Decomposition of *Eichhornia crassipes* by Different Fungal Species in Chitwan Annapurna Landscape Nepal

M.Sc. Dissertation submitted for partial fulfillment of Master's Degree in Botany
(Plant Pathology and Applied Mycology)

Submitted by
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Letter of Recommendation

This is to recommend that the thesis entitled "**Decomposition of *Eichhornia crassipes* by Different Fungi in Chitwan Annapurna Landscape Nepal"** has been carried out by Mr. Sagar Khadka for the partial fulfillment of the Master of Science in Botany with special paper "Plant Pathology and Applied Mycology". This is his original research work and has been carried out under our supervision. To the best of our knowledge, this thesis work has not been submitted for any other degree in any institutions. I recommend the thesis to be evaluated and accepted for the degree of Master of Science in Botany, Tribhuvan University, Kathmandu.

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Sagar Khadka
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Abstract

Water hyacinth (*Eichhornia crassipes*) is considered as one of the most noxious invasive aquatic weed in almost all tropical as wells subtropical lakes or wetlands in Nepal and has negative effect on environment. The main objective of this study was to assess the effect of *Eicchornia crassipes* and utilize soil fungi *Trichoderma*, *Rhizopus* and *Aspergillus* in composting as a cheap effective management options. In addition, the compost was used to study its effectiveness on germination and growth of wheat *(Triticum aestivum)* and compared with cow dung and organic manure. A study survey was done in Ghailaghari, Begnas and Rupa Lakes of Nepal. Compost was prepared using soil and water hyacinth in three different proportions in plastic bags, i.e., 50:50, 25:75 and75:25 in which 10^7 cfu/ml unit spore suspension of three fungi, i.e., *Trichoderma*, *Rhizopus* and *Aspergillus* from each 10 ml PD broth and *Trichoderma* from the market was inoculated. Initially, soil fungi were isolated using soil plate method and then transferred to PD broth for spore suspension. The compost was analyzed for C, N, P, and K.

Most of the people believe that water hyacinth has adverse environmental impacts as well as socio-economic impacts. The C, N, P, K were found higher in the compost with ratio 75:25. *Rhizopus* and *Trichoderma* treated compost showed highest percentage of Carbon, Nitrogen, Potassium and Phosphorus. Fungal species of *Aspergillus*, *Fusarium*, *Trichoderma*, *Mucor*, *Penicillium* and two unidentified species were employed in the composting at the control set of water hyacinth compost. Cow dung followed by *Trichoderma* treated pots showed highest growth of the test plant.

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C	Carbon
cfu	Colony forming unit
cm	Centimeter
g	Gram
ha	Hectare
K	Potassium
kg/ha	Kilogram per hectar
km	Kilometer
LIBIRD	Local Initiatives for Biodiversity, Research and Development
m	Meter
ml	Milliliter
N	Nitrogen
P	Phosphorus
PD broth	Potato Dextrose broth
PDA	Potato Dextrose Agar
RFRLC	Rupa Lake Restoration and Fishery Co-operative

1. INTRODUCTION

1.1 Background

Water hyacinth, *Eichhornia crassipes* (Mart.) Solms, is regarded as one of the troublesome weed that belongs to the family Pontederiaceae. This is a perennial monocot native to Brazil and Ecuador region. It is also listed as one of the '100 world's worst invasive alien species' by IUCN (Lowe *et al.*, 2000; Bhattacharya and Kumar, 2010; Rakotoarisoa *et al.,* 2015). This plant is considered as favorite ornamental plant for artificial pond due to its attractive flower and foliage (Barber and Hayne, 1925). It is a free floating macrophyte that prefers growing in fresh water ponds and slow moving waterways (Parolin *et al.*, 2012). It can double its biomass and invades water bodies by destroying ecosystems. (Lata and Veenapani, 2011).

Its use as an ornamental plant has led to accidental and intentional introductions to non-native water bodies and spread as aquatic weed to new catchments (Villamagna *et al.,* 2009). Due to lack of insects, viruses and other enemies as its pest, this weed has been successful in the introduced native environment (Wolverton and Mcdonald, 2010). It appears as floating islands of plants that can double within 4-7 days (Navarro and Phiri, 2000). The mats are dispersed by means of wind and most of the biomass production is allocated for root development during the mat formation (Center *et al.,* 2005; Katagira *et al.,* 2011). It is often spread during the flood events (Penfound and Earle, 1948; Ghosh, 2010).

The plant is responsible for environmental degradation like drastic changes in the plant and animal community, light and oxygen diffusion, degraded water quality and reduction in water movement etc. It causes clogging of irrigation, hydropower and water supply ways, hindrance to water transport, blockage of canals and rivers that results in flooding. The infestations of this weed is also responsible for the diseases like Malaria, Bilharzia and Cholera (Ruskulis, 1998; Navarro and Phiri, 2000;; Bhattacharya and Kumar, 2010).

Mechanical removal is short term solution that is less effective to the large area since plant grows back again and chemical uses also have environmental and health hazards (Mangas-Ramirez and Elias-Gutierrez, 2004). There is a need for a new perspective to manage this species by alternative uses in biofuel crop, traditional medicine, biogas production, mushroom bedding material, making of ropes, production of fiber boards, as animal fodders and fish feed, green manure, compost and as an ornamental plant (Calvert, 2002; Bhattacharya and Kumar, 2010; Jafari, 2010). Water hyacinth is rich in protein, hemicellulose and cellulose hence it can be used as feed for duck, goats, pigs and fishes (Nigam, 2002; Tham, 2015). In those places where water hyacinth is found abundantly, it acts as source for bioethanol and biogas production due to presence of low lignin (Chanakya *et al.,* 1993; Kurup and Prabhu, 2005; Bhetalu *et al.,* 2012).

It has medical value and used for goiter and wound healing by Indian tribes (Ghosh, 2010). Harmful metals like Cd, Mn and Zn are absorbed by this weed and purify water (Aisien *et al.,* 2010). Roots act as low cost bio-–sorbent for absorption of Uranium (Kouraim, *et al.*, 2015). Water hyacinth compost is rich in nutrients like N, P, K which reduces the use of chemical fertilizer and plays vital role in the nutrient recycling (Prasad *et al.*, 2013; Osoro *et al.,* 2014). Bacteria, fungus and Actinomycetes play vital role in bioconversion of weed biomass into organic manure (Geetha, 2009). Most of the fungi are saprophytic, capable of degrading cellulose and lignin (Thorn *et al.,* 1996; Sivaramanan, 2014). The decomposition of organic materials into stabilized compounds is achieved by microorganisms although the precise role of certain microbes in unknown (Fuchs, 2010). The C:N ratio and water holding capacity was increased on the fungal inoculated samples of cellulolytic wastes (Hart *et al.,* 2002; Sivaramanan, 2014). Its manure consists of acceptable composition of N, P, K, pH which is beneficial for agricultural lands to grow crop plants (Kafle *et al.,* 2009).

1.2 Justification

Water hyacinth causes several social, economic and environmental effects and its mat leads to the degradation of aquatic ecosystem (Barrett, 1989). Many lakes and ponds of Nepal are facing problems due to water hyacinth. In Nepal, almost negligible research on this plant has been done. Hence, this study has been conducted to use this weed as compost, by using some decomposing soil fungi.

The improvement of traditional method of composting through the uses of cellulolytic microorganism plays role in hastening the decomposition of plant biomass (Singh and

Sharma, 2002; Abdulla and El-shatoury, 2007; Mahanta *et al.,* 2014). As water hyacinth is easily available it can be used as source of macronutrients like Nitrogen, Potassium, Phosphorus that can be applied to the plants like maize and common beans (Kwabiah *et al.,* 2003; Wasonga *et al.,* 2008; Osoro *et al.,* 2014).

Scientists believe that the proper utilization of this weed is one of the possible management options. The best one is composting the weed for agricultural use. Such study like preparation of compost from water hyacinth using the soil fungi has scope in Nepal. If farmers prepare the compost using this weed and apply in their agriculture land, there will be dual benefits. The first one will be management of water hyacinth and another will be application of composting their crop fields to increase the yield.

Present study, hence, focuses the management of water hyacinth disposal problems by means of composting using inoculation of soil fungi like *Trichoderma*, *Rhizopus* and *Aspergillus*. Soil fungi are present naturally in soil that decomposes plant biomasses. Some local people living near to lakes have been using degraded biomass of water hyacinth in the field.

1.3 Objectives

The main objective of the study was to know the effect of *Eicchornia crassipes* and utilize soil fungi involved in composting as cheap effective options for management. The specific objectives were:

i. To enhance the degrading process of compost of *Eichhornia crassipes* by using species of soil fungi like *Trichoderma*, *Rhizopus*, *Aspergillus*.
ii. To study effect of compost from Water hyacinth on growth and development of wheat.

1.4 Limitations

- Only soil fungi were used for decomposing the water hyacinth compost.
- C, N, P and K were used as parameters for analyzing the nutritional quality of compost prepared using different fungi. Other parameters like productivity of plant could not be taken into consideration due to the time and resource constrain.

2. LITERATURE REVIEW

2.1 History

Amazon Basin is considered as the origin place of water hyacinth (Center *et al.*, 1999). It is now wide spread in five continents including warm areas of Asia, Australia Africa, New Zealand, Central America, North America (Jafari, 2010; Agunbiade *et al.*, 2009). During the late 19^{th} and early 20^{th} centuries water hyacinth was introduced into many countries including Africa and now this weed creates environmental and cultural problems (Center *et al.*, 2002). Water hyacinth was first reported in Egypt in 1880s, but was not noticed in waterways until 1932 (Navarro and Phiri, 2000). After introduction of this weed in 1879 to Europe, it was unable to survive due to post and prolonged winter. Later it was introduced in 1880. Its journey as an ornamental plant to different countries started in at 1895 in Australia, 1910 in Malaysia, 1937 in Zimbabwe and 1952 in the Republic of the Congo (Kadono, 2004; Bhetalu *et al.*, 2014).

The history of water hyacinth in China can be trace back to early 1900s. In 1903, it was originally introduced from Southeast Asia as ornamental plant into Taiwan. Soon it spread due to its use as animal food resulting damages to biodiversity in 1980s and 1990s (Ding *et al.*, 2001). Water hyacinth was introduced as ornamental plant in Japan in 1884, i.e., early Meiji era.that caused serious weed problem (Kadono, 2004). It was introduced by British colonies in India during 1890 and often known today as as Bengal terror (Ghosh, 2010). In Nepal, this weed was mentioned in 1966 by Hiroshi Hara in Flora of East Himalaya and the first herbarium was reported from Nepalgunj (Tiwari *et al.*, 2005).

2.2 Reproduction

Mostly water hyacinth depends upon vegetative reproduction for its prolific multiplication and growth which is through stolon production or budding. Colonial growth of water hyacinth is due to the production of seeds by sexual reproduction (Penfound and Earle, 1948; Barrett, 1980b; Ghosh, 2010; Zhang *et al.*, 2010; Adebayo *et al.*, 2011).

2.3 Biomass production

Depending upon location and time water hyacinth can easily produce 140 dry material ha^{-1}year$^{-1,}$ hence is regarded as potential source for biomass (Masami *et al.,* 2008; Patel and Patel, 2015). The entire surface of water is usually covered by the perennial mats of this weed which can double within a week under the favorable growth (Penfound and Earle, 1948; Kurup and Prabhu, 2005). Generally, water hyacinth has capacity of producing double biomass within 12 days., Barrett (1989) states that twenty five plants can produce two million plants occupy 10,000 sq m of water surface and having weight equivalent to jumbo plane.

2.4 Impacts

Invasion of this weed in new aquatic ecosystem leads to drastic change in light availability in water body, reduction in flow of water, interference with fisheries, recreational activities as well as side effects on human health (Villamagna and Murphy, 2010; Adebayo *et al.,* 2011). Physicochemical parameters like temperature, pH, dissolved oxygen, carbon dioxide concentration and nutrient composition can be altered in water infested with water hyacinth (Rai and Munshi, 1979; Tellez *et al.,* 2008). There are several reports jointly on deterioration of water quality due to death and decay of large masses of vegetation (Mironga *et al.,* 2011; Ndimele *et al.,* 2011; Patel, 2012; Theuri *et al.,* 2013).

Water hyacinth can compete with crop plants if become established on the irrigated land (Gay and Berry, 2017). The breeding grounds for mosquitos are created due to the choking of water bodies (Barrett, 1989; Ghosh, 2010). Increasing mats provide food, shade and oxygen thus creating favorable conditions for snails bearing schistosomes (Filmalter *et al.,* 2013). Heavy infestation provides coverage for reptiles and poisonous snake that leads to incidents like attack of crocodiles (Mironga *et al.,* 2011; Ndimele *et al.,* 2011; Patel, 2012).

2.5 Management strategies

2.5.1 Mechanical removal

Mechanical removal is temporary and short term solution that can be applied within small areas. Small recovery of water quality occurs and the plant grows back again (Mangas-Ramirez and Elias-Gutierrez, 2004).

2.5.2 Chemical control

Chemicals like 2, 4-D; Diquat and Glysophate are the most commonly used to control during small infestation of water hyacinth and it has shown good success rate. Along with the glyphosate, citric acid, propionic acid, acetic acid and formic acid performed well as to control of water hyacinth. This method is less successful in large areas and environmental and health issues are raised due to these herbicides (Calvert, 2002; El-shahawy, 2015).

2.5.3 Biological control

Introduction of natural enemies is considered as effective alternative method of eradication of this aquatic weed. After raring the insects species like *Neochetina eichhorniae* Warner, *N. bruchi* Hustache (Coleoptera. Curculionidae) and *Sameodes albiguttalis* (Warren) (Lep. Pyralidae), these were released as a part of mass culture and release program in early 1979. *Neochetina weevil* was highly successful in comparison to moths like *S. albiguttalish* (Irving and Beshir, 1982; El Tayeb, 2012).

In natural conditions, Chilo an insect borer has been found attacking *Eichhornia crassipes*. The Qalumid mite is also considered lethal since it damages the leaf tissue and slow down reproduction by vegetative means. The most promising insect for damaging water hyacinth appears to be an aquatic grasshopper, *Gesonula punctifrons* in India. In Puerto Rico snail *Marisa cornuarietis* was considered to be used for controlling the massive growth of water hyacinth. Fungus *Cercospora piaropi* infestation in South Africa was discovered in 1987. *Neochetina eichhorniae* was established as a part of effective control measure for growing water hyacinth in South Africa (Bennett, 1967; Cilliers, 1991).

Myrothecium advena and *Fusarium pallidoroseum* (Cooke) Sacc are considered as promising bio control agents of water hyacinth in Kerala since these are responsible for 50% infection to water hyacinth (Praveena and Naseema, 2004). On the basis of performance in natural environmental conditions, fungus like *Alternaria*. *alternata*, Alternaria. *chartarum*, *Fusarium chlamydosporium*, *Fusarium equiseti* and *Pythium ultimum* could be used as effective bio-control agents against water hyacinth (Tegene *et al.*, 2012).

2.5.4 Alternative uses

There are reports on application of water hyacinth in production of fiber boards, basket work, yarn and ropes (Gajalakshmi and Abbasi, 2002; Gunnarsson and Petersen, 2007; Raji *et al.*, 2008).

Paper: For paper production stems of water hyacinth has been used. In countries like Philippines and India, projects on paper production from water hyacinth on small scale have been successful. Low quality paper has been produced from this weed fiber which was generally used for making boxes and folders. Water hyacinth is a good potential source for a long fiber pulp which can be used for paper production and it acts as absorbent for sodium silica sulfur and chlorine from waste water of paper industries (Widyanto *et al.*, 1983; Calvert, 2002; Jafari, 2010).

Fiber boards: Water hyacinth fiber has been used for fiber board production by House and Building Research Institute in Dhaka. The pulp obtained from stalk can be mixed with other waste paper pulp for board making. These properly dried fiber board are considered as low cost roofing material and can be used for indoor partition walls and false ceiling (Calvert, 2002; Jafari, 2010).

Basket work: Stalks of properly dried water hyacinth can be used in making traditional basket. It's used as domestic purpose in Philippines while in India it is used to demonstrate to tourism (Calvert, 2002; Jafari, 2010).

Yarn and ropes: The stem fiber of water hyacinth is very useful for making ropes. Fiber is exposed by shredding the stalk of plant which is left to dry for several days the finally treated with sodium metasulphide. Rope is made in Bangladesh by furniture manufacturer, winds the rope around the cane using metal frame (Calvert, 2002; Jafari, 2010; Tumbaga *et al.*, 2013).

Feed for animal and fishes: Water hyacinth ensilage serves as alternative to other high quality diets for cattles in dry seasons. Diets produced from berseem and water hyacinth fiber residues used in twenty four buffalo calves showed optimum level of protein as required by the ruminants (Borhami *et al.*, 1992; Tham and Uden, 2013). Moreland *et al.* (1991) concluded that water hyacinth is palatable and does not hampers the growth when used along with alfalfa as feed ration for rabbit. Due to high level of energy source like cellulose and hemicellulose, it can be used as food source for cattle and fishes (Nigam, 2002). Leaves of water hyacinth consist of high protein which make them suitable fodder for cows, goats, pigs, ducks and tilapia fingerlings (Abdelhamid and Gabr, 1991; Tham, 2015).

Charcoal briquetting: The water hyacinth briquettes can be alternative to firewood since these have high combustible value (Davies *et al.,* 2013). It provides income as well as reduces the pressure on other fuel resource biomass (Ndimele *et al.,* 2011). Fuel briquettes sewage sludge mixed with water hyacinth and sewage mixed with sedge were checked for quality like water content, calorific value and compressive strength to be used as alternative energy source. The briquettes made from water hyacinth and plantain peels are suitable for domestic as well as industrial uses since these can be reliable, durable and can stand mechanical handling as well as transportation (Davies and Abolude, 2013; Supatata *et al.,* 2013).

Bioethanol and biogas: Study on water hyacinth indicates it as suitable for bioethanol production (Kurup and Prabhu, 2005; Patel and Patel, 2015). Liquid ethanol was successfully produced from water hyacinth using yeast fermentation and two-sequential steps of acid hydrolysis. *Pichia stipitis* has been used along with water hyacinth hemicellulose acid hydrosylate for bioethanol production. As these plants have high growth rate and low lignin, these can be used either as bioethanol for vehicles or biogas in generating electricity (Nigam, 2002; Isarankura-na-ayudhya *et al.,* 2007; Bhetalu *et al.,* 2012)**.**

In the areas where water hyacinth is abundant, it acts as feedstock for production of biogas. Volatile fatty acid of water hyacinth along with cow dung slurry in biogas digester yields 22% more biogas per unit feed. It was found that with the increase in amount of paper along with cow dung slurry and the plan, the production of biogas was found to be decreased. Solid state fermentation of fresh and dry plant biomass and garbage from market can also be effective in biogas production thus being economically beneficial (Chanakya *et al.,* 1993; Ganesh *et al.,* 2005; Verma *et al.,* 2007; Yusuf and Ify, 2011; Njogu *et al.,* 2015).

Phytoremediation: Toxic metals such as Cd, Pb and Zn in the water bodies are absorbed and accumulated by the plant directly from the water (Aisien *et al.,* 2010; Lu *et al.,* 2004) .Water hyacinth can help in cleaning up Zn, Cd, Cu and Cr from waste water bodies due to its small size and large surface area (Alluri *et al.,* 2007; Yapoga *et al.,* 2013).

Meristematic root cells of water hyacinth shows its potential for mitigation of mercuric pollution. Also uranium can be adsorbed by the roots of water hyacinth hence is considered as low cost absorbent (Wolverton and McDonald, 1978; Lenka *et al.,* 1990; Sadeek *et al.,* 2014; Kouraim *et al.,* 2015). The culture of water hyacinth is responsible for water purification with the reduction of 8.9% of BOD, 9.2% of COD, 45.5% of nitrate, 37.8% of phosphorous and 7.5% of faecal coliform (Wolverton and McDonald, 1978; Aremu *et al.,* 2012).

Compost: Some aquatic weeds can produce large amount of biomass. Composting of water hyacinth is easy to work with soil due to the decomposed structure and acts as alternative treatment with dried water hyacinth (Geetha, 2009; Dhal *et al.*, 2011). The vermicompost of this weed was used for testing growth and reproduction of *Crossandra undulaefoliae* and other species of vegetation which had beneficial impact (Sannigrahi, 2009; Gajalakshmi and Abbasi, 2002).

The agitated pile compost process was efficient with addition of optimum cattle manure along with the plant material itself (Prasad *et al.,* 2013). In the vermicomposting experiment of *Eisenia foetida* using toxic weeds like congress grass (*Parthenium hysterophorus* Linn.), water hyacinth (*Eichhornia crassipes*) and bhang (*Cannabis sativa* Linn.) there was high increase in nitrogen, potassium, phosphorus and a high decrease in organic carbon, C/N, C/P (Chauhan and Joshi, 2010). Summer season actually enhances the degradation of water hyacinth biomass using indigenous earthworm species *Perionyx excavatus* rich in N, P, K, Ca, Mg, Fe, Mn, Zn etc (Deka *et al.*, 2013).

Composting using rotatory drum is efficient for water hyacinth, cow dung, sawdust, rice husks, etc. and it helps in reduction of heavy metals during composting (Singh *et al.,* 2012; Singh and Kalamdhad, 2016). Water hyacinth can be used as a source for macronutrients like N, P and K for the crops due to its high availability in rivers and ponds in eastern Africa. Application effects of green manure *Tithonia diversifolia* (Hemsley) A. Gray) and water hyacinth compost increase nutrient status like N, P, K, Ca, Mg, Cu, Zn, Mn, Fe, nitrate and nitrite compounds, pH and organic C in soil thus promoting the use of organic amendments rather than chemical fertilizers (Chukwuka and Omotayo, 2008; Ansari and Rajersaud, 2011).

The mineralization of organic matter was found to be increased with the increase in water hyacinth quantity in the compost of sewage sludge (Morales-Linares *et al.*, 2015). The productivity of Lagos spinach tends to increase with application of water hyacinth compost (Viveka and Grace, 2009; Sanni and Adesina, 2012). The compost of water hyacinth has potential to increase fertility and when applied at higher rate has a tendency to accumulate heavy metals beyond permissible level of human consumption in cabbage plant (Mashavira *et al.,* 2014).

2.6 Compost using microorganism

Numerous microorganisms are employed in composting process. Their efficiency is often influenced by substrate particle, temperature and competition among themselves (Fuchs, 2010). Conversion of cellulolytic products into biodegradable products using microorganism is a real environmental challenge (Petre *et al.,* 1999). There are many reports on microorganism degrading polyester polyurethane especially fungi hydrolyzing the ester bond by esterase enzyme (Nakajima-Kambe *et al.*, 1999; Ibrahim *et al.,* 2011).

Cooperative action of fungi, bacteria and other micro flora plays vital role in complete degradation of complex agricultural biomass. Composting process can be enhanced using hydrolytic enzymes of cellulolytic bacteria and fungi. pH and electric conductivity increased in three month old compost in a cemented tank when inoculated with a fungi and bacteria. Fluid containing microorganism as activator was used to shorten the composting process of rice straw and water hyacinth (Mishra and Nain, 2013; Danuwikarsa, 2015).

The degree of humification and maturation processes in composting improves with the inoculation of microbes (Wei *et al.,* 2007). The N, P, K of lignocellulolytic wastes increased during pre-decomposition of bio-inoculants like *Pleurotus sajor-caju*, *Trichoderma harzianum*, *Aspergillus niger* and *Azotobacter chroococcum.* C/N ratio in compost of shredded straw supplemented with pig manure was found higher in the one with higher microbial mass (Eiland *et al.,* 2001; Singh and Sharma, 2002).

Different species of *Trichoderma* enhanced the rate of decomposition of wastes by 18% (Rahman *et al.,* 2011).

Large amount of biomass coming from aquatic weeds acts as excellent source of organic manure with bioconversion process by micro flora like bacteria, fungi and actinomycetes. Geetha (2009) used different additives like urea, cow dung, *Trichoderma* and *Pleurotus* in composting of water hyacinth and African payal. Fungi species like *Trichoderma viridae* and *Trichoderma harzianum* were employed for composting water hyacinth which showed better growth of plant *Abelmoschus esculentus* (Lekshmi and Viveka, 2011).

Water hyacinth can be used as both green manure as well as compost since microbial decomposition helps to break down fats, lipids and proteins. There are experimental evidence of increased yield and growth due to effective microorganism (EM), cattle manure and molasses separately in composting of water hyacinth on a common bean (Osoro *et al.,* 2014). About half a ton of residues as fertilizer can be obtained from one hector of water hyacinth biomass using microbial anaerobic degradation (Ghosh, 2010).

From the literature review of several scientific articles it is concluded that water hyacinth is a weed having multipurpose uses. The most beneficial use is composting since it is easily available and can be applied for agricultural benefits. Few research on use of effective microorganisms for composting showed that it is most effective method for providing nutrient to soil using water hyacinth and other types of biomass.

3. MATERIALS AND METHODS

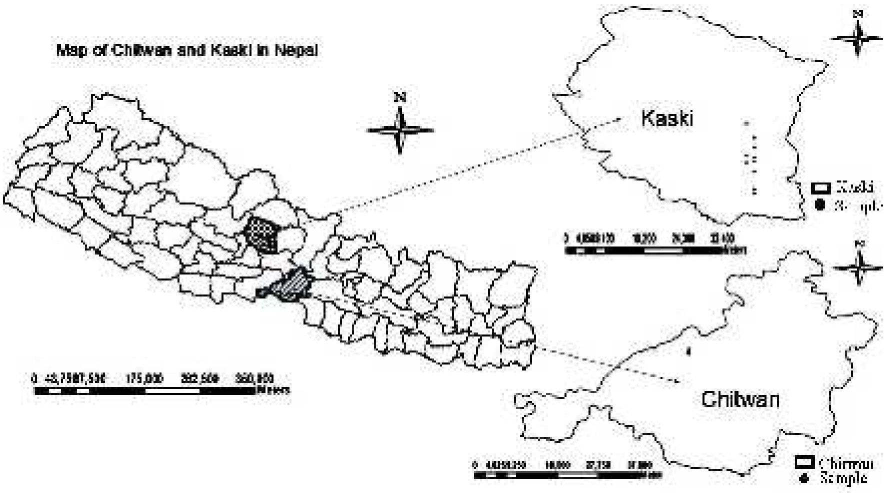
3.1 Study area

Chitwan Annapurna Landscape (CHAL) was the study area for socio-economic survey and collection of water hyacinth. CHAL occupies an area of 32057 sq km area includes six major river systems (River names) and 19 districts of Central Nepal. This area exhibits climatic diversity of tropical, subtropical to cold dry regions of Trans-Himalayan region (Nepal, 2013; Shrestha and Gautam, 2014). Three sites in CHAL were selected for the study of water hyacinth, i.e., Ghailaghari pond of Chitwan and Begnas and Rupa lakes of Kaski (Fig. 1). Ghailaghari pond of Chitwan is present in the Jagatpur VDC.

Begnas lake is located in the south east part of Pokhara which is a fresh water lake. The lake is considered as the second largest lake of Pokhara which is situated in Leknath Municipality and consists of a dam constructed in 1988. Surface area of the lake is 3.28 km^2 (Rai, 2000; Swar and Gurung, 1988). Rupa lake is situated 6 km east from Pokhara valley. It is considered as the third biggest lake in Pokhara valley. It covers an area of 115 hectare (Rai, 2000; Udas *et al.,* 2007). The geographic co-ordinates and altitude of the study area is shown in Table 1.

Table 1: Geographic co-ordinates of study area of study site

Sites	**Longitude/Latitude**	**Altitude (m)**
Begnas Lake	N 28°10’16.6” E084°06’52”	678
Ghailaghari Lake	N 27°33’34.2” E084°19’58.8”	151
Rupa Lake	N 28°08’40.3” E084°06’29.1	613



(a)

Figure 1: (a) Map showing study areas

3.2 Methods

3.2.1 Questionnaire survey

Field work was conducted from April 2017. Data were collected by using questionnaire (Annex III) related to water hyacinth. A set of questionnaire was prepared to conduct the household survey. Information like the water hyacinth impact on livelihood, means of management preferred by local people and alternative use known by the local people etc. At least 15 consultations from each site were interviewed. Location by GPS and description of the pond were recorded. The invasive property of water hyacinth was observed in each study area. Six sample points at each direction representing location of plants in different direction were taken.

3.2.2 Laboratory work

3.2.2.1 Preparation of biomass and isolation of fungal strains

Fresh biomass of water hyacinth (WHB) was collected from the study areas were washed thoroughly to remove dirt and dust on them. Biomass was chopped into 2 cm to 4 cm pieces and dried in shade for three days. Garden soil from Central Department of Botany, Tribhuvan University was dried in green house and then sieved to remove unnecessary stone particles.

Potato Dextrose Agar (PDA) media was used for the fungal culture (Johnson and Sekhar, 2012). About 4 g soil from the bottom of kitchen waste was taken for isolation of soil fungi. Fungal isolation method was carried out using soil plate method (Warcup, 1950). Then soil was transferred into the sterile petridishes. A pinch (0.005-0.15 g) of soil was scattered at bottom of petridish on which molten cooled (40-45°C) PDA was added. After that the soil particles in the media were dispersed by rotating the petridish gently. The petridishes were incubated at 23±2°C for the growth of fungal colony.

Trichoderma, Rhizopus and *Aspergillus* were identified on the basis of microscopic and macroscopic morphological characters and isolated from pure culture. A single hyphae was transferred to the petridish containing PDA for the pure culture (Watanabe, 2002). Triplicate for each pure culture was maintained, which were inoculated in PD broth media for spore suspension. The inoculated PD broth was incubated at 23±2°C which helped to increase spore of the respective fungi. Suspension of *Trichoderma viride* from seed market was also used for composting.

3.2.2.2 Sterilization of plant material and preparation of compost with different fungal inoculation

Chopped water hyacinth was mixed with garden soil in three different proportions in plastic bags, i.e., 50:50, 25:75 and 75:25 and each weighing 800 g. These were autoclaved at 121°C in 15 lb/in ^{2} pressure for the purpose of sterilization (Johnson and Sekhar, 2012). Ten mm (10^7 cfu/ml) of spore suspension of each fungus was inoculated in three different composition of compost. Fungal cfu/ml unit was determined by counting spores using Haemocytometer. Total 45 samples (triplicate for each 15 treatments) were maintained. A total of 9 samples without any fungal inoculation and sterilization were considered as the control sets. Samples were left for composting in green house from September 6 to November 7, 2016.

3.2.2.3 Nutrient analysis of the compost

The chemical quantity of C, N, P, K in the two months old compost after inoculation were determined (King, 1932; Hald, 1947; Bradstreet,1954; Schulte,1995) following the standard protocol at Agricultural Technology Centre (ATC), Lalitpur, Nepal.

Methods for Chemical and physical Analysis of slurry/compost

I. Total nitrogen (N%)-Kjeldahal Methods: Organic matter was oxidized by treating soil with Conc. Sulphuric acid. It was facilitated by digestion mixture. The digestion solution liberate the ammonia on treating with alkali, zinc and thiosulphate which was collected in the boric acid solution and titrated with standard acid using mixed indicator.

II. Phosphorus determination (P_2O_5%): Using chlorostannous reduced molybo-phosphate-phosphoric blue colour method by colorimetric determination of P.

III. Potassium determination (K_20%): The potassium content in the fusion extract with 6N HCL. The extract is measured by flame photometry.

IV. Organic carbon (O.C%): Using Walkey-Black method (assumes 75% oxidation) followed by titration.

3.2.2.4 Exploration of fungi present on control set of experiment

In control treatments with different compost proportion, inoculation of fungi was discarded. Plant material and soil used for these compost proportions were also unsterilized. The aim was to explore fungal species involved in degradation of biomass of the water hyacinth and soil when there was no external inoculation of degrading fungi.

Soil plate method recommended by Warcup (1950) was used for colony observation of different fungi. The procedure was same as mentioned above but in this case 0.005-0.15 g of compost material of each composition was used instead of soil. The criteria used for identification process was the colony characteristics and microscopic morphological details of the fungi (Watanabe, 2002). Nine plates were used as tripilicate for each compost proportion. This whole work was conducted in November 2016. Frequency was calculated by diving total number of occurrence by total number of plates. Relative frequency of fungi occurring on the samples was calculated by dividing frequency of each fungus by total frequency and was multiplied by 100 to convert into percentage.

3.2.2.5 Greenhouse experiment

Green-house experiment was carried out from November 20 to December 18, 2016 in Central Department of Botany, Tribhuvan University, Kirtipur to assess the effect of different compost treatments on growth and development of wheat (*Triticum aestivum* L). The effect of different compost treatments wheat was evaluated. Ten seeds were sown in each 54 equal sized pots. With these treatments, cow dung and organic manure were also used. Three pots were used as control without compost, cow dung and organic manure. Each pot containing 1.5 kg soil were applied with 150 g of respective compost treatments except control.

Five plants from each pot were removed after counting seed germination. Parameters taken for the plant with different treatments was germination percent, shoot length, root length and biomass. The shoot length was measured from day 7 to day 28 after seed sowing. Five plants from each pot were selected for these parameters. The root length was measured after 28 days. The germination percentage of seed was calculated using the following formula

$$\text{Seed germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

3.3 Data Analysis

All the data were entered in MS Excel 2013 and analyzed using IBM SPSS version 20. For C, N, P, K analysis of different treatments and field data, one-way ANOVA with Duncan test was used to compare compost nutrients quantity and wheat growth parameters.

4. RESULTS

4.1 Nutrient analysis of the compost

The nutrient analysis of compost was done in form of C, N, P, K analysis of compost. In this case, soil fungi were used for nutrient conversion from water hyacinth biomass. More the richness of nutrients in the compost better the performance for its suitable use to enhance the growth and development of agricultural plant. The description is explained below.

4.1.1 Nitrogen

Nitrogen level was found to be increased in 75:25 in comparison to others (P<0.05). The increasing order of nitrogen content in different composts with different inoculation was in this order: *Rhizopus* > *Aspergillus* > *Trichoderma* (Commercial) > *Trichoderma* (Soil) > control for 75:25. The highest percentage of nitrogen was in *Rhizopus* 75:25 **(**2.66 ± 0.16%) and the lowest percentage was in Control 25:75 (0.82± 0.02%) (Table 2).

Table 2: Nitrogen in water hyacinth compost having different treatments of fungi.

Treatments	**Nitrogen (%) Compost proportions**		
	25:75	**50:50**	**75:25:**
Control	0.82± 0.02	0.93± 0.03	1.06± 0.03
Trichoderma (Soil)	1.25 ± 0.01	1.15 ± 0.03	1.97 ± 0.06
Aspergillus	1.01 ± 0.02	1.06± 0.03	2.14± 0.02
Rhizopus	1.06 ± 0.07	1.04 ± 0.01	2.66 ± 0.16
Trichoderma (Commercial)	1.01 ± 0.01	1.07 ± 0.04	2.41 ± 0.12

4.1.2 Carbon

The increase in carbon content in different fungal inoculation was in this order: *Rhizopus* > *Trichoderma* (Commercial) > *Trichoderma* (Soil) > *Aspergillus* > control (Table 2). The highest percentage of carbon was 4.50 ± 0.05% in *Rhizopus* (P<0.05) and lowest percentage was (0.82± 0.02) in control 25:75 (Table 3).

Table 3: Carbon in water hyacinth compost having different treatments of fungi.

Treatments	**Carbon (%)**		
	Compost proportions		
	25:75	**50:50**	**75:25**
Control	2.14 ± 0.07	1.92± 0.01	2.90 ± 0.07
Trichoderma (Soil)	2.12 ± 0.07	2.07 ± 0.05	3.33 ± 0.06
Aspergillus	1.93 ± 0.04	2.76 ± 0.38	3.39 ± 0.06
Rhizopus	……………	4.03 ± 0.12	4.50 ± 0.05a
Trichoderma (Commercial)	1.52 ± 0.03	……………	2.53 ± 0.21

4.1.3 Phosphorus

The phosphorus (%) ranged between 0.72 and 2.36% with the highest quantity in *Trichodema* treated compost. The content of phosphorus in different treatment of compost was: *Trichoderma* (Soil) > *Rhizopus* > *Aspergillus* > *Trichoderma* (Commercial) > Control. In this case too, the phosphorus increased with increase in proportion of water hyacinth ($P<0.05$). The highest amount was found in *Trichoderma* 75:25 (2.36 ± 0.04%) and in Control 25:75 (0.72 ± 0.02%) (Table 4).

Table 4: Phosphorus in water hyacinth compost having different treatments of fungi.

Treatments	**Phosphorus (%)**		
	Compost proportions		
	25:75	**50:50**	**75:25**
Control	0.72 ± 0.02	0.90 ± 0.00	1.03 ± 0.02
Trichoderma (Soil)	1.78 ± 0.11	1.89 ± 0.07	2.36 ± 0.04
Aspergillus	1.03 ± 0.01	0.99 ± 0.01	1.91 ± 0.04
Rhizopus	1.01 ± 0.02	1.14 ± 0.07	1.95 ± 0.03
Trichoderma (Commercial)	1.01 ± 0.02	1.29 ± 0.02	1.81 ± 0.04

4.1.4 Potassium

The potassium content was found to be as follows: *Rhizopus* > Control > *Trichoderma* (Soil) > *Aspergillus* > *Trichoderma* (Commercial) ($P<0.05$). Potassium level was found high in *Trichoderma* 75:25 (2.52 ± 0.06%) while lowest value was in Commercial *Trichoderma* 25:75 (0.99 ± 0.04%) (Table 5).

Table 5: Potassium in water hyacinth compost having different treatments of fungi

Treatments	**Potassium (%)**		
	Compost proportions		
	25:75	**50:50**	**75:25**
Control	1.05 ± 0.04	1.16 ± 0.03	1.68 ± 0.02
Trichoderma (Soil)	0.98 ± 0.01	1.20 ± 0.03	2.52 ± 0.06
Aspergillus	1.02 ± 0.04	1.12 ± 0.03	2.10 ± 0.10
Rhizopus	1.15 ± 0.05	1.32± 0.02	2.52 ± 0.04
Trichoderma (Commercial)	0.99 ± 0.04	1.74 ± 0.03	2.31 ± 0.02

4.2 Isolation and identification of fungi involved in control set of experiments

Different fungi naturally occurring in soil were reported. Fungi identified were the species of *Aspergillus*, *Fusarium*, *Trichoderma*, *Mucor*, *Penicillium* and *Verticillum*. The most occurring fungus was *Trichoderma* with the highest relative frequency (20%) than other. *Aspergillus flavus* and *Fusarium* species showed frequency (16%). The relative frequency was as follows: *Trichoderma* > *Aspergillus flavus*, *Fusarium* > *Mucor*, *Aspergillus niger* > *Penicillium*, *Verticillum* (Fig. 2).

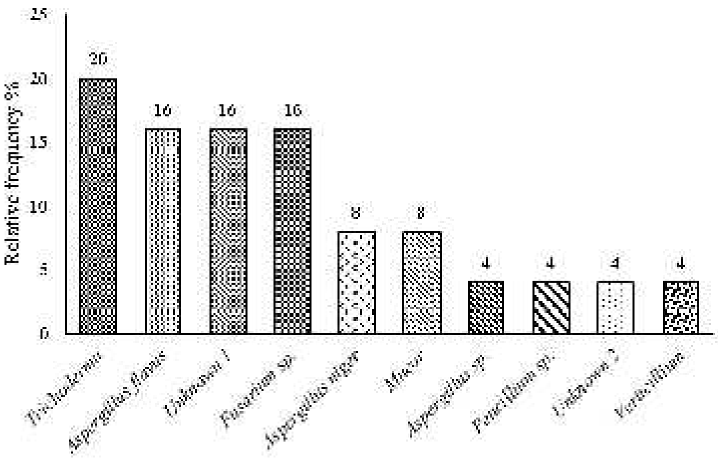


Figure 2: Fungi involved in composting the water hyacinth in control

4.3 Germination of wheat seeds on pots having different treatments

Wheat seeds showed higher percentage of germination on the compost treatments as well as on the cow dung and organic manure treated pots, (P<0.05). The highest percentage of germination was 83.33% in control 50:50 compost-soil ratio, *Aspergillus* 50:50 and *Aspergillus* 75:25 than other treatments (Fig. 3).

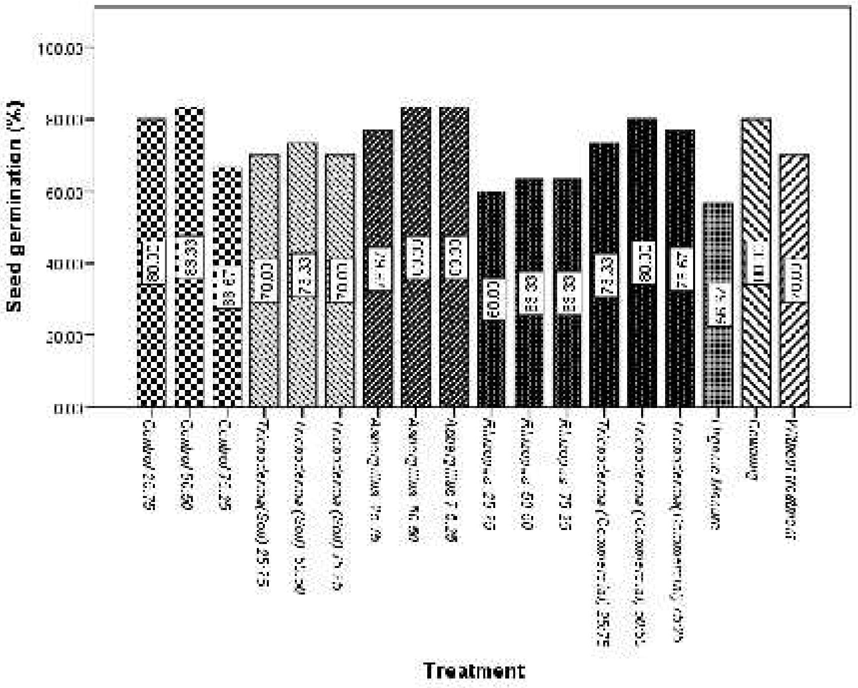


Figure 3: Germination of wheat in different fungal treatments in water hyacinth compost

4.4 Effect of compost on shoot length of wheat

Root length, shoot length and biomass was found to be increased in compost treated plants than in control treatments. This analysis was done by comparision of compost treated plants with organic manure, cowdung and control.

Shoot length at day 7: At day 7, the growth of plant was found to be increased with the application of the compost. The growth of the plant was found to be increased in the compost having higher proportion of the water hyacinth, (P<0.05). Plant growth with application of manure formed with treatments of different fungi can be shown as: cow dung > *Trichoderma* (Soil) > *Rhizopus* > *Trichoderma* (Commercial) > *Aspergillus* > Organic manure > without treatment. The longest shoot was 9.80 cm in cow dung followed by *Trichoderma* 75:25 and the in control the shoot lenth was the least i.e. 5.40 cm (Fig. 4).

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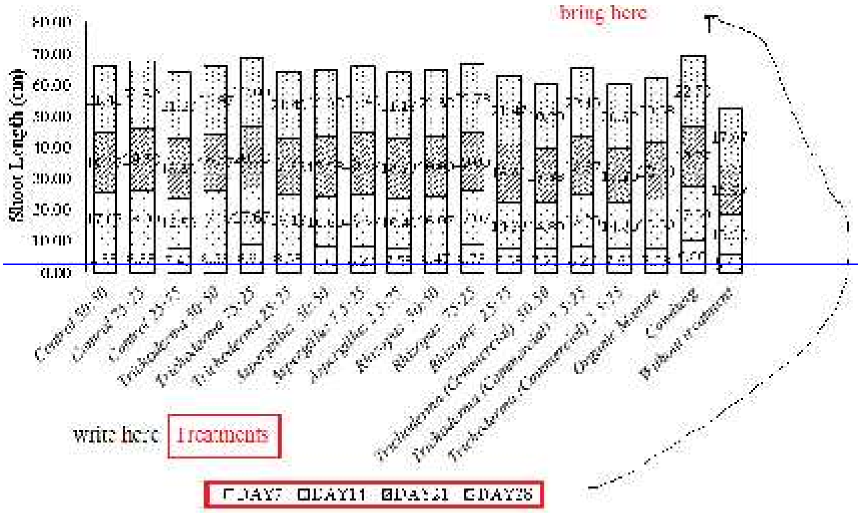
~~Duncan test showed significant differences between treatments of subset 1 and subset 6 at day 7.~~



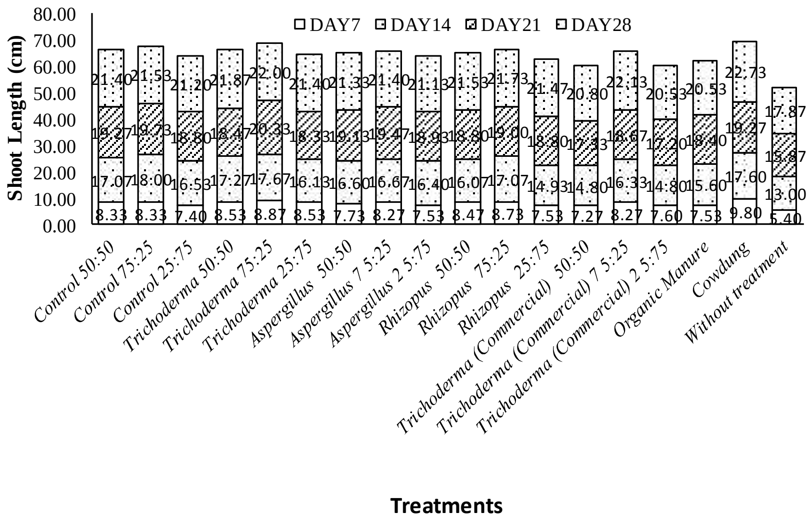


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Figure 4: ~~Growth of s~~Shoot length of wheat at different time duration after sowing

~~Growth of s~~Shoot length at 14 days: The growth of the plant ~~after 7~~at 14th days of ~~initial measurement was found to be increased. The growth was found to be maximum in pot with cow dung followed by *Trichoderma* (Soil). The growth~~ was also comparatively lower in the pots without any treatment. The highest ~~value~~ shoot length was ~~17.60 cm~~ in cow dung treated plants (17.60 cm) ~~treated and~~ whereas the shoots were shorter in control ~~lowest value was~~(13 cm) ~~in control treatement~~ (P<0.05) (Fig. 4). ~~The significant difference between subset 1 and subset 5 was observed in post hoc Duncan test at day 14.~~

~~Growth of s~~Shoot length at day 21: ~~In this case~~At the day of 21^{st} the growth of plant ~~on~~ in the compost treated by *Aspergillus* ~~treated pot~~ showed improvement. The ~~growth~~ shoot length was found higher in cow dung ~~treatment~~ and the compost with 75:25 proportion~~,~~ (P<0.05). The ~~highest value~~longest shoot was ~~19.27 cm~~ in cow dung (19.27 cm) ~~and~~ while the shoot length was ~~lowest value was~~ 15.87 cm in ~~without any treatment~~control (Fig. 4). ~~Duncan test revealed significant differences between subset 1 and subset 5 in different treatment at Day 21.~~

~~Growth of s~~Shoot length at day 28: ~~Growth rate of plant in different pots differed with slight difference in length.~~ The ~~increasing order of growth~~shoot length at 28^{th} day after sowing in the differents treatments ~~can be shown~~was found to be the longest in cow dung and shortest in control which was as follows: ~~as~~ cow dung > *Trichoderma* (Soil) > Control > *Rhizopus* > *Trichoderma* (Commercial) > Organic Manure > soil . The shoot length ~~highest value was 22.73 cm~~ in cow dung treatment was 5 cm longer than ~~and 17.87 cm in pots without any treatment~~control (P = 0.006, Fig. 4).

~~The p-value from one way ANOVA test showed value of 0.006 less than 0.05. The significant differences between subset 1 and subset 3 of different treatments at day 28 was revealed using Duncan test.~~

Effect of compost on root length ~~in different compost~~of wheat: In case of root length ~~of the wheat plant treated with different compost was measured. I~~it was observed that ~~the length of the~~ roots in the pot applied with cow dung ~~had higher value~~were longer in comparison to others (P<0.05). *Trichoderma* (Soil), *Rhizopus* and *Aspergillus* also showed better growth than the pots without any treatment (Fig. 5). ~~The rate of r~~Root length was increased with ~~the~~ increase ~~in~~ of amount of compost ~~having~~ with higher proportion of ~~the~~ water hyacinth, i.e., 75:25 ~~(P<0.05)~~. The ~~highest value~~longest root was ~~8.80 cm~~ found in cow dung (8.80 cm) and ~~8.53~~ in *Trichoderma*

75:25 (8.53 cm).~~The lowest value was 5.87 cm~~Root length in pots without any treatment was 5.87 cm (Fig. 5).

~~Duncan test showed significant differences between subset 1 and subset 4 of rootlength in different treatments.~~

Effect of compost ~~treatments~~ on biomass: The dry biomass of ~~grown~~ plants was higher in compost treated pots. ~~The b~~Biomass in compost with increasing proportion of water hyacinth ~~compost~~ was also found to be increased in all treatments. ~~The increase in biomass with the increase in proportion of the water hyacinth~~Decreasing order of biomass was found as follows: ~~can be shown as:~~ 75:25 > 50:50 > 25:75. Fungus *Trichoderma* was found beneficial for the biomass ~~and growth~~ in comparison to others. The biomass was the highest ~~value was 0.65 gm~~ in cow dung (0.65 gm) while it was the lowest ~~value was 0.27 gm~~ in control pots ~~without any treatment~~(0.27 gm) ($p<0.05$, Fig. 6). ~~The significant differences between subset 1 and subset 5 of biomass in different treatments was observed using Duncan test.~~

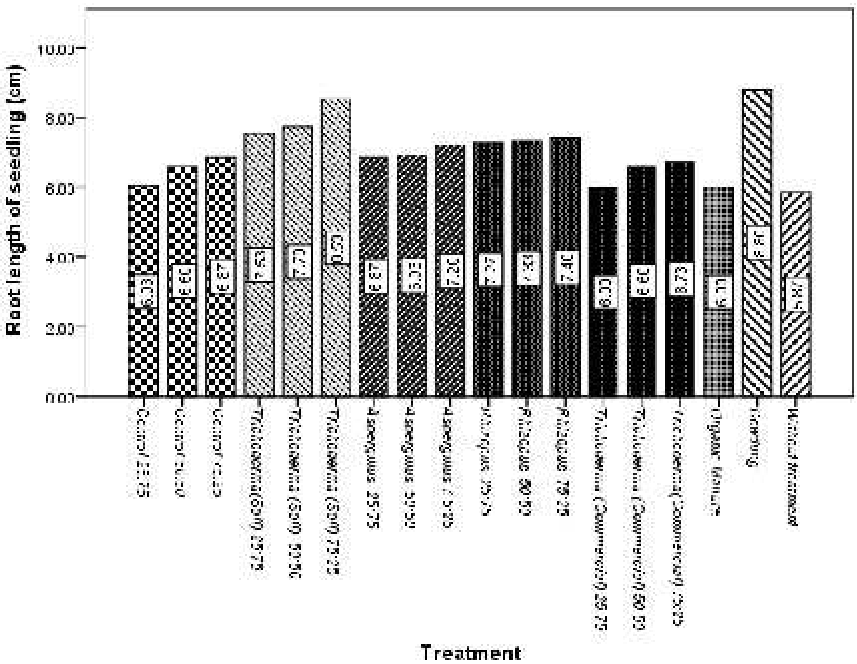

Figure 5: Root length of wheat ~~plant on 28th day~~ in different treatments of compost

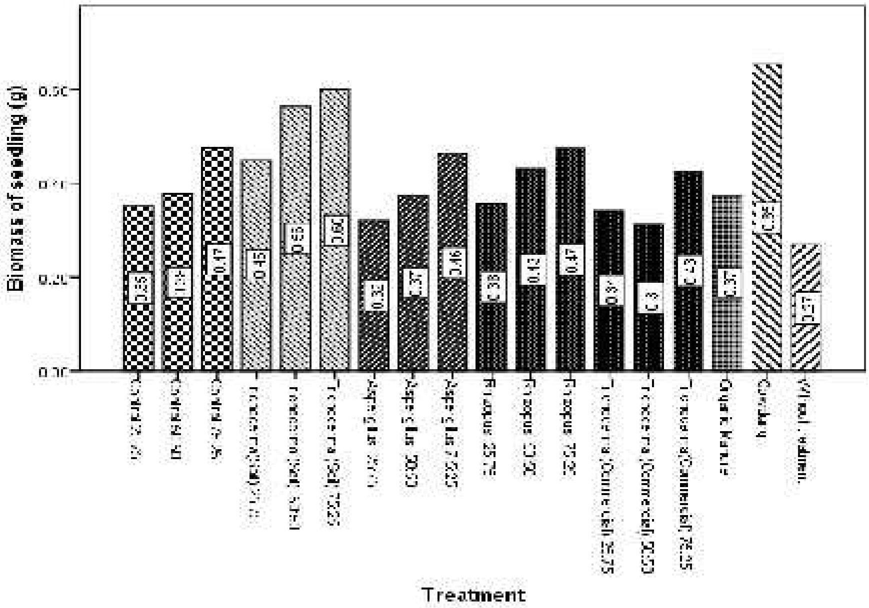

Figure 6: Biomass of wheat plant in different treatments of ~~water hyacinth~~ compost

4.5 People's perception about water hyacinth

Most respondents considered that water hyacinth is a problematic weed. It has ~~N~~negative impact on ~~the~~ irrigation ~~was considered by only~~according to 27% ~~twenty seven percent~~ of respondents. The weed was also used as manure in agricultural field and about ~~thirty three percent~~33% –participants had no idea on effect of water hyacinth manure on agricultural yield. Farmers do not use water hyacinth as fodder. Cattle might feed on the growing water hyacinth on the field but it was never used as fodder by ~~the~~ local people ~~locally~~. Only ~~two percent~~2% respondents agreed that water hyacinth ~~was~~ can be used to feed cattle. Ninety eight percent respondents agreed that water hyacinth ~~was~~ is expanding in the area (Fig. 7).

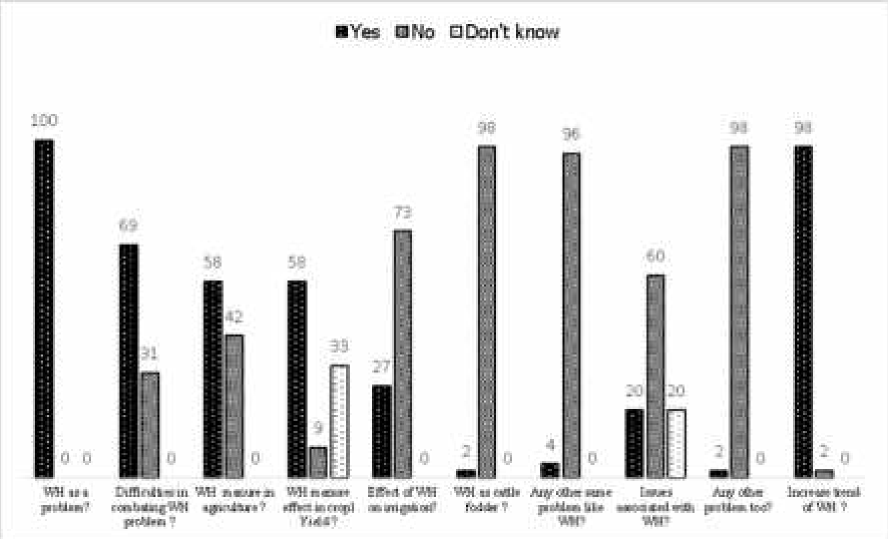

Figure 7: ~~Result of questionnaire survey in study area~~Respondents view on impacts of water hyacinth

4.6 Problems associated with water hyacinth

Water hyacinth was linked with different impacts on environment as well as livelihood ~~issues~~ of the people. Respondents mentioned ~~the~~ following problems~~,~~ i.e., disturbance in irrigation (2% respondents), damage to boats (22% ~~Respondents~~), expansion and control of weed hyacinth (2% ~~respondents~~), hindrance in fisheries (2% ~~respondents~~), discouraging recreational activities (18% ~~respondents~~) and environmental degradation (42% ~~respondents~~) (Table 6).

Table 6: Problems associated with water hyacinth

Study area	Social		Economic			Environmental	
	Irrigation (%)	Boating (%)	Fishery (%)	Tourism (%)	Costly to remove (%)	Water pollution (%)	Aquatic lives and fishes (%)
Chitwan	0.00	0.00	0.00	0.00	0.00	6.66	93.33
Begnas	6.66	66.66	6.66	53.33	0.00	26.66	13.33
Rupa	0.00	0.00	0.00	0.00	6.66	93.33	0.00

4.7 ~~Most e~~Effective methods of water hyacinth control and management

Ninety six percent of respondents considered mechanical removal as the effective management of water hyacinth rather than using chemical treatment and only 4.4% favored chemical treatment. Respondents referred different alternative uses of water hyacinth as follows: Use for handicraft (11.11% respondents), for biogas (13.33%), alcoholic production (2.12% ~~respondents~~), biogas or alcohol (15.53% ~~respondents~~) (Table 7).

Table 7: Effective methods recommended by local people for water hyacinth management

Study area	Mechanical removal (%)	Chemical treatment (%)
Chitwan	86.66	13.33
Begnas	100.00	0.00
Rupa	100.00	0.00

	Alternative use (%)						
Study area	Handicraft	Manure	Unknown	Handicraft, Manure	Biogas	Alcohol	Biogas, Alcohol
Chitwan	0.00	0.00	100.00	0.00	0.00	0.00	0.00
Begnas	33.33	13.33	46.66	6.66	0.00	0.00	0.00
Rupa	0.00	0.00	6.66	0.00	40.00	6.66	46.66

5. DISCUSSION

5.1 Nutrients in compost

The nitrogen is essential for ~~the fungus~~ fungi since it is used up by fungi to build up cells during degrading the plant biomass (Jusoh *et al.*, 2013). Mahanta *et al.*, (2012) reported 1.82% ~~percentage~~ nitrogen in *Eicchornia* biomass using cellulose degrading microorganism while during present study, the higher percentage was found to be 2.66% in *Rhizopus* treated compost. The nitrogen content was found to be increased with the increase in the proportion of the water hyacinth which indicates that more the biomass, the higher the nitrogen availability for microorganisms for the effective degradation.

Carbon as the element in the protoplasm and source of energy for microorganisms helps to increase the metabolic activities (Danuwikarsa, 2015). Carbon percentage during nutrient analysis of present study showed 4.50 ± 0.05% in *Rhizopus* ~~(Commercial)~~ treated compost which was similar than carbon percentage result of Lata and Veenpani (2011) in water hyacinth manure, i.e., 4.42%. The carbohydrate, cellulose, hemicellulose content are more degradable and easily lost in form of carbon (Kuo *et al.*, 2004). In this case the ~~overall~~ higher carbon content was shown by the fungi *Rhizopus* inoculated compost than other inoculated compost. The carbon content was lesser than other nutrients since plant sample used for composting was shade dried for only three days.

Mineralization process by microorganisms plays vital role in release of the phosphorus. The higher content of phosphorus is an indication of the higher microbial activity (Prasad *et al.*, 2013). In present study, phosphorus ~~percentage~~ was found to be 2.36% but in composting of water hyacinth using *Trichoderma viradae* and *Trichoderma harzianum* by Lekshmi and Viveka (2011) it was 0.11% and 0.12%, respectively. The higher degradation activity of fungi resulted the mineralization of the phosphorus from the different proportion of soil and water hyacinth. The phosphorous content was found to be higher with increase in proportion of water hyacinth biomass.

Potassium is one of the micronutrient which is easily available with the mineralization of organic matter (Louisa and Taguiling, 2013). It is the element which is easily leached out (Jusoh *et al.*, 2013). Potassium in *Trichoderma* inoculated compost in this research work showed ~~percentage of~~ 2.52% that can be compare with composting of water hyacinth using *Trichoderma viridae* and *Trichoderma harzianum* having percentage of potassium 0.15% and 0.16%, respectively (Lekshmi and Viveka 2011). The potassium content was higher in *Rhizopus* and *Trichoderma* inoculated compost with high proportion of water hyacinth. i.e 75:25

The ~~overall experiment~~results show~~s~~ ~~the~~ increase in the nutrient content in the ~~given~~ compost of different proportion. According to ~~Anonymous~~ NARC (2071), the recommended Nitrogen, Phosphorus and Potassium for the rice plant is 100 kg/ha, 30 kg/ha and 30 kg/ha while for wheat, it is 100 kg/ha, 30 kg/ha and 25 kg/ha. N, P, K requirement for maize is 60 kg/ha, 30 kg/ha and 30 kg/ha .The N, P, K percentage of water hyacinth manure in this experiment was converted to kg/ha. The highest N, P, K value of water hyacinth manure is 3759.39 kg/ha, 1694.91 kg/ha and 1190.47 kg/ha, respectively showing that it can be applicable to any cereal crops. The most nutrient rich proportion is the manure with the higher content of the water hyacinth, i.e., 75:25. *Rhizopus* and *Trichoderma* can be considered as ~~one of~~ the effective fungi to be used in degradation of the biomass since nutrients was found to be increased in compost treatment. The improvement of soil nutrients in agricultural land cannot be fulfilled by Cow dung alone in context of Nepal. Water hyacinth produces higher biomass that is easily available and cheaper hence can be used as alternative to cow dung.

5.2 Role of fungi in composting

From the samples of soil containing *E. crassipes*, species of *Aspergillus ~~niger~~*, *Penicillium* ~~spp.~~, *Trichoderma* ~~sp.~~, *Fusarium* ~~sp.~~, *Chaetomium* ~~sp.~~ and *Rhizopus* ~~sp.~~ in which *Aspergillus* and *Trichoderma* ~~were~~ are considered as potentially cellulose degrading micro fungi (Sharma and Ramendra, 2015). The occurrence of ~~different~~ these fungi indicates their involvement in cellulose degrading activity and mineralization of plant biomass into organic nutrients. Those fungi and bacteria involved in decomposition of cell wall polysaccharides employs enzyme systems including cellulases, hemicellulases and other glycoside hydrolyses including the polysaccharide lysases and carbohydrate esterases (Himmel *et al.*, 2010). ~~Different~~

~~f~~Fungi differ~~s~~ among themselves in degradation capacity and enzymatic activities. Most of these fungi identified are known as decomposers and fungi which are involved in degrading food stuffs, plant biomass and help~~s~~ to clean the environmental wastes. Fungi like *Trichoderma* has been known to enhance biomass production and growth of plant as well as provide resistance to abiotic stress (Contreras-Cornejo *et al.*, 2009; Harman *et al.*, 2004). Mohanan *et al.* (2014) found that monoculture of *Trichoderma* used for degradation of ~~kitchen~~ wastes released from kitchen and water hyacinth showed improved enzymatic activities. Fungi secretes various enzymes that are capable of degradation of cell wall structures like cellulose, hemicellulose, lignin etc (Perez *et al.*, 2002). These fungi are present in soil and have their role in decomposing waste matter converting them into organic nutrients thus increasing the soil fertility. More the involvement of different types of fungi, faster becomes the process of degradation.

5.3 Effect of compost treatments on plants growth

The shoot length, root length and biomass ~~was~~ were taken as parameters to analyze the effect of the compost. Composting of water hyacinth reduces the chemical fertilizer application in field and plays vital role in nutrient recycling as well as reduces the chemical fertilizer application in field (Prasad *et al.*, 2013). With the application of compost from water hyacinth, the seedling growth ~~as well as length and biomass~~ of wheat plant was ~~under taken into consideration~~observed. Water hyacinth tends to increase the productivity of Lagos spinach, tomato plant, *Amaranthua viridis* and *Brasicca Junea* with increase in rate of application (Kafle *et al.*, 2009; Lata and Veenapani, 2011; Mashavira *et al.*, 2015; Sasidharan *et al.*, 2013). The compost treatments were compared with cow dung, organic manure from market and pot without any manure application. The growth of shoot length, root length and biomass tends to be higher in compost treatments than the pot plant without any treatment. The highest growth ~~rate~~ was however found in plants with cow dung showing it is effective for growth of crop plants. *Trichoderma* enhances the uptake of nutrient and crop productivity (Harman *et al.*, 2004).*Trichoderma* treated plants however showed more effective growth than the plants containing the inoculations of the *Aspergillus* and *Rhizopus*. According to Vidya and Girish (2014), the length of shoot at day 15 was found to be 15.99 cm which is nearly similar to the highest value of shoot length of this experiment, i.e., 17.60 cm at day 14. The growth parameters ~~under taken~~ tend~~s~~ to be increased in the wheat plants treated with water hyacinth compost than control showing that N, P, K and C increases due to the microbial degradation of inoculated fungi that helps to improve soil quality and enhances the plant growth. Cow dung alone cannot fulfill the demand of nutrient required in agricultural land. Water hyacinth manure can serve as alternative to cow dung that can be degraded by soil fungi and decomposers in natural conditions. Strains of different soil fungi can be provided for composting.

5.4 Perception of the people on water hyacinth

Socio-economic survey revealed that invasion~~,~~ history and management issues of water hyacinth. It tends to ~~which~~ appear 10 to 12 years ago in all study areas due to human activities. The negative impacts includes blockage of irrigation, difficulty in boating and degraded water. The affected aquatic lives includes plants like *Nelumbium nuciferum*, *Nymphea alba*, *Myriophyllum* sp., *Potomogaton* sp. and *Vallisneria* sp. etc and fishes like *Aristichthys nobilis*, *Clenopharyngodon idella*, *Cyprinus carpio* and *Labeo rohita* etc.

Institutions like Mother Club, Boating Association, Fishery Association, Hotel Association, Municipality Office, Rupa Lake Restoration and Fishery Co-operative (RLRFC), Seed Foundation, District Forest office, National ~~Tal~~ Lake Development and Conservation Committee, Seed Foundation, LIBIRD, ~~District Forest~~ etc were involved in management of water hyacinth in Kaski district while local fishermen were involved in mechanical removal of this weed in Ghailaghari pond of Chitwan. Alternative methods preferred by the respondents includes handicraft, manure, biogas and alcohol. The water hyacinth is increasing day by day even with all the management strategies hence the weed must be deposited away from the bank or nearby areas to prevent its further spreading.

6. CONCLUSION AND RECOMMENDATION

Mechanical removal is considered as the most appropriate means for management of water hyacinth on the basis of people perception. *Rhizopus* and *Trichoderma* is considered as the effective fungi to enhance the composting process for water hyacinth biomass however *Trichoderma* treated compost is also beneficial for the growth and development of plant. *Trichoderma* and *Aspergillus* are the dominant species in compost in natural conditions as observed in control sets.

Other cellulose degrading microorganisms like bacteria can be used for composting. The effect of inoculated fungi on cellulose, hemicellulose degradation of water hyacinth needed to be studied in Laboratory. Properly dried plant materials can be used to increase carbon content in compost. The plant productivity along with seedling growth with the application of compost is needed to be studied in field the effective result of compost.

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Annexes

Annex I: Key for identification of fungi

1a. Mycelium rarely septate: asexual spores in sporangia…......... Zygomycetes (3)

1b. Mycelium septate; conidia borne on conidiophores ……………........……… (2)

2a. Spores in a sac, as ascospores ……………………......………….. Ascomycetes

2b. Spores as conidia on conidiophores of various form, not in asci ....… .... Deuteromycetes (4)

3a. Sporangia spherical, sporangiospores unbranched, at the nodes opposite the rhizoids …………...…………………………………………………… *Rhizopus*

3b. Sporangiospores branched or unbranched, zygospores in special hyphae, not in coremia nor sporodochia ………………………………………………… *Mucor*

4a. Conidiophores not in coremia nor sporodochia and conidia clear and bright colored …………………………………………………….... Moniliaceae (5)

4b. Conidiophores in a sporodochium; conidia more than two celled, fusiform, curved …………………………………………..… Tuberculariaceae (*fusarium*)

5a. Branching of conidiophores confined to the tip; conidia in heads ....……… (7)

5b. Branching of conidiophores not confined to the tip …………………....…… (9)

7a. Conidiophores distinctly swollen at the tip, conidia not in chain, not enclosed in mucus ……………………………………………................... *Trichoderma*

7b. Conidia in chains …………………………………………………….………… (8)

8a. Conidiophores distinctly swollen at the tip, foot cells also prominent *Aspergillus* (A).

8b. Conidiophores not swollen at the tip, or only slightly, foot cells not differentiated; tips verticilliately branched ………………………………….. (9)

9a. Conidia not enclosed in mucus; conidia with basal ring and pore …. *Penicillium*

9b. Conidiophores branching principally in whorls, all branches bearing spores, spores not in chains, spore globose, ellipsoidal/ oval, conidia not enclosed in mucus, conidia on branchlets occurring singly …………………….. *Verticillium*

Key to species

(A) Aspergillus

1a. Conidiophore pitted, often appearing rough; conidial heads yellow green …… ……………..……………...……………..……………......…………….. A. flavus

1b. Conidial heads black, phialides in two series ……………..…………… A. niger

(B) Trichoderma

1a. Setae like hyphae lacking conidia globose …………… *Trichoderma harzianum*

1b. Setae like hyphae lacking conidia not globose phalides mainly verticillate, conidia ellipsoidal, phialides thinly arranged ………… *Trichoderma harzianum*

Annex II: Description of fungus

Rhizopus

It is popularly known as black mould since the entire mycelieum appears blackish. The well-developed mycelium is differentiated into the three different types of hyphae, i.e., sporangiophores, stolons and rhizoids. The tufts of sporangiophore develops from the mycelium that later develops sporangium. Sporangium are the swelling knob like structure at the tip of conidiophore which later produces non-motile round black spores called sporangiospores (Sharma, 2011).

Mucor

The mycelium of *Mucor* is similar to *Rhizopus*, except that there is no differentiation of holdfast and stolons. Also there is absence of holdfast or rhizoids. Sporangiophores are erect, sporangium bearing hyphae which may arise singly or in groups from mycelium. Sporangiospores are round non-motile spores that may give rise to multinucleate, coenocytic hyphae (Sharma, 2011).

Fusarium

The mycelium consist up of colorless branched and septate hyphae that turns brown at maturity. It consist up of three kinds of spores, i.e., macro conidia, micro conidia and chlamydospores. Macroconidia consist of pointed ends on both sides. These are sickle shaped bodies developed from cells known as phialides and may produce separately or in sporodochia. Microconidia develops on branched or unbranched conidiophores which are uni or bicelled small oval bodies. Round ovalthick walled cells from terminal or intercalary cells develops singly or in chain that germinates by means of germtubes known as chlamydospores (Sharma, 2011).

Trichoderma (Persoon) harz

Sterile hyphae creeping, septate, forming a flat, firm turf. Conidiophores erect, arising from short, branched side branches, branching usually opposite, not swollen at the apex and bearing terminally the conidial heads. Conidia small, mostly globose, bright coloured or hyaline (Gilman, 1967).

Trichoderma harzianum

Conidiophores are erect hyaline and branched bearing short, thick hyaline structures called phlialides. From the apical end of the phialides develops unicelled, globose, subglobose or ovate conidia. Chalmydospores are subglobose and brown in coloration (Watanabe, 2002).

Aspergillus

Mycelium consist up of interwoven mass of branched and septate hyphae. From the somatic hyphae develops long, aseptate and branched conidiophores. Foot cell is the hyphal cell that gives rise to conidiophores which terminates into a bulbouse head, called vesicle. A layer of conidiogenouse cells called sterigmata or philiades develops from the entire surface of vesicles. These are multinucleated bottle shaped structures which gives rise to globose .unicellular bodies called conidia. The development of these conidia are in basipetal sucession. Sometimes two layer stigmata develop upon one another. In this cases secondary sterigmata develops on primary sterigmata (Sharma, 2011).

Aspergillus flavus

It is recognized by light yellow-green olive brown spherical or columnar spore heads. Conidiophores usually are 1 μm in length which is heavy walled hyaline, coarsely roughened, there is presence of conidia bearing cells which may be of two series, i.e., chiliads and supporting cells. Conidia are spherical to sup spherical,sometimes oval or pear shaped having diameter of 3-6 cm (Navi *et al.*, 1999).

Aspergillus niger

Spore heads are compact spherical or columnar with greenish black, purplish black or carbon black in coloration. Conidiophores are 3 μm in length which is smooth and hyaline. Heads usually consist of two series of conidia-bearing cells, i.e., supporting cells and phialides. Conidia are dark coloured with conspiciouse longitudinal strains which are often spherical rough and spiny at maturation (Navi *et al.*, 1999).

Penicillium

The mycelium consist up of several hyphae which are tubular, hyaline, septate and branched. These mycelium can break into fragments that develops into a new mycelium. Conidiophore may be developed from any cell from the mycelium except any specialized foot cell like *Aspergillus*. These are erect, tubular and branched. When the conidiophore is branched, these terminates into a clusters of phialides or sterigmata while in the case of unbranched condition, philiades develops directly on the tip. Tufts of philiades containing branches is called metulae and lower branches supporting the metulae is called rami (Sharma, 2011).

Verticillium sp.

Conidiophores are erect hyaline bearing simple, verticillate and alternate, opposite phiallides which are tapering from base towards apex. These bears spore masses known as conidia which are hyaline, philaposporous and one one celled. Chylamydospores are subglobose and pale brown in colour (Watanabe, 2002).

Annex III: Questionnaire form

a) Name : ______________________________

b) Age : ______________________________

c) Address : ______________________________

d) Occupation : ______________________________

e) Male/female : ______________________________

f) Education : ______________________________

Questions

1. Is WH perceived as a Problem? Yes/No

2. What institutions and communities are affected by or concerned about WH in the area?

3. What is the general trend of WH problem?

   Increase/Decrease/No change/No knowledge

4. How many which institutions or organizations are working on WH in this area?

5. What is the history of WH in the area? Are the problems associates with water hyacinth locally?

   a) Social? Specify type.

   b) Economic? Specify type.

   c) Environmental? Specify type.

6. Were/are there any difficulties in responding to a growing WH problem in the area? Yes/No

7. If yes, what are the reasons? Is there a coordinated institutional framework handling the WH problem? Yes/No

8. Are there any farmers, social workers, people representing govt and non-govt institution, scientists dedicated to control of WH?

9. What are the alternative uses of WH by local people in their daily lives?

10. Is WH used as manure in agricultural farms?
11. Has WH manure increase the agricultural Yield?
12. Is there any negative impacts of WH on irrigation?
13. Is WH palatable and consumed as cattle fodder?
14. Which method do you think is most effective? Mechanical Removal/Chemical Treatment/Alternative use by local people?
15. What suggestions do you have to improve the management of WH in your area?
16. Besides WH, have you felt any other same problem?
17. If WH is not managed properly what will be the extra cost to remove this?
18. What types of aquatic plants disturbed due to invasion of WH?
19. Any story/issue/points associated with WH?
20. First time when have you seen the WH here?
21. Like WH have you seen any other problem too?

At least 15 consultations from each sites.

Annex IV: Lab report on nutrient analysis of compost

ATC AGRICULTURAL TECHNOLOGY CENTRE

Pulchowk, Lalitpur, Nepal
Tel. No. : 01 5525956
E-mail: agritech1993@gmail.com

Soil Sample Analysis Report

Report No. : 13/2074 Sample Received Date: 25/04/2074

Entry No. : ATC/13/2074 Date Completed : 31/04/2074

Client : Sagar Khadka Sampled By : Client

S.N.	Lab. No.	Sample Identification	N %	P2O5	K2O %	Organic Carbon %
1	074/ 187	S.No. I- Control 50:50	0.96	0.89	1.1	1.91
2	188	II	0.89	0.9	1.19	1.91
3	189	III	0.97	0.9	1.2	1.95
4	190	S.No. I- Control 75:25	1	0.99	1.65	2.93
5	191	II	1.06	1.05	1.7	2.99
6	192	III	1.11	1.06	1.69	2.77
7	193	S.No. I- Control 25:75	0.8	0.72	1	2.11
8	194	II	0.81	0.75	1.02	2.05
9	195	III	0.85	0.69	1.12	2.27
10	196	S.No. I- Trichoderma 50:50	1.15	1.95	1.15	2.02
11	197	II	1.11	1.98	1.2	2.17
12	198	III	1.2	1.75	1.25	2.03
13	199	S.No. I-Trichoderma 75:25	2	2.42	2.4	3.4
14	200	II	1.85	2.36	2.54	3.22
15	201	III	2.05	2.29	2.61	3.38
16	202	S.No. I-Trichoderma 25:75	1.25	1.7	1	2.12
17	203	II	1.23	1.65	0.98	2.24
18	204	III	1.26	1.99	0.95	1.99
19	205	S.No. I-Aspergillus 50:50	1.02	0.99	1.09	2.36
20	206	II	1.1	1.01	1.1	2.4
21	207	III	1.05	0.98	1.18	3.53
22	208	S.No. I-Aspergillus 75:25	2.09	1.9	2.3	3.28
23	209	II	2.16	1.85	1.95	3.5
24	210	III	2.17	1.99	2.03	3.38
25	211	S.No. I-Aspergillus 25:75	1	1	0.95	1.98

Agricultural Technology Center 1993 Lalitpur, Nepal

AGRICULTURAL TECHNOLOGY CENTRE

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Contd…

S.N.	Lab. No.	Sample Identification	N %	P2O5	K2O %	Organic Carbon %
26	212	II	1.05	1.05	1.01	1.95
27	213	III	0.99	1.03	1.1	1.86
28	214	S.No. I-Rhizopus 50:50	1.05	1.2	1.35	3.91
29	215	II	1.02	1.01	1.29	3.91
30	216	III	1.04	1.21	1.32	4.26
31	217	S.No. I-Rhizopus 75:25	2.74	2.01	2.45	4.54
32	218	II	2.9	1.95	2.6	4.4
33	219	III	2.35	1.89	2.52	4.57
34	220	S.No. I-Rhizopus 25:75	0.95	0.98	1.08	5.12
35	221	II	1.2	1	1.11	8.03
36	222	III	1.04	1.06	1.25	7.93
37	223	S.No. I-Commercial Trichoderma 50:50	1.05	1.3	1.8	8.38
38	224	II	1.15	1.25	1.72	9.37
39	225	III	1	1.31	1.69	8.67
40	226	S.No. I-Commercial Trichoderma 75:25	2.65	1.89	2.29	2.31
41	227	II	2.3	1.75	2.35	2.33
42	228	III	2.28	1.79	2.3	2.96
43	229	S.No. I-Commercial Trichoderma 25:75	0.99	1.02	0.92	1.57
44	230	II	1	0.98	0.99	1.52
45	231	III	1.03	1.04	1.05	1.46

Agricultural Technology Center 1993 Lalitpur, Nepal

D. Laxmi

Soil Scientist

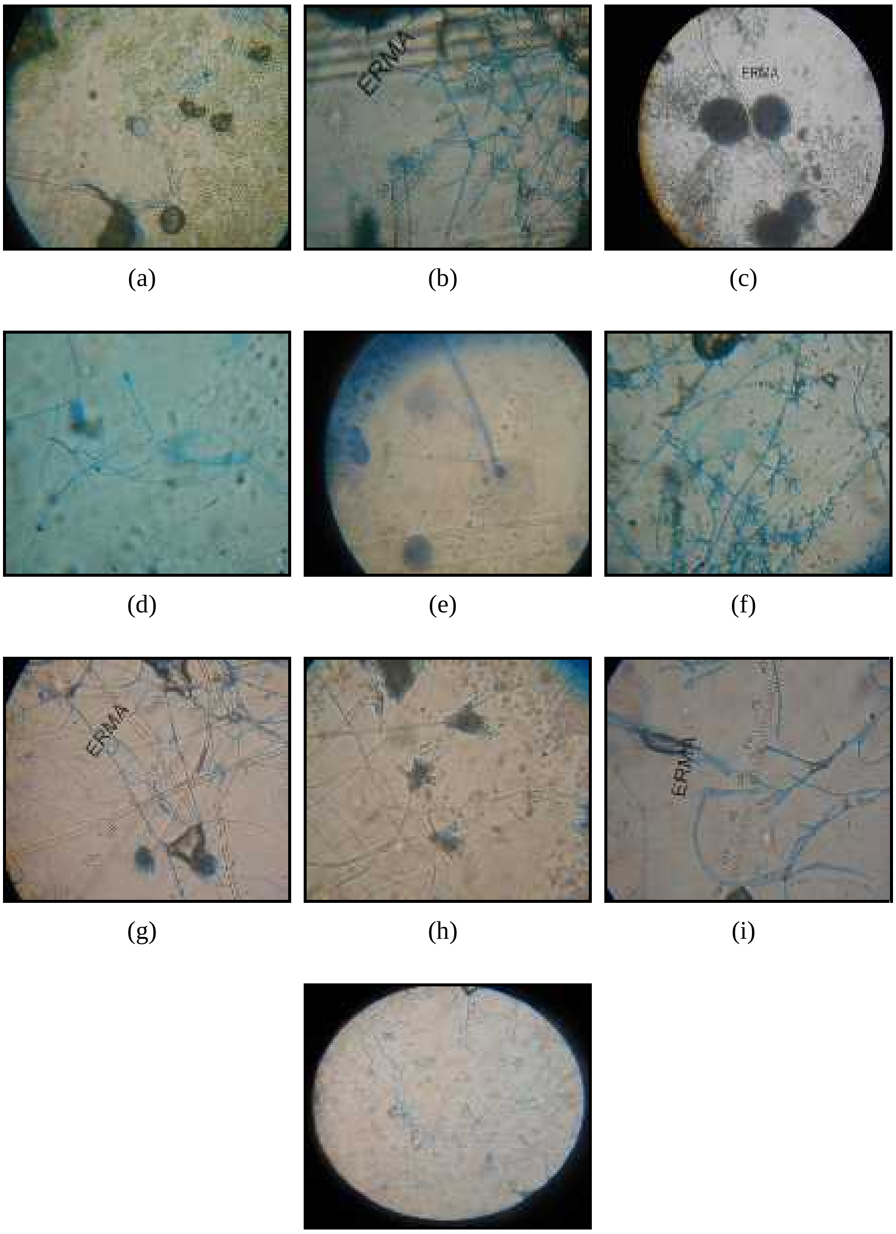
Note: 1. The issued report refers only to the sample received and tested.
2. This report is neither to be reproduce wholly or partially and nor can be used as an evidence in the court of Law.
3. Samples will be kept stored for a month only.

Photoplate 1: Pure culture of *Trichoderma*, *Aspergillus* and *Rhizopus*

Photoplate 2: Composting process and nutrient analysis of water hyacinth in laboratory

(a) (b) (c)

Photoplate 3: Fungal colony observed from compost samples: (a) Control 50:50, (b) Control 75:25, (c) Control 25:75 (Observations taken after 7 days)



(a) (b) (c)
(d) (e) (f)
(g) (h) (i)
(j)

Photoplate 4: Fungi species recorded during the study– (a) *Aspergillus flavus*, (b) *Verticillium*, (c) *Aspergillus niger*, (d) Unknown species I, (e) *Aspergillus* sp., (f) *Trichoderma harzianum*, (g) *Mucor*, (h) *Penicillium*, (i) Unknown species II, (j) *Fusarium*

(a)

(b)

(c)

(d)

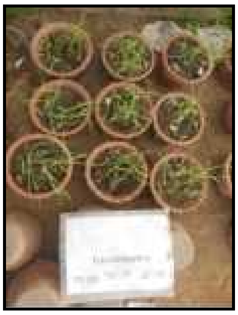
(e)

(f)

(g)

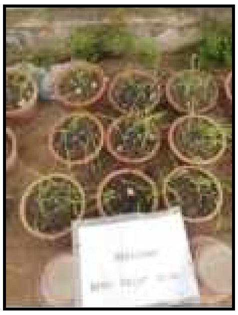
(h)

Photoplate 5: Pot plant experiment using different treatments before seed germination- (a) Control, (b) *Aspergillus* (c) *Trichoderma* (d) *Rhizopus* (e) *Trichoderma* (Commercial) (f) without treatment



(a)

(b)



(c)

(d)

(e)

(f)

(g)

(h)

Photoplate 6: Pot plant experiment using different treatments after seed germination at day 28 – (a) Control, (b) *Aspergillus*, (c) *Rhizopus*, (d) *Trichoderma*, (e) *Trichoderma* (Commercial), (f) Organic manure, (g) Cow dung, (h) without treatment