilima, Situka-1, Staha, and TMV-1. Plots were established and maintained in the same field for the two growing seasons. Field experiments were laid out in Randomized Complete Block Design (RCBD) in three replications. The trial was conducted in paired blocks with *E. turcicum*inoculated and Mancozeb fungicide treatments (Harlapur, 2005). Four maize seeds were planted per hole and thinned to stand density of about 68,000 ha^{-1}, in a plot size of 3 m × 5 m, separated by 75 cm, 30cm and one meter inter-row, intra-row and inter plot/replicates, respectively. Thereafter, ten stands each were randomly selected at the two middle rows of each plot and tagged. Such stands were used for disease assessment.

The protected block was sprayed with 0.25% Mancozeb (Dithane M45, 80% WP) at 1.68 kg/ha (Patakyet al*.,* 1998;Harlapur, 2005) using 15-L-knapsack sprayer while the unprotected block was inoculated with pure culture of *E.turcicum*isolates, mass produced in sorghum seedsand used for maize inoculation using the whorl placement technique (Adipalaet al., 1993; Veerabhadraswamyet al., 2014). Plants in the two middle rows were inoculated by placing about 10 infected sorghum seeds on five whorls of each stand (50 seeds per stand). Inoculation was done twice at 35 and 45 DAPat 1600 h and thereafter spread with water using a 15-L-knapsack sprayer (Harlapur, 2005) to disperse conidia for infection.Five fungicide sprays were done, starting from 35 DAP and thereafter, maintained at intervals of 7 days. To avoid inter-plot dispersal of inoculum and drift of the fungicide, three rows of tall and late maturing local variety of maize presumed to be resistant to northern leaf blight were planted between the protected and inoculated blocks. Blanket application of Dimethioate insecticide was applied twice, 30 and 45 days after planting at commercial recommendation in the two blocks to avoid insect damage. Agronomic recommendations for maize production were observed.

Northern leaf blight assessment

Disease severity were recorded based on percent leaf area infected at the silk dry stage using visual scales of 0-5 as described by CIMMYT (1985) and Durrishahwar et al. (2008) with modification. Disease severity rating was as follows; 0 = leaves free from infection, 1 = a few restricted lesions on the lower leaves (≤ 5 %), 2 = several small and large lesions on many leaves (5.1-10%), 3 = numerous small and large lesions on many leaves (10.1-25%), 4 = many enlarged and coalesced lesions on many leaves above the cob (25.1-50%) and 5 = several coalesced lesions, leaf showing wilting, tearing and blotching typical blight symptoms (> 50%). Severity scores were converted to percent disease index as described by Wheeler(1969).After harvesting, grain yield and 1000-grain weight were calculated from weight of hand threshed maize and converted to tons/ha and g/plot after adjusting to 15.5% moisture content with "Mini GAC Moisture tester" by Dickey-John Corporation Auburn, Illinois, USA. Such data were used to determine yield losses.

Data analysis

Data were subjected to combined analysis of variance (ANOVA).Means that showed significant differences were compared using Turkey's-Kramer simultaneous test for data at P≤ 0.05 (Steel et al., 1997). Data on grain yield and 1000-grain weight were used to evaluate grain yield losses and grain yield enhancement of Mancozeb fungicide over the inoculated plots for each variety in the two growing seasons. *E. turcicum* inoculated treatments were expressed as a percentage of Mancozeb treated plots as described by Harlapur (2005).

$$Percent\ yield\ loss = \frac{Vp - Vu}{Vp} \times 100$$

Where, Vp = Value of protected plot, Vu = Value of unprotected plot.

Yield enhancement by the Mancozeb fungicide sprays on each variety was calculated by the modified method of Veerabhadraswamyet al. (2014) as described below;

$$Percent\ enhancement\ of\ yield = \frac{Yield\ differences}{(Yield\ of\ untreated + Yield\ of\ treated)} \times 100$$

Crop loss assessment model

Crop loss assessment models were developed for each of the five varieties using grain yield (tons/ha) and 1000-grain weight (g/plot) in 2013 and 2014. The grain yield, 1000-grain weight values and the percent disease index (PDI) values were used to study the relationship between northern leaf blight severity index and losses in the maize varieties used (Nwanosikeet al., 2015a). Critical point models for northern leaf blight of maize were developed using simple linear regression functions;

$Y = a + bx$

Where; Y = the yield loss, 'a' = constant, 'b' slope and 'x' = per cent disease index (PDI). Yield expressed as a percentage of the average yield of Mancozeb treated plots were used as the dependent variable. Selection of the best fitting models were based on correlation coefficient (r), which showed the relationship between dependent and independent variables, 2) coefficient of determination (R^2), which indicated the proportion of the total variation explained by the model and 3) F-statistics, which tests the significance of the regression model ($p < 0.05$) as described by Perkins and Pedersen (1987). Genstat 14th edition (PC/window7, 2013), IBM SPSS statistics 20 and XLSTAT 2015 version software statistical packages were used for analysis of data.

RESULTS

Northern leaf blight(NLB) severity index indicated significant differences (p ≤0.05) in Mancozeb treated and *E. turcicum* inoculated plots with means of 23 and 88.9%, respectively (Table 1). Severity index of NLB ranged from 78.7% in Situka-1to 95.7% (Bora) in the *E. turcicum* inoculated plots, with relatively low disease severity index in Mancozeb treated plots (23%).The study revealed high *E. turcicum* pressureattributed tofavourable climatic factors in Mbeya.

In terms of grain yield, Mancozeb significant increase yield (3.31 tons/ha) over the 1.51 tons/ha recorded in *E. turcicum* inoculated plots (Table 1). When yield was measured in1000 grain weight,there were no significant differences (P ≤ 0.05) in both Mancozeb treated and *E.turcicum* inoculated plots.However, Mancozeb sprays indicatedhigh grain weights (340.3 g/plot) compared to*E.turcicum*inoculated plots (219.8 g/plot). Such results indicated grain yield enhancement of 30% in TMV-1 and

Table 1. Effect of Mancozeb and *E. turcicum* inoculation on development of northern leaf blight and yield of maize in Mbeya during the 2013 and 2014 growing seasons.

Variety	% Disease severity index		Grain yield				% Grain yield loss	
	Mancozeb treated	*E. turcicum* inoculated	Grain Yield (tons/ha)		1000-grain wt (g/plot)		Grain yield	1000-grain wt
			Mancozeb	*E. turcicum*	Mancozeb	*E. turcicum*		
Bora	28.3[a]	95.7[a]	3.15	1.28[b]	350	214.3	58.2	38.7
Kilima	20.0[b]	88.3[ab]	3.22	1.68[a]	336.7	217.6	45.3	35.3
Situka-1	20.0	78.7[b]	3.47	1.61[a]	343.3	233.6	51.7	31.9
Staha	26.7[a]	87.3[a]	3.43	1.25[b]	340	214.3	62.8	36.9
Tmv-1	20.0[b]	94.7[a]	3.23	1.74[a]	331.7	219.2	46.0	33.8
Mean	23.0	88.9	3.31[ns]	1.51	340.3[ns]	219.8[ns]	52.8	35.3
Cv	18.8	7.2	17.8	6.7	4.1	6.3	-	-

Means followed by the same letter in the same column are not significantly different according to Turkey's 95% level of confidence.

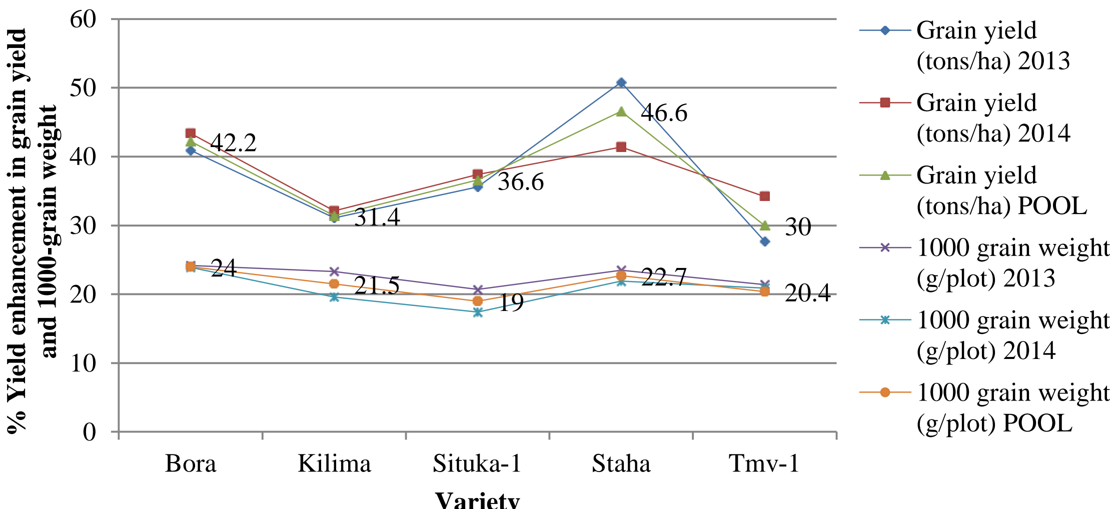

Figure 1. Maize percentage of grain yield enhancement (tons/ha) and 1000-grain weight (g/plot) of Mancozeb fungicide over the *E. turcicum* inoculated plots, with pooled data as the mean yield in 2013 and 2014.

46.6% in Staha. Similarly, yield was also enhanced with the 1000-grain weight and ranged from 19% (Situka-1) to 24% in the variety Bora (Figure 1). Although all the varieties were susceptible to NLB disease under artificial inoculation, Bora and Staha consistently recorded high disease index and high grain yield enhancement when Mancozeb fungicicde was used.

Northern leaf blight disease significantly ($P \leq 0.05$) and adversely reduced grain yield of the maize varieties used.Results (Table 1) showed grain yield losses of 46 to 62.8% with an average loss of 52.8 and 31.9% (Situka-1) to 38.7% (Bora)with mean loss of 35.3% when yield was measured 1000-grain weight. The variety Staha recorded 62.8 grain yield loss followed by the variety Bora which gave 58.2%. Kilima recorded the least yield loss of 45.3% in 2013 and 2014 maize growing season in Tanzania. It was similarly found that, the variety Bora showed yield loss of 38.7% followed by Staha (36.9%) while Situka-1 recorded lowest loss of 31.9%, considering the 1000-grain weight. Statistically, there were no significant differences ($P \leq 0.05$) in NLB severity index of the varieties Bora, Staha and TMV-1 in the *E. turcicum* inoculated plots, but there were variationsin yield losses both in grain yield and in 1000-grain weight of the varieties. Such inconsistenciessuggested that grain losses of maize due to NLB do not only depend on the level of susceptibility but also on variety tolerance to the disease.

Crop loss assessment model for each of the five varieties using grain yield, 1000 grain weight and the

Table 2. Crop loss models, correlation and regression coefficients between severity index of northern leaf blight and grain yield of five maize varieties during the 2013 and 2014 growing seasons in Mbeya.

S/No	Variety	Grain yield		1000-Seed weight	
		2013	2014	2013	2014
1	BORA	y = 4.09 - 0.0293 PDI r = - 0.9244 (0.008) R^2 = 0.8545	y = 3.82 - 0.0266 PDI r = - 0.9561 (0.003) R^2 = 0.9141	y =419.47-2.1296 PDI r = - 0.9741 (< .001) R^2 = 0.9489	y = 391.93 - 1.8443 PDI r = - 0.9778 (<.001) R^2 = 0.9561
2	KILIMA	y = 3.97 - 0.0239 PDI r = - 0.9540 (0.003) R^2 = 0.9100	y = 3.32 - 0.0202 PDI r = - 0.7617 (0.078) R^2 = 0.5801	y =381.57-1.8697 PDI r = - 0.9759 (< .001) R^2 = 0.9523	y = 355.12 - 1.5015 PDI r = - 0.9257 (0.008) R^2 = 0.8569
3	SITUKA-1	y = 4.17 - 0.0312 PDI r = - 0.8296 (0.041) R^2 = 0.6883	y = 3.90 - 0.0292 PDI r = - 0.9310 (0.007) R^2 = 0.8667	y =388.41 - 2.008 PDI r = - 0.9807 (< .001) R^2 = 0.9619	y = 367.67 - 1.6267 PDI r = -0.9704 (0.001) R^2 = 0.9417
4	STAHA	y = 5.28 - 0.0474 PDI r = - 0.9901 (< .001) R^2 = 0.9803	y = 3.55 - 0.0253 PDI r =- 0.9662 (0.002) R^2 = 0.9336	y =401.58-2.2164 PDI r =- 0.9300 (0.007) R^2 = 0.8649	y = 381.53 - 1.7988 PDI r = - 0.9837 (<.001) R^2 = 0.9676
5	TMV-1	y = 3.70 - 0.0222 PDI r = -0.9018 (0.014) R^2 = 0.8132	y = 3.70 - 0.0222PDI r = - 0.9018 (0.014) R^2 = 0.8132	y =355.19-1.4268 PDI r = -0.9699(0.001) R^2 = 0.9407	y = 355.19 - 1.4268 PDI r = - 0.9699 (0.001) R^2 = 0.9407

Figures in bracket are the t- test F-probability, r = correlation coefficient, R^2 = coefficients of determination, PDI = percent disease index.

percent NLB disease index (PDI) values were used to determine the relationship between NLB severity index and losses in the maize varieties. The cumulative effect of the disease epidemics indicated high negative significant correlation coefficients ($P \leq 0.05$) between NLB severity index and grain yield (tons/ha and 1000-grain weight). Correlation coefficients ranged from - 0.83 (Situka-1) to 0.99 (Staha) in 2013 and - 0.76 (Kilima) to - 0.97 (Staha) in 2014in grain yield (tons/ha). In the 1000-grain weight, correlation coefficients range of -0.83 (Situka-1) to -0.99 (Staha) in 2013 and -0.76 (Kilima) to - 0.97 in Staha (Table 2) were also observed. The results confirmed that increase in the NLB severity index reduced grain yield in the five varieties of maize used in the study.

Coefficient of determination varied from R^2 = 0.69 - 0.99 in 2013 to R^2 = 0.58 - 0.96 in 2014 in grain yield (tons/ha) and ≥ 0.86in 1000-grain weight for the two growing seasons. The study confirmed that 69-99% and 58-96% variation in grain yield were attributed to NLB in 2013 and 2014, respectively and more than 86% in 1000-grain weight. It is therefore, clear that critical point models using percentage leaf area affected by NLB indicated good fit in the five varieties. Therefore, the validity of the relationship between grain yield and severity index at silk dry stage showed strong evidence and varied from variety Kilima (r = - 0.7617, R^2 = 0.580, $P \leq 0.078$) in 2014 to Staha (r = - 0.9901, R^2 = 0.9803, $P \leq 0.001$) in 2013. Similarly, observation was made in terms of yield measured in 1000-grain weight. However, the minimum relationship was very strong in variety Staha (r = - 0.9300, R^2 = 0.8649, $P \leq 0.007$) in 2013 (Table 2). The study confirmed that the predicted grain yield (tons/ha and 1000 grain weigh) loss values in varieties due to NLB indicated good fit.

The linear regression coefficients indicated negative slopes, - 0.02 to -0.04 and - 0.02 to - 0.3 in 2013 and 2014, respectively (Table 2). The varieties Kilima and TMV-1 consistently showed - 0.02 slope coefficients. Bora, Situka-1 and Staha differed and ranged from - 0.03 to - 0.04, particularly in 2013 grain yield. In slope coefficients using 1000-grain weight, variety TMV-1 recorded -1.4 while Staha, Bora, Kilima and Situka-1 ranged from -1.5 to -2.2. Such indicated that grain yield loss in every unit increase in NLB intensity. However, losses varied among the varieties due to different levels of tolerance to NLB.

DISCUSSION

Northern leaf blight (NLB) disease progressed faster and

was very high (88.9%) in the maize inoculated with *E. turcicum,* than in Mancozeb treated plots. This was evident inthe low severity index (23.0%) in Mancozeb treated plots. The study agreed with those of Kachapur and Hegde (1988), Raid (1990), Pandurangegowda et al.(1993), Harlapur et al. (2007) and Wathaneeyawech et al. (2015) who reported that Mancozeb sprays at 0.25% significantly reduced NLB and increased grain yield of maize. The high disease index was associated with favourable climatic conditions that prevailed during the growing seasons (Raid, 1991; Nwanosike et al., 2015b). Pataky (1992) also reported that under favourable environmental conditions, maize hybrids reacted differently to NLB.

Timely application of Mancozeb also increased grain yield by 30 to 42.2% in grain yield and 20 to 24% in 1000-grain weight over the inoculated plots. However, the consistently high disease index and grain yield enhancementobserved in the varieties Bora and Staha indicated that even among susceptible maize varieties, NLB reacted differently. Raid (1991) earlier reported low NLB disease development in maize due to application of Mancozeb, such management approach should be prompt and not when NLB had reached epiphytotic level before initiation of fungicide control particularly for susceptible hybrids. This is because increase in corn residues serves as a source of primary inoculum (Wise and Mueller, 2011), and the disease severity has a profound effect on yield losses of maize (Veerabhadraswamy et al., 2014).

Previous reports have shown that NLB significantly and adversely reduced grain yield of the maize varieties and hybrids (Pataky, 1992; Solomonovish et al., 1992; Adipala et al., 1993; Harlapur, 2005). Yield losses of 45.3% (Kilima) to 62.8% (Staha) in grain yield and 33.8 to 38.7% in 1000-grain weight suggested differences in the genetic background and levels tolerance among the maize varieties to NLB disease. Earlier, reports have shown that yield losses due to NLB in maize varied among genotypes based on resistance or susceptibility of hybrids (Pataky et al., 1998; Shivankar and Shivankar, 2000; Babuet al., 2004). Nwanosike et al. (2015a) reported grain losses of 23.9-40.4% in grain yield and 11.2-36.1% in 1000-grain weight in Morogoro, Tanzania. Perkins and Pedersen (1987) reported that reduction in 500-grain weights of maize affected yield losses due to loss of active leaf area.

The highly significant ($P \leq 0.05$) negative correlations between NLB severity index and grain yield (r = -0.76 to -0.99) and in 1000-grain weight (r = -0.92 to -0.98) indicated that increase in severity index of NLB reduced yield in the five varieties of maize. Harlapur (2005) also reported high negative correlation (-0.97) between two susceptible maize genotypes (CM-202 and Deccan-103). Similar high negative correlation of -0.59 to -0.98 between NLB and five susceptible varieties of maize were also reportedin Morogoro, Tanzania (Nwanosikeet al., 2015a). Pataky et al. (1998) reported that yield measured as weight of ears and number of marketable ears decreased with increase in NLB disease severity index. Highly positive significant correlation (r = 0.76 - 0.94) have been reported between NLB and percentage of unmarketable ears of susceptible maize genotypes (Campbell and Madden, 1990; Raid, 1991; Pataky et al., 1998).

Significant coefficient of determination (R^2 = 0.58 to 0.98) in grain yield and 1000-grain weight (R^2 = 0.85 to 0.96) suggested that 58-98% and 85-96% variation in yield could be attributed to NLB severity index at harvest. Hence the study confirmed that the predicted grain yield losses in the varieties Bora, Kilima, Situka-1, Staha and TMV-1 indicated a good fit. This was also evident in the negative slope coefficients (Table 2), which indicated considerable yield loss per every unit increase in NLB disease severity index. Reports have shown good predictions on grain yield reduction of susceptible maize genotypes due to NLB disease, using critical point model. (Perkins and Pedersen, 1987; Bowen and Pedersen, 1988; Adipalaet al., 1993; Patakyet al., 1998; Nwanosikeet al., 2015). Negative slope coefficient observed between NLB severity index and yield of maize have been documented (Chenulu and Hora, 1962; Fisher et al., 1976; Pataky, 1987, 1992; Harlapur, 2005).

Conclusion

The study therefore, reveals that Mancozeb fungicide sprays against NLB disease enhanced grain yield of the maize varieties particular in high susceptible varieties (Bora and Staha). For cultivation of such highly susceptible varieties, fields must be adequately scouted for NLB, and initiation of Mancozeb sprays must be routinely timed prior or as soon the disease is detected in the field to suppress the disease. It was also established that, under artificial inoculation of *E.turcicum*, the maize varieties Bora, Kilima, Situka-1, Staha and TMV-1 were highly susceptible and resulted to grain yield losses. The crop loss models for individual variety indicated good fitness with strong and reliable validity. Such models can be used to estimate potential losses of maize caused by NLB disease in Mbeya Region. However, being the first report on the relationship between maize grain yield and northern leaf blight disease in Mbeya Region of Tanzania, there is a need for further studies, mostly using leaf position to confirm the developed crop yield loss models for sustainable management of northern leaf blight in such endemic and high maize production region.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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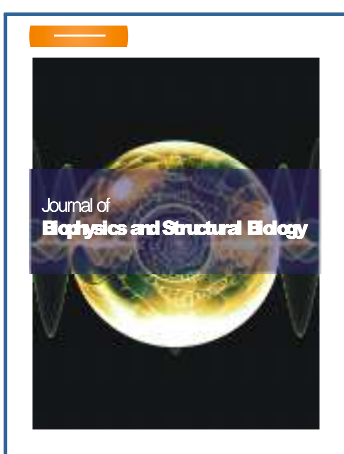

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