Screening , Isolation and Identification of some Wood Inhabiting Fungi on Five Different Forest Trees

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Dedication

TO THE MEMORY OF MY MOTHER AND MY FATHER

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All prayers and gratitude to ALLAH without whose help this work would not have been accomplished .

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List of Abbreviations

Abbreviation	Meaning
ANOVA	Analysis of Variance
ASTM	American Society for Testing and Material
AUMC	Assiut University Mycological Center
CO2	Carbon dioxide
CODIT	Compartmentalization of Decay in Trees
CWA	Coarse Woody Debris
ITS	Internal Transcribed Spacer
LSD	The Least Significant Difference
MS Excel	Microsoft Office Excel 2010
PCP	Pentachlorophenol
PDA	Potato Dextrose Agar
PH	Potential Hydrogen OR Power of Hydrogen
P-value	Probability Value
R^2	Resistance to Heat Flow in Wood
S^2	Secondary Cell Wall Layers
SD	Standard Deviation
SPSS	Statistical Package for the Social Sciences
WL	The Weight Loss

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Abstract

The objective of this study to investigate wood decay ability and determination rate of decay in five different types of forest trees ; *P. halepensis* , *C. sempervirens* , *P.atlantica, C. siliqua* and *E. macroryncha* against four types of basidiomycetes fungus; *S. hirsutum* , *I. hispidus* , *H . fasciculare* and *A. polytricha* . The assessment of mass loss conducted according to the *ASTM D 143-94*. In this study the experiment were conducted based on five steps; cutting wood samples for fungal testing , preparation of media for culture , drying the specimens , distribution of test specimens and exposure to fungi and examination of the samples exposed to fungi .

The mass loss of wood blocks was analyzed after five periods 2 ,3, 4, 5 and 6 months respectively. Whereas, all the fungus that were tested completely invaded woodblocks within two weeks . Our results showed variability in the wood degrading abilities . Interestingly, the natural resistance of the heartwood of *C. sempervirens* was highly resistant to all fungus that were tested. Whereas, the results of *C. siliqua* showed very low resistance to fungus degradation. Whoever, the most active and effective fungus among the tested species for *C. siliqua* was *H . fasciculare* which showed 31.94% loss . On the other hand, *A. polytricha* showed lowest activity on blocks degradation, which ranged between (5.528 to 16.91). The results of the present study reveal that there were differences in the wood decay capability of blocks according to the type of fungus were used .

Chapter One

Introduction

On land , fungi are important microorganisms and major recyclers of nutrients. In forest ecosystems the decomposition of forest litter is essential to nutrient recycling . The humus in the lower litter layer and the soil are very much a product of the complex relationships between the carbon sources (dead plants and animal tissues) and organisms that decompose these compounds . Fungi are able to decompose material as rigid as wood and reduce it to a soft almost paper-like substance . Fungi have been included in many groups over the years since the detailed study of them began . Initially they were studied by botanists because they were often found associated with plant habitat or the plants themselves. For many years fungi were considered "lower" non-photosynthesizing plants . In fact , some plant pathology textbooks written since 1980 still consider them as such (Johnson , 1990) .

In wood decay, the wood structure of trees is very important, as well as the enzymatic potential of the fungi . Trees can differ not only in the anatomical structure of their wood but also down to structural differences of individual cell-wall layers . All possess differing 'attractiveness' for fungal enzymes to break them down , this being manifested by the diverse patterns of wood decay observed . Beyond the purely visual changes , this has far-reaching consequences for the mechanical properties of the fungus-infected wood , such as its strength or stiffness (Francis *et al* . , 2000) .

Although the whole cross section of the tree serves as a mechanical structure, only those cells arranged in the outer growth rings of the xylem conduct and transport water and minerals . The latter tissue is called sapwood , while the inner dysfunctional wood is termed heartwood. New cells formed in the spring are called early wood are thin-walled and predominantly conduct water and minerals . Late wood cells are often thick-walled and provide strength and stability. The abrupt change between the late wood and early wood is visible to the eye as an annual or growth ring .

Softwood is typically composed of 90% to 95% tracheas , i.e. softwood wood cells . The storage and transport of nutrients in the radial direction take place within the

parenchyma cells , which are typically arranged radially or axially as xylem ray or axial parenchyma . The secreting elements in conifer wood are epithelial cells , which surround the resin canals in spruce . These canals appear as minute axial and radial cavities within the tissue of most softwoods . The structure of hardwoods is more complex than that of softwoods , but the concepts are analogous .

 Fiber cells , which represent the most common strengthening cells , consist of two types : libriform wood fibers and fiber tracheas. Hardwoods also contain vessels , which are longitudinally aligned and are the main conducting elements of water and minerals in the xylem . The parenchyma cell content of hardwoods is , on average , much greater than that of softwoods . The xylem rays are broader and occupy a greater volume in hardwoods than softwoods. The exact chemical composition of wood varies not only for each tree species , but also within different tissues (root , stem or branch) , the geographic location and the soil conditions . (Boddy *et al* ., 2008) .

 There are two major components within wood : lignin (18-35%) and carbohydrates (65%-75%) . The carbohydrates consist of cellulose (crystalline structure with high degree of polymerization) and hemicelluloses (mixture of polysaccharides) . Cellulose is a long , linear homopolymer consisting in an arrangement of glucose units . It has a very high degree of polymerization and a high degree of crystallinity (60 to 70%) . The surface of the microfibrils is surrounded by hemicelluloses , which provide cohesion between the microfibrils in the cell wall layer. Hemicelluloses are polymers based on various pentose and hexose sugar units , and possess a much lower degree of polymerization (Rowell , 2005) .

 As a constant feature of all vascular plants , lignin provides stem tissues with both mechanical strength and durability. It is a phenolic substance based on three alcohol precursors . These molecules are irregularly linked with ether and carbon-carbon bonds to form the lignin. In soft wood lignin, the dominant precursor is the conferral alcohol and are both precursors of the hardwood lignin. The random polymerization of these phenoxy radicals takes place through two dehydrogenation steps of the initial alcohols (Melanie Spycher , 2007) .

In the concept of fungi degradation action . Fungi are the primary degraders of lignocellulose (Rabinovich *et al* . , 2002) . In addition to secreting enzymes that are critical to lignocellulose decomposition , fungal growth on lignocellulose is promoted by the formation of mycelia that allow filamentous fungi to transport nutrients , including nitrogen and iron , to the carbon-rich lignocellulosic substrate (Hammel *et al* . , 2002) . Many fungi are also more resistant to wood-derived biocides that limit bacterial growth. These compounds include tannins and various phenolic compounds (terpenes, stilbenes, flavonoids and tropolones) that are particularly abundant in the heartwood of fallen trees .The majority of wood-degrading fungi that have been characterized to date are members of the phylum Basidiomycota ,and are characterized by either brown-rot or white-rot decay (Sonam Mahajan , 2011).

Wood rot diseases , caused by a wide variety of wound colonizing fungi, produce decay of the trunk , large branches , and roots of practically all woody plants . Decay usually develops slowly over a period of many years and may not noticeably shorten the life of an affected tree or shrub, although it causes huge annual losses of timber for building and wood products . The annual loss to wood decay is estimated at 20 billion board feet in the United States (or about one-third of the timber cut annually) , an amount more than that caused by fire , insects, and various other natural catastrophes combined. This annual loss represents enough lumber to construct a wooden sidewalk a mile wide . Fortunately, this loss is decreasing as we learn to better manage our forests and as we continue to harvest trees at progressively younger ages . (Francis and Schwarze , 2007) .

On the other hand , discoloration and decay are much more common and serious in over mature trees and poorly managed stands than in young trees or well managed stands . In living trees , most of the decay is confined to the older, central wood (heartwood) of roots , trunks , or branches . Once the tree is cut , however, the outer wood or sapwood is also colonized by the wood decay fungi , as are the wood products made from the tree , if moisture and temperature conditions are favorable for growth of the fungi . When deep wounds or cuts are present , discoloration and decay often spread into the outer wood , and the entire tree , especially if it is a hardwood , loses its economic value .

Fungi can only cause serious damage to wood when the moisture content is above the fiber saturation level (26% to 32% of the dry wood weight , depending on the species) . The microbiological degradation can only occur if the wood has a moisture content exceeding 20% of its oven dry weight . Although this is substantially below the approximate 30% minimum required for fungal decay , a lower moisture content is still advisable , because this provides a margin of safety in the event that the material does not dry uniformly (Anna Hyvönen *et al* . , 2005) .

Wood-decay fungi spread through wood as microscopic strands called hyphae, which begin as either germinating spores or bits of hyphae carried to the wound by many factors such as , (insects , wind , Birds , Bark beetles) . Wood-decay fungi are aerobic organisms producing CO2, water and energy from wood by respiration . They break lignin by oxidoreductase and the degradation of cellulose and hemicelluloses is predominantly by hydrolases . Transport mechanisms are essential for the growth of filamentous fungi and their hyphae in a mycelial network (Fuhr *et al*. , 2011) .

These strands of wood-decay fungi often secrete oxidizing enzymes that discolor wood and reduce its resistance to decay . In nature we can see differences in the response of live sapwood and in the formation of decayed wood. Wood-decay fungi need higher levels of oxygen . Pioneer fungi associated with the wood-decay fungi during the initial discoloration phase have small spores . They may be present in healthy sapwood , but not active until wood is exposed by wounding . They may also be carried to the wound by insects , or they may grow into the wood from the bark . Some are dark and stain the wood; some are not . Some are pathogenic and spread into living sapwood; others are not and become active only after living cells of wood have died . If the oxygen content of wood is relatively high , these fungi grow as mycelium and produce spores in the infected wood if the oxygen content is low, the small spores bud to form colonies that look like bacterial colonies , but mycelium is not produced (Walter and Dudzik , 2011) .

Wood shrinks as it loses moisture and swells as it gains moisture . This is partially true . Actually, wood will change dimension only between two moisture conditions . One condition is when the wood is void of moisture . This is termed the oven-dry condition . The second condition is when the wood fibers are saturated with moisture .

This point usually occurs at about30 % moisture content . As wood is dried from an original green condition , sometimes more than 100 % moisture content , moisture is first lost from the cell cavities . No shrinkage will occur until the wood reduces to a moisture content of about 30 % (fiber saturation point) . If drying continues below 30% moisture content , water is removed from the cell walls and shrinkage occurs.The amount of shrinkage or swelling depends on the species , density and board direction . The dimensional stability of different wood species is affected by width and density differences between early wood and late wood in the growth rings . For example , in species having wide , dense latewood bands and low-density early wood bands , the differential shrinking and swelling of the bands with changes in moisture content can cause large stresses in the wood that can result in raised grain and a defect known as shelling . Raised grain will tend to be more pronounced on flat grained lumber (Todd Shupe *et al* ., 2008).

To be more specified ,more than 1,000 species of fungi can cause wood deterioration and decay. Most of the fungi that cause serious wood rot are Basidiomycetes (brown rot and white rot fungi) .The fungi grow inside the wood cells and produce enzymes that digest the cell wall components for food and energy . Wood-rotting Basidiomycetes generally remain confined to the discolored cylinder and are unable to attack the new growth . The decay within the discolored column continues until the wood is completely disintegrated (Nancy, 1999) .

1.1 TYPES OF WOOD DECAY FUNGI

Brown-rot basidiomycetesits the largest group of fungi that degrades wood is the basidiomycetes . It has been calculated 1600-1700 species the primary mycelium developed after spore germination is also haploid . Dikaryotic mycelium can be identified by the presence of clamp connections at the septum . Wood-rotting basidiomycetous fungi are usually divided into white-rot and brown-rot fungi . They are taxonomically closely related, and white-rot and brown-rot fungi can be found in the same genera . Most wood rottes belong to the orders Agaricales and Aphyllophorales . Brown-rot fungi mainly decompose the cellulose and hemicellulose components in wood . Brown-rotted wood is dark , shrink , and typically broken into brick-shaped or cubical fragments that easily break down into brown powder . The

brown color indicates the presence of modified lignin in wood . (Sylwia Solarska , 2009) .

   White-rot basidiomycetes the only organisms capable of mineralizing lignin efficiently are basidiomycetous white rot fungi and related litter-decomposing fungi different white-rot fungi vary considerably in the relative rates at which they attack lignin and carbohydrates in woody tissues. Many white-rot fungi colonize cell lumina and cause cell wall erosion . Eroded zones coalesce as decay progresses and large voids filled with mycelium are formed . This type of rot is referred to as nonselective or simultaneous rot . Some white-rot fungi preferentially remove lignin without a substantial loss of cellulose, and cause white-pocket or white mottled type of rot . There are also fungi that are able to produce both types of attack in the same wood (Annele Hatakka , 2001) .

   Mycelial cords, the main transport pathways in mycelial networks of those wood land fungi not confined to a single resource unit develop in a way that suggests each cord-forming species has evolved a different foraging strategy. With cords developing early in growth to form long supply lines from the large wood food bases this organism prefers . Advance only halts when the mycelium meets , and pauses to exploit , a substantial new wood resource . Cords perform a remarkable range of transport functions , carrying carbon sources including sugars and polyols , amino acids, phosphate and water . Substances can transition towards the growing margin and back to base at the same time , (Watkinson *et al* . , 2005) .

   Fungi in the first growth phase use spare substances (low molecular mass) gathered in wood . Hemicelluloses and cellulose with low polymerization degree are the next wood components which are decomposed by fungi . These compounds undergo fast decomposition from the reason of their chemical composition , significantly lower polymerization degree in comparison to alpha-cellulose and their distribution outside cellulose micelles . There is no accordance among different authors about the rate of particular wood components decomposition (Piotr *et al* . , 2012) .

   Fungal invasion and decay of wood occur mostly in the nonliving , structural wood fibers and nonfunctional water –conducting tissues (also known as the apoplastic tree

or the part of the tree made up to of nonliving cells without protoplasma) , decay may not directly affect the biological health of the tree (also known as the symplastic tree , which is formed by the connection of living cells with protoplasma) . Importantly , the health of foliage is usually not a good indicator of the potential structural condition of a tree , because the symplastic tree is not directly dependent on the apoplastic tree for function . Wood decay fungi grow as microscopic hypha (single , segmented strands of a fungus) between cells in lumen , or center , of wood cells and within cell walls . Hypha release enzymes that break down cellulose and lignin (Christopher and Luley , 2006) . Observation of reaction zones that form ahead of advancing decay suggests that a generalized active response to decay occurs in sapwood that is not limited to the defined walls of CODIT . Reaction zones are areas of discolored sapwood that might have induced alteration of cell walls , elevated levels of antifungal compounds such as polyphenols , and other chemical changes (Pearce ,1996) .

Another theory suggests that decay is limited to heartwood and inner sapwood because of the presence of high sapwood moisture .The resulting low oxygen and high carbon dioxide levels are thought to prohibit growth of decay fungi in functional sapwood (Boddy and Rayner ,1983). Most decay fungi spread via airborne basidiospores or ascospores that are released from fruiting structures produced on living or dead trees . A single conk can release millions of spores . Removal of conks will eliminate one source of spores but will have no impact in decay inside the tree . root decay fungi can spread via contact between adjacent trees (Christopher and Luley, 2006) .

Wood decay fungi are the primary biotic decomposers of wood. In forest ecosystems, they play an important role in carbon and nitrogen cycling and help to convert organic debris into the humus layer of the soil . They colonize downed timber and slash on the forest floor, lumber, and even wood in service . Wood decay fungi can also attack living trees, acting either as true pathogens responsible for root rots and stem cankers or as rotting agents of heartwood and sapwood .Wood decay is a major source of loss in both timber production and wood use , and importantly for

landscape trees , in parks and urban forests, it can cause a tree to be hazardous (Paolo and Giovanni , 2007).

Wood decay leads to loss of tree vigor and vitality, resulting in decline, dieback, and structural failure . Wounds play an important part in this process since they are the primary point of entry for wood decay pathogens . Bark , which serves to protect tree tissues , is the first line of defense against wood decay organisms. Whenever bark is broken , a wound results. Wounds that penetrate bark expose underlying tissues to invading pathogens , that cause rot or decay . Wounded trees do not technically "heal" since they are not capable of repair or replacement of damaged tissues . Instead, trees close over their damaged tissues with wound wood callus tissue . Trees also wall-off injuries by producing chemical and physical barriers to pathogens . Organisms that are able to overcome these protective barriers can then colonize and invade wounded tissues . Among the most aggressive of these organisms are the wood decay fungi. The ability of trees to compartmentalize decay differs between woody plants . (Nicole Ward Gauthier *et al* . , 2015) .

1 . 2 Aim of the study

1- Study the wood decay fungi in *vivo-vitro* of certain forest trees .
2- Isolation and identification of wood decay fungal parasites .
3- Investigation the rate decay in vitro on five different kinds of wood .
4- Comparison studies between the five types of wood inoculated by isolated fungi .
5- Measuring the sensitivity of the wood to these attackers .

Chapter Two

Literature Review

Emerhi and Ekeke , (2008) carried out a study on the biodegradation effects of some rot fungi on *Pinus caribaea* wood. they proposed in their study that there is an effect of two species of white rot fungi and two species of brown rot fungi on *Pinus caribaea* , besides the possibility of controlling the fungi with chemical preservatives . They followed special procedure , where they collected wood samples from a ten-year old plantation of *Pinus caribaea* , from Nigeria , and then divided the samples into two groups . one group was inoculated with two species of white-rot fungi ; *Corioliopsis polyzona* and *Pleurotus squarrosulus* . Whereas the second group was inoculated with two species of brown rot fungi ; *Lentinus lepideus* and *Gleophyllum striatum* . Owing to the biodegradation , they noticed that the wood samples lost their weight by factors that varied from 1.5 to 48.1% for *Corioliopsis polyzona* , 9.6 to 58.% for *Pleurotus squarrosulus* , 40.4 to 78.1% for *Lentinus lepideus* and 6.8 to49.2% for *Gleophyllum striatum* . Also the wood decay varied along the tree bole(trunk) while the height parts were not affected . They came to the result that the biodegradation by rot fungi differs in strength depending on the fungus species . According to this result , they suggested that preservative impregnation and retention are the best way to control the rots to make *P. caribaea*a utility wood .

In the concept on biodegradation , Rina and Kishore , (2015) studied the anatomical characterization of wood decay pattern in *Azadirachta indica* caused by the white rot fungi . They attempted to investigate the amount of cell wall damage caused by *Irpex lacteus* and *Phanerochaete chrysosporium* on sapwood of *Azadirachta indica* . They inoculated the *A.indica* wood with *P.chrysosporium* and *I.lacteus* . However , the first fortnight of completely invasion of the wood blocks by both fungi showing no considerable weight loss , they noticed that the weight loss took a place 120 days later and became faster , recording 28-32 % weight loss . The study showed selective delignification characterized by separation of fibers , cell wall thinning ,formation of erosion holes on fiber walls , and the axial and ray parenchyma cells . Also the observed "U" shaped erosion troughs on the fiber wall , which indicated that both strains caused simultaneous pattern of white rot .

Robert , (1984) studied the wood decayed by white rot fungi for special lignin degradation . He followed a screening procedure that uses scanning electron microscopy. To ensure the presence of only one decay fungus he made isolations from all samples collected in the field . He made radial and tangential sections from each collection of decayed wood and fixed them on aluminum stubs. And used a desiccator to dry the sections and coated them with 40% gold-60% palladium in a vacuum evaporator, and a Philips 500 scanning electron microscope to observe the specimens. Furthermore ,he made lignin and wood sugar analysis on field-collected samples. He observed areas of delignified wood seen as white zones ,which he removed by fine-pointed forceps. He broke up the samples into small pieces that to pass through a 40-mesh screen and analyzed wood meals for sulfuric lignin and individual wood sugars using high-pressure liquid chromatography. The gained results showed the presence of 26 different fungi that caused delignification of wood samples , a simultaneous removal of cell wall components ,and also that wood from different trees may degraded differentially by the same fungus .

On the other hand , Mongkolsuk , *et al* . , (2008) studied the possibility of using white-rot fungi to remediate soils that are contaminated with hazardous compounds. First they run their study in laboratory then they conducted their field study in summer 1989 . They described the fungi , their ligninolytic system ,and the compounds that they degrade . They used Pentachlorophenol as a target chemical . The study suggested that white rot fungi have potential for use in the remediation of soils contaminated with toxic compounds ,including PCP .

However , Anna , (2007) conducted a study on coarse woody debris (CWD) . The aim of this was to examine the importance of CWD as habitat for wood-inhabiting fungi in the wet sclerophyll forests of Tasmania . The study observed how the fungal species richness , fungal community composition are affected by changing tree age . She divided the trees into three age-classes (69 ,105 ,and >150 years old) . Six living *E* . *oblique* trees in each of the classes were cut down . Samples were made from specific points , and the decay profile was mapped . From each sampling point , in the rotten wood , fungi were isolated , and from control samples of clear heartwood and sapwood . Based on their color and texture , she classified the collected samples of each decayed wood type . She could isolate ninety-one species of wood-inhabiting

fungi from the examined trees. There was a enormous difference in the community composition of wood inhabiting fungi between the oldest age-class examined trees and the younger two age-classes . more than half of all species were only . These studies are among the first to examine wood-inhabiting fungi in mature *E . obliqua* trees and logs in Tasmania .

The efficacy of stilbenes and pinosylvins against White –Rot and Brown –Rot fungi was studied by Catherine *et al . ,* (1999) . they examined the composition of stilbenes , (a class of phenolic compounds) , in pine cones and evaluated the fungi toxicity of pinosylvins and its methyl derivatives on white rot and brown rot fungi . They , as a first step, used a traditional bioassay media (2% malt extract agar) and soil-block bottle tests to extract three stilbenes , pinosylvin , pinosylvin monomethyl ether and pinosylvin dimethyl ether from white spruce *Picea glauca* , jack pine *pinus banksiana* , and red pine *pinus resinosa* pine cones . Then they used a mixture of these stilbenes with two different concentrations (ei ,0.1% and 1.0%) to examine their fungal inhibitory activity , using two bioassay methods . The study they conducted demonstrated that extracts from conifer seed cones contain stilbenes that may exhibit antifungal action against white rot fungi and brown rot fungi . This action varied with the stilbenes , the concentration , the fungi and the bioassay method . The results they gained showed that stilbenes may help to protect pine tissue and prevent the fungi to reach free water in the cell , and hence contribute to wood decay resistance against brown rot fungi .

Furthermore , Rasmina *et al .* , (2012) compared the ability of two species of white rot fungi , *Pycnoprorus sanguinedus* and *Oxyporus latemarginatus* , to degrade lignin in kenaf chips . They isolated the white rot fungi used in their study from the Forest Institute of Malaysia center . Then they grown the isolates in Potato dextrose agar slants , and incubated at 28 °C for 6 days in anamorphic stages . Then they inoculated the kenaf chips with white rot fungus for a period of 1,2,3,4,8 and 16 weeks . Using scanning electron microscope and chemical analysis they observed the mixture , and noticed that all the cells of fibers, vessels ,and ray parenchyma are decayed by the white rot fungi . Also , detected that kenaf chips were markedly degraded during the first week of pretreatment , and extensively degraded during the incubation period to 16 weeks . After treating kenaf chips with the tow white rot fungi ,they observed an in

increase in degradation as the incubation period increased , with a greater activity of *O . latemarginatus* than *P . sanguines* to degrade lignin .

An investigation was conducted by Juan *et al .* , (2005) to search the degradation of lignin in pulp mill wastewater by white rot fungi . They aimed from their work to compare the ability of five white rot fungi , *Phanerochaete chrysosporim* , *Pleurotus ostreatus , Lentinus edodes , Tramestes versicolor* and S22, to degrade lignin , and to investigate the effects of pH , concentrations of carbon, nitrogen and trace elements in the wastewater medium to remove lignin . The procedure they follow was to grow the five mentioned fungi on a porous plastic rings , which serve as an effective medium for supporting attached growth of the fungi . Then they used the attached –growth fungi individually to treat black liquor from a pulp and paper mill . They got results that conclude that the five white-rot fungi , *Phanerochaete chrysosporim* , *Pleurotus ostreatus , Lentinus edodes , Tramestes versicolor* and S2 , grown on the medium they used (porous plastic). Also more than 71% of lignin was completely removed from the wastewater. additionally they discovered factors that had significant effects on the growth of the fungi, degradation of lignin. These factors were concentrations of carbon , nitrogen and trace elements in wastewater. They advised in their study by recommending an addition of 1g/l glucose and0.2g/l ammonium tartrate. This mixture was beneficial for the lignin degradation by white-rot fungi. Also they noticed that three white-rot fungi , *P chrysosporim* , *P ostreatus* , and S2 , showed high capability of lignin degradation at pH 8.0-11.0, which suggested that the white –rot fungi were able to grow and degrade lignin even in the strong alkaline medium.

The degradation of cellulose and hemicelluloses by the brown-rot fungus , *Piptoporus betulinus*-production of extracellular enzymes was studied by Vandula and Petr , (2006). They aimed from their study to estimate the activity of glycosylhydrolases of the brown-rot fungus ,*Piptoporus betulinus*. And tried to identify these activities in the natural substrate and characterize the main cellulolytic enzymes . When grown on wheat straw , *P. betulinus* caused 65% loss of dry mass within 98 days .

Angela and Andre, (2001) investigated the hydrolytic and oxidation effects of enzymes that produced from white-rot brown-rot fungi during *Eucalyptus grandis*

rotting I solid medium . They described through this study the enzymatic activities produced by two brown-rot fungi and four white-rot fungi grown on *E. grandis* wood chips under soli-state fermentation . They found that the profiles of extracellular enzymatic activities produced during wood decay varied among the fungi . However , all fungi produced hydrolytic activities , brown-rot fungi produced higher levels of cellulose and xylanase than white-rot fungi did . Whereas , phenoloxidase were only found in the white –rot fungal extracts . Also found that the capability of white –rot fungi to degrade wood , which determined by weight loss , appeared to be correlated with levels of oxidative activities only after long biodegradation periods . Although the two brown-rot species were similar in the hydrolytic activities , *Laetiporeus sulfureus* demonstrated a very limited degradative capacity , in contrast with *Wolfiporia coco*s , which induced an effective decay . Moreover , they discovered that the fungi providing the highest levels of lignin loss were responsible for the highest values of polyoses removal . Depending on these results , they explained the removal of lignin caused the wood cell wall more permeable , which assist the xylanases diffusion into the wood cell wall .

A survey run by Sally *et al* . , (1999) to investigate the species abundance pattern of two wood basidiomycete communities in Australia . They estimated the species diversity of wood decay basidiomycetes in two sites of remnant native vegetation. The first site ,which was Kyeema Conservation Park , has a continuous over story of *Eucalyptus baxteri* and *E* . *obliqua* and has non-sandy soil . In contrast the , other site, which was Cox Scrub Conservation Park , has patches of *Eucalyptus baxteri* and *E.obliqua* and sandy soil . The researchers made samples from the two patches over a period of two years . They found thirty six species of wood decay basidiomycetes in both sites of research . Some species were common in both sites. They stated that the discovered species in this survey were not located at Australia only , but also have been described from other countries . Hence the fungal communities discovered in these two sites in Australia are not unique . Their survey showed differences in the assemblage of fungal species in two patches relatively close together. They reasoned that there were some factors that may affect the fungal communities . These factors were host (*E.baxteri vs E.obliqua*) , rainfall , vegetation cover, soil type and landscape topography. Noticed that *Eucalyptus baxteri* grows on dryer , sandy soil , therefore

they suggested that any fungal survey testing host specificity would be confused by other factors .

The fungi the cause decay of *Pinus radiata* specifically in New Zealand , were studied by Chee *et al* . , (1989) . They described the isolation of basidiomycete fungi from a number of sources and made their reports on the results of two laboratory assays to determine decay potential . They collected basidiomycete fungi from *P.radiata* wood and fruiting bodies . They used wood block assay and cellulose assay to screen fungi for decay potential and to identify those suitable for further study. Regression analysis was used to determine any correlation between the wood block and the cellulose assays. The study resulted in that wood weight loss caused by the test fungi ranged between 0.1 and 27.1%. The 13 isolates causing the highest weight losses were all brown rot fungi (7.7-27.1%). Mean wood weight loss by the brown rot fungi (13%) was apparently higher than the white rot fungi (3.8%) . The researchers concluded their study with the result that there was no correlation between the wood block assay and the cellulose assay. Although , the potential of the test fungi to cause weight loss in wood was not related to their ability to produce cellulase in liquid culture, both assays were carried out under one set of conditions.

Also the decaying of wood that was caused by brown rot fungi was studied by Douglas *et al* . , (1991) , and the changes that occurred in pore structure and cell wall volume were studied as well . Since brown rot fungi destroy wood by selectively degrading the hemicelluloses and cellulose without extensively changing the lignin, and based on results that demonstrated that chemical treatment of wood can have a substantial effect on cell wall pore volume and size distribution ,the researchers in this study aimed to determine the pore volume in sound wood cell wall , does the attack of brown rot fungi result in a sudden increase in cell wall volume and whether the decay of wood opens up the pore structure of the cell wall in a manner that allows access by large molecules. They first examined the accessibility of volume in sound wood to water and the solutes of increasing molecular size. Then they determined the effects of decay on these accessibilities to various probes. The sweet gum wood they used in their study had been dried, and the brown rot fungus used was *Postia Placenta.* They incubated blocks of volumes (2.5 X2.5 X.3 cm^3) with the fungus for up to 4 weeks in soil blocks cultures. Using the solute exclusion technique, previously developed by

other researchers, they determined the pore volume. They found that the pore size was nearly equal to 15Å , and the cell wall volume was 0.35ml /g. At 35% weight loss, they noticed that the cell wall volume doubled to about 0.7ml/g. they reasoned this increase in cell wall volume to two effects : the creation of new openings in the cell wall or erosion of pre-existing pores in the range of 12Å to38Å, and the swelling of cell wall as a result of fungal action.

   Piotr *et al* . , (2012) found the correlation between the mass-loss of wood decayed by brown and white rot fungi species and the structural wood components content . They studied the qualitative and quantitative changes of the pine wood chemical composition during a decay process caused by white and brown rot . They collected Scots pine wood *Pinus Sylvestris* (L) samples from sapwood zones ,and used two species of testing fungi as testing biological materials. The testing materials were *Coniophora puteana* ,brown rot fungi ,and *Trametes versicolor* ,white rot fungi. The researchers examined the effect of fungi activity on chemical composition changes. They came to the results that indicated decomposing all wood structural components-cellulose ,hemicelluloses and lignin by white rot fungi *Trametes versicolor* ,with similar rates of cellulose and lignin decomposition . Whereas brown rot fungi had no decomposition effect on lignin ,but its effect was only on carbohydrates. Back to white rot decay ,the content of small-molecular carbohydrates did not change their level ,which indicates uniform cellulose depolymerization and exploitation of decomposition products by fungus. Also they discovered that cellulose decomposition, whatever the fungi kind was, occurred from the beginning of fungi development in wood .

   Whitney and Denyer , (1968) studied ,in field ,the rates of decay by *Coniphora puteana* and *Polyporus tomentosus* in living and dying white spruce. They investigated the effect of moisture content of white spruce heartwood on the rate of decay by two root decaying fungi , *C. puteana* and *P. tomentosus* . They altered the moisture gradients in heartwood by varying soil moisture content , and investigated also decay rates of the two fungi in living and dying trees . Their conclusion stated that *C. puteana* caused more decay in heartwood of dying or dead trees than it did in inoculated healthy trees , while the other fungi , *P. tomentosus* caused the significant decay in living tress. Additionally ,they noticed that the percentage of infection by

C.puteana was higher in trees that were predisposed to die at the time of inoculation than those remained alive and healthy during the experiment. They found no correlation between the rate of decay by either fungus and the heartwood moisture content . From their point of view , it seemed that more reasonable that changes in the heartwood resulting in more favorable growing conditions for *C .puteana* were responsible for the increased infection and increased decay in dead or dying trees rather than changes in moisture alone .

Bjorn and Heidi , (2001) studied the wood-decay fungi in hazel wood . They hypothesized that older stands and fallen hazel wood positively affect species richness, suggesting that old age of stands should be carefully treated that to preserve and manage fungal biodiversity in this habitat. Researchers investigated eight hazel stands in south east Sweden , founding a total of 140 species of wood-decay fungi . 60 of them were pyrenomycetes and 80 basidiomycetes . The relationship between structural variables and species richness showed that the total richness was higher in young hazel stands and stands with low concentration of woody debris . On the other hand , sites of high percentage of dead wood had low number of species per stem . The study discovered that the total species richness was not correlated to the age of stands for unknown reason . They concluded their research with the advice that any research on other forest type should be applied critically to the management of fungal biodiversity in hazel stands .

Weijie *et al* . , (2013) designed a study to quantify wood debris decay rates and their variability and to explore decay rate variability that is linked to environmental (such as temperature and wood moisture content) and other factors and that not related to those variables . Their study was conducted in a large area of undistributed subtropical moist forest located in the Ailao Mountains Nature Reserve .They measured environmental factors and wood debris decay from three tree species using CO_2 release rates to investigate variations through time. The species that were examined in this study were *Lithocarpus chintungenesis , Lithocarpus xylocarpus* and *Schima noronhae* . The examination was run six time during two years . They defined three decay classes for each of these species ,producing nine groups . The variability in wood debris decomposition rates was measured by the research team and their results supported previous studies that suggested that half or less of the variation

could be explained by environmental factors . Predictions were made on individual pieces of wood debris in each wood species and decay group based on different criteria . For instance ,those predictions of release rates (R)based on temperature and moisture together had the higher R^2 values that ranging from 0.25 to 0.57, while those based on moisture alone their R^2 values ranged from 0.16 to 0.35 and R^2 values that based on temperature only were seen to be from 0.07 to 0.35 . what's more , the study indicated there was no correlation between wood density and surface area and CO_2 release rates . Furthermore , the researchers found that the predictions of wood debris CO_2 release rates from temperature varied widely among groups . Those for *Lithocarpus xylocarpus* , were consistently strong for the three decay classes . Whereas , predictions for *Schima noronhae* all were weaker and those for *Lithocarpus chintungenesis* were variable . The annual total CO^2 release from wood debris groups was estimated to have average exponential decomposition rate (R) equal to 0.09 per year.The importance of environmental factors in determining wood debris decomposition was supported by this study .

Hamid *et al* ., (2015) studied the fungal trunk pathogens that associated with wood decay of pistachio trees grown in Iran . The goal behind this study was to identify the different fungal species associated with trunk disease (die-back or decline symptoms), by means of morphological and molecular examinations , and evaluate the pathogenicity of different fungi in one *pistachio cultivar* planted in Iran . To run this study the researchers made samples from 32 symptomatic pistachio trees from different orchards . Then they isolated fungal trunk pathogens , observing the common symptoms of the disease , which included : yellowing , die-back ,shoot canker and plant death . They also noticed some internal symptoms includes central necrosis , watery necrosis , internal wood discoloration with brown to black . They found ,from this study , five kinds of wood necrosis , and that pistachio trees represent a "catch crop" for species of the genus *Phaeoacremonium (Pm. parasiticum , Pm. aleophilum , Pm. cinereum* and *Pm . viticola*) , which are species that cause dieback or decline symptoms on woody hosts .The most common species isolated from orchards were *Pm . parasiticum* and *Paecilomyces variotii* , with occurrence of *Pm. aleophilum , N. parvum , B. dothidea , Pm. cinereum , and Pm. viticola* . Based on the pathogenicity tests they did on pistachio trees the results showed that all inoculated species used were pathogenic on host , and the *N. parvum* species who causes trunk

lesions had longer action than caused by other species. Also they discovered that all isolates used in their study caused longer basipetal than acropetal lesions on pistachio.

Mohammed *et al* ., (2017) studied the physical , mechanical and natural durability properties of *Pinus halepensis* (Mill) located in the Mediterranean area. They aimed from this study to describe the physical , mechanical properties of Aleppo Pine wood in Tunisia . Also to study the natural decay and termite resistance of Aleppo Pine wood from Morocco , that to identify its potential valorization . They concluded their study with that most Pine species showed a low durability towards *Coniophora puteana* and *Gleophyllum trabeum* , slightly durable toward *Poria placenta* invasions, and sensible against termites . Moreover , the wood ,physically and mechanically ,was very stable .

Manel *et al* . , (2015) studied the natural durability of Algerian pistacia. Their main goal of this study was to contribute to the quality of timber production that is widespread in Algeria . Hence , to evaluate the natural durability , they used the gravimetric method that includes testing for fungal attacks and measuring the sensitivity of the wood to these attacks . They used standardized sapwood and heartwood samples from *Pasticia Atlantica* trees grown in two different areas in north western Algeria . And four *Basidiomycetes fungi Coriolus versicolor* , *Gloeophyllum trabeum* , *Coniophora puteana* and *Poria placent* . The findings from this research showed that the durability of the heartwood and sapwood from the samples tested was identical , and no difference was observed , between the two areas of study , for the same type of wood. finally they concluded that *Pastacia atalntica* (Desf) . timber could be considered as very durable .

Abbot *et al* . , (2015) studied the saptrophic wood degrading abilities of *Rigidoporus*. The researchers mainly focused on assessment of saprotrophic wood decay ability of a tropical rubber tree pathogen (*Rigidoporus microporus*) .

González *et al* ., (2009) found that the vineyards affected by esca disease were surveyed in the provinces of Alicante and Valencia (south-east of Spain) . The presence of resupinate , hymenochaetaceous basidiocarps was observed on the trunks of vines that displayed typical esca symptoms as well as in apparently healthy plants.

Vines showing fruiting bodies represented 40% of the total esca-diseased plants and were randomly distributed in the several vineyards surveyed. Using classical and molecular identification methods , the fungus producing the observed basidiocarps was recognized as *Inonotus hispidus* , during this period , no basidiomycetes excluding *Stereum hirsutum* (Willd.) Pers.) , *Inonotus hispidus* were found .

Giordano *et al* . , (2015) studied The extent to which the presence of wood decay fungi in standing trees is underestimated when diagnosis is based on the visual inspection of trees was studied and whether the rate of underestimation may vary depending on the environmental context (urban vs. forest sites) and the fungal species was tested . A total of 903 broadleaf and conifer standing trees(including *Cupressus* spp , *Pinus halepensis*) were inspected for the presence of fruiting bodies . fallen decaying trees are species-rich environments , and deadwood has been recognized as providing resources for a variety of living organisms   such as *Inonotus hispidus* (Bull.) wood decay fungi significantly reduce the quality of timber in production forests .

Michael and Vicente , (2015) Reported that   considerable number of basidiomycetes are on living *Vitis vinifera* .They detailed information about this relation dates back to the 19th and early 20th century. More recently, several basidiomycetes have been discussed as being associated with Grapevine Trunk Diseases, especially esca. On a European scale, they listed 24 basidiomycetous taxa. Ten species are treated with respect to "life strategy and symptoms", "host plants", "geographic distribution", "transmission and vectors", "fruitbody characters" and "diagnosis". Fourteen additional species are listed with their main features only. Also showed that the References are given both to data provided in the literature and from our observations. *Fomitiporia mediterranea*, and, to a lesser degree, *Inonotus hispidus* are discussed as primary pathogens. The remaining taxa, including *Stereum hirsutum*, *Trametes hirsuta*, and *Schizophyllum commune*, they are considered as subordinate only, mainly causing white rot on already dead parts of grapevine trunks. With the data at hand, the ecological significance of *Auricularia auricula-judae* remains uncertain .

Heilmann and Boddy , (2005) Investigated the effects of exudates from uncolonized and from partly decayed beech wood on the extension rates of 16 later stage decay fungi were. They said the partly decayed wood had been colonized by the pyrenomycete *Eutypa spinosa*, or the basidiomycetes *Fomes fomentarius*, *Stereum hirsutum*, and *Trametes versicolor*, all known as common early decay agents in European beech forests. Sterilized wood pieces were they placed onto 0.5% malt agar, opposite to small agar plugs containing the test fungi. The presence of uncolonized wood stimulated extension rates in many species . whereas, the four previously decayed wood types had variable stimulatory or inhibitory effects. Their resulted showed that the wood decayed by *S. hirsutum* reduced extension rate, delayed growth, or total inhibition in the majority of species, thus it is suggested that this species uses secondary metabolites in a defensive strategy. A single species was, however, stimulated in the presence of *S. hirsutum*-decayed wood. In contrast, also the presence of wood decayed by *F. fomentarius* was stimulatory to 45% of the species. The other previously decayed wood types generally resulted in more variable responses, depending upon species.

Milenko *et al* ,(2012) Investigated from the deteriorating of impact on the mass of Sessile- and Pedunculate Oak by mass - loss test ,with four common decaying Basidiomycetes fungi as follows:*Stereum hirsutum,Chondrostereum purpureum, Stereum rugosum* and *Xylobolus frustulatus*. They were isolate the mycelia of tested fungi from fresh fruit bodies growing on oak wood and collected from forest stand in locality of Majdanpek . Their tests have been performed with two geographically different strains of fungus *Stereum hirsutum*, due to its` importance and common appearance on Oaks. Second strain of this fungus originated from fungi culture collection of Institute for Wood biology and Wood protection in Hamburg – Germany. Cultivation of fungi have been performed in sterile plastic Petri – dishes (D= 90 mm) in standard climatic conditions of temperature and humidity . they found that the fungus *Sternum hirsutum* causes white rot of death wood of broad leaf trees, with- or without bark, but also attacks physiologically weakened trees and branches. It appears as saprophyte on death broad leaf trees (Oak, Beech and Birch), rarely on conifers. Fungus *Stereum rugosum* appears on death, upstanding or felled timber (with or without bark) of broad leaf trees (Beech, Oak, Birch, and Hazel – wood) as saprophyte, but also as parasitic. It is dangerous causer of Oak cancer and white rot of

sapwood. These fungus attacks a numerous of broad leaf trees causing very fast deterioration of wood. This is the reason why it is usually used as test fungus in testing of decaying capability of the other wood - decaying fungi .

Rimvydas *et al* , (2006) .Their objective were to investigate the ability of three wood-decay fungi, *Phlebiopsis gigantean Phlebia centrifuga* and, to colonize fine roots of conifer seedlings. Each fungus, mycorrhizal syntheses they were attempted with *Picea abies* and *Pinus sylvestris* . After 24 weeks , their findings from the isolation of fungi and direct sequencing of fungal internal transcribed spacer (ITS) were carried out from healthy-looking surface-sterilized root tips that yielded both pure cultures and ITS sequences of each inoculated strain. Mycelial mantle of *Hypholoma fasciculare* , *P. gigantean* were frequently formed on root tips of *P. abies*, and microscopical examination has shown the presence of intercellular hyphae inside the roots. Their results provide evidence of the ability of certain wood-decay fungi to colonies fine roots of tree seedlings.

James *et al.,* (1996) They tested 98 isolates (78 species) of lignicolous fungi followed by chemical and ana-tomical analyses . and they showed that the isolates of *Auricularia polytricha* caused a white rot, with high weight losses and unusual, branching microcavities that were oriented longitudinally in the S2 cell-wall layer and among white-rot fungi on birch , make a relationship between strongly selective delignification and strongly selective utilization of mannose . They collect the fruiting bodies from the field. they said ,when the fruiting structures were small and cultural characters unknown , spore isolations were also made for verifi-cation . Cultures were grown at 25C on malt extract agar (1.25% malt extract, 1.5% agar) and stored at 4C. block decay tests were conducted as described in a standard method . they used the sapwood of yellow birch (*Betula alleghaniensis* Britton) and southern pine (*Pinus taeda* L.) in their experiment . Their deductive in the Auriculariales, members of the Exidiaceae caused weight losses in-dicative of decay caused high weight losses are *Auricularia polytricha* .

Chapter Three

Materials and Methods

3.1- The study Area

The Specimens under research were collected from five species ; *Pinus halepensis* (Miller) , *Cupressus sempervirens* (L) , *Pistacia atlantica* (Desf), *Ceratonia siliqua* (L) and *Eucalyptus macroryncha* (F. Muell .ex Beuth) , (Fig. 3.1) ,which are widespread trees at the study area (ALBAKOR) . The study site is an open wood land and located between 32.31.26°N and 20.37.29 E. Geographically it is to the north of Touchera , and to the south of Farzoogha and Lahmeda villages. The climate of the study area is similar to that of Aljabal Alakhdhar ,which is soft and dry in summer , warm and rainy in winter. The annual mean air temperature is usually 20.2 °C , and sometimes 26.7 °C in August. Wind directions varies seasonally , where that comes from north west blows at 43.7 , and that comes from south blows at 24.4 . During summer the south eastern wind is the most dominant ,with a degree of 20.3%. Moreover , the annual mean wind speed reaches 10.1 knots ,reaching its maximum speed in April. On the other hand, the rainfall rate in the study area is in the range of 261.2 mm. (Al-Masri , 2009). Because of the evaporation from the sea, the humidity in the study area attains its height in summer (July −August), between 70-80% (Beneneh Meteorological Station , 2004).

3.2- Field Samples

The trees from which the samples were collected were chosen randomly from the whole study area . After being specified by marking the studied trees with a paint , 5 samples were collected from each tree species . Branches with diameters bigger than 5cm^2 ,and are free of any apparent injuries ,such as wounds and cankers ,were originally collected. Each branch was 60cm long ,and wood debris 5- 10cm^2 width . A handsaw was used to cut the branches from the trees. The handsaw was being sterilized with Clorox© after each cut process that to avoid any contamination. The slots and cut branches terminals were wrapped by KOLLAN© (ARBOKOL) , (Fig. 3. 2), which is a gummy preservative material that is used to wrap grafting sites on

trees to prevent them from contamination (Fig. 3.3) . Before being dried each branch was weighed with electronic balance ,defined by an ID card that contained the species and weight. the branches were organized in groups ,each group had a number that identifies the tree species (1.1,1.2,1.3,…). Then the samples were kept in well-sealed black bags at room temperature (20-30°C) and stored in the lab for 8 weeks , that to reduce its moisture content (Fig. 3.4). After this storage period ,the samples re-weighted ,to ensure the loss of moisture content.

3.3- Wood samples examination and Preparation of wood discs

This step was done after the storage and re-weighing procedure. The aim of this process was to ensure that the unavailability of endophyta fungi, and the procedure was as follow: Each collected branch was cut into discs using a sterilized handsaw (Fig. 3.5), followed by, arbitrarily , taking small parts from the center of each disc and growing them on culture of (PDA) previously prepared . After that The cultures were kept at 20°C for a tow weeks. Thereafter , each culture dish was inspected to discover whether or not the presence of interior fungi (endophyta), (Okino *et al* . , 2010) , (Jennifer and Angela , 2007) .

3.4- Preparation of wood blocks

After being investigated and the absence of endophyta fungi were ensured , the assessment of mass loss conducted according to the methods described in the American Society for Testing and Materials *ASTM* D 143-94 , (Standard methods of laboratory testing , small clear specimens timber and test of natural decay resistance of woods) . wood blocks were prepared from every samples species of trees used in this research ,from the same branches. Using the formerly described handsaw , the branches were cut into small blocks with dimensions of (2x1.5x1.5cm^3) in the longitudinal directions (L) , radial (R) and tangential (T) (Fig. 3.6) . The part from which the blocks were made was Heartwood (Emerhi *et al* . , 2008) ,(Abdalla , 1995) , (Manel *et al* . , 2015) . Specimens from every plant species were selected , and examined with four wood decay fungi, which were previously isolated from the same trees species being studied and also identified . The prepared blocks were divided into groups , according to the tree types . Each consisted of five blocks (20 blocks for each

tree type , *e i* . 100 blocks total). Subsequently, the blocks were weighed again to determine the initial dry mass of wood samples , or relative density. They were sterilized in an autoclave .

3.5- Preparation of culture media

In this experiment ,the essential nutrient medium used was PDA (Potato Dextrose Agar). This medium consists of (potato 200 g/L, dextrose 20 g/L, agar 15 g/L) , at pH5.6 ±0.2. in order to grow up the fungi a mixture of 39 grams of basic PDA powder was dissolved in 1000 ml of distilled water in 2 liters flask ,heated on water bath at 100 °C , to be completely dissolved and homogeneous and then autoclaved at 121 °C for 15 minutes at 1 bar pressure . The mixture was then divided into two parts. The first part was poured in Petri dishes (with 9 cm diameter) left to cool until became jelly. These Petri dishes will be later used in the experiment . The second part of the mixture was poured in 500 ml necked conical flasks and was kept in a refrigerator to be used later (Fig. 3.7) .

3.6- Preparation of wood block baits

The sterilized blocks were then transferred and distributed over necked conical flasks that contain nutrient media (PDA) . The media were colonized by wood decay fungi, which were intended to be used in this study (Fig. 3.8). With blocks set flush on the surface of the culture, the flasks were incubated in a dark place at room temperature (20-27 °C) for five period times (2, 3, 4, 5 and 6 months) ,that for accuracy . Each tree species blocks were kept with one fungus type. Then after each determined period , each formerly- weighed block was oven-dried at 100°C for five hours , and then was re-weighed to find out the weight loss rate. The weight loss (WL) , during the experiment, was expressed as a percentage of the initial relative density of the wood sample according to the formula :

$$\% WLg = (\frac{Initial\,,density - final\,,relative\,,density}{initial\,,relative\,,density}) \times 100$$

3.6.1 - Preparation of wood block inocula

All formerly prepared blocks, for all species from *P. halepensis* , *C. sempervirens* , *P. atalantica* , *C . siliqua* and *E . macroryncha* after being weighed (initial relative density) and autoclaved at 121 °C for two hours were then kept in necked conical flasks. The 500 ml flasks were prepared with (PDA) culture media that was colonized by the targeted fungi . Each fungus was being individually isolated in a flask ,and incubated at room temperature 20 °C , and kept in dark conditions for two weeks . Each individual blocks were set on a flush to the surface of the culture in flasks . These flasks and their contents were incubated in dark place at 20°C for five periods (2, 3, 4, 5 and 6 months) .

3.6.2 - Incubation Periods

Two months since the flasks were incubated , the treated blocks with the four used fungi were removed and their surfaces were scraped free of mycelium and nutrient medium by using lancet (Fig. 3.9) , oven-dried at 100 °C for five hours, then re-weighted (final relative density) , that to ascertain the begin of decay process ,and to determine the loss rate . Next period were persisted for three months , in which the blocks were removed , scraped ,oven-dried at 100°C for five hours and re-weighted to find out the weight loss rate . In a third period Using the same previously stated procedures , the blocks were dried , re-weighted. This period was done after four months of incubation . And in this period , the scraping , drying at 100 °C and re-weighting processes were made after five months of incubation . The last period of incubation lasted for six months(the fifth period) . All blocks were removed from the flasks , and have their surfaces scraped carefully, that not to be crumbled in fingers , oven –dried at 100°C and re-weighed again that to ; explore the continuity or discontinuity of the decay process , at which fungi species this period stopped and determine whether there was an increase in the rate of weight loss or not (Rasmina *et a*. , 2012) .

After the period of this test were done all the weights were recorded and the loss rates were calculated , after each fungus treat with the tree specimens. where the lowest and the highest weight loss was determined for each fungus treat . Then after a

comparison of the rate of weight loss was made between the four fungi species that treated with the same tree species after each period of incubation intervals . This comparison determined which fungus species that had the highest effect in the decay process on the individual tree species , and compared it with the other fungi . To decide which tree species that had the best natural durability property when exposure to these fungi , the rates of weight loss among the tree specimens were compared , and compared it with the other plant species under study . In the same time , the rates of weight loss among the tree specimens were compared that to decide which tree species that had the best resistance property or sensitive to specified test , when exposure to these fungi .

3.7- The preparation of isolates fungus test

The fungi species used in this experiment were randomly isolated from infected parts on the same used trees species , (deadwood) , (Fig. 3.10). Whereas , wood from these infected parts and was fragmented to small pieces , transferred to surface-sterilized prepared media in petri dishes . These isolates were then incubated on PDA culture at (20-27°C) for a tow weeks , that to allow the fungi to grow and colonize the medium on the Petri dishes . However , to gain the pure culture serial transfer technique was established (Rina and Rajput , 2015) . Each pure fungus species was transferred , Using a cork porar instrument (small disks taken from the pure culture colonized by tested fungi), to a 500 ml necked conical flask contains a sterilized PDA , and sealed with cotton (Fig. 3.11) . 20 flasks for each sample within the individual fungi species used . These flasks were later used in the experiment , where in which the previously sterilized and prepared different tree blocks were incubated . (i.e. the blocks from *P . halepensis* were placed with fungi *S . hirsutum* , fungi *I . hispidus* , fungi *H . fasciculare* and fungi *A. polytricha* separately , and so on with other sample blocks) . Afterward they were incubated between 20-27°C and a relative humidity between 65 and 75%, for five interval periods .

3.8- The statistical Analysis for the whole values

MS Excel 2010 was primarily used for two main statistical goals ; to estimate the mean values for samples weight during incubation periods ,that to make sure the

presence of differences in weight loss rates among the tree species used ,and to determine the standard deviation which guarantees these mean values. On the other hand , SPSS was used to find out the contrast between mean values and to make a comparison among the mean average of weight loss for those utilized tree species and treated with the employed fungi species in the study.
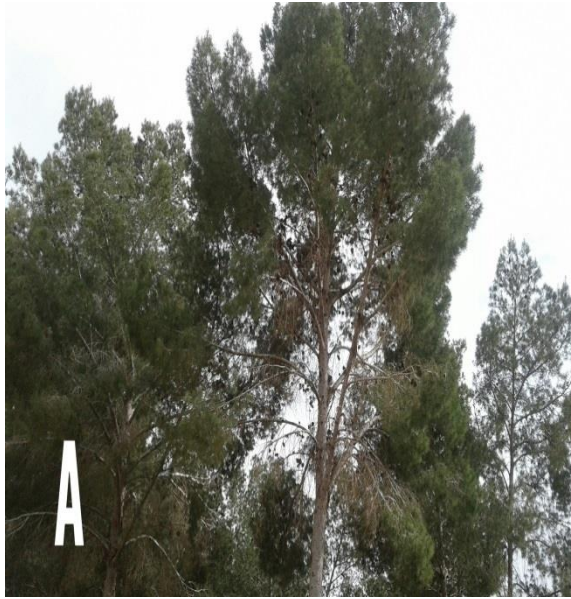
(Figure 3.1) Show the five different forest trees that widespread in study area.
A-*Pinus halepensis* .
B-*Cupressus sempervirens* .
C-*Pistacia atlantica* .
D- *Ceratonia siliqua* .
E-*Eucalyptus macroryncha* .
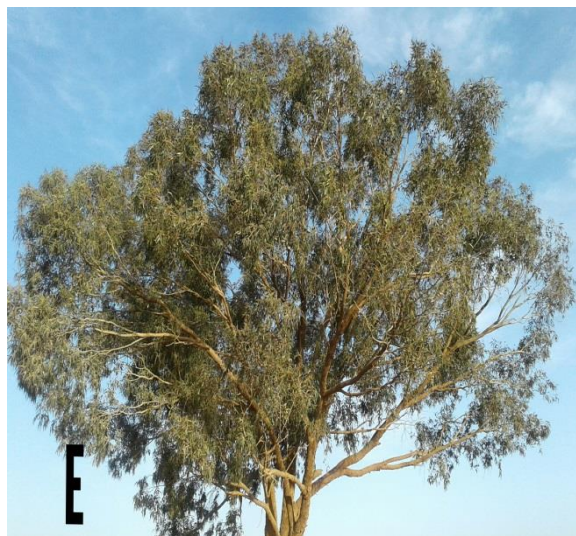
(Figure 3.2) Gummy preservative material that is used to wrap grafting sites on trees to prevent them from contamination .

(Figure 3.3) Shows wrap grafting sites on trees to prevent them from contamination by using gummy preservative material ,1- *P. halepensis* , 2- *C. sempervirens* , 3 - *P. atlantica* , 4 – *C . siliqua* and 5- *E . macroryncha* .

(Figure 3.4) The samples were kept in well-sealed black bags at room temperature (20-30°C) and stored in the lab for 8 weeks , to reduce the moisture content   after weighted and organized in groups .
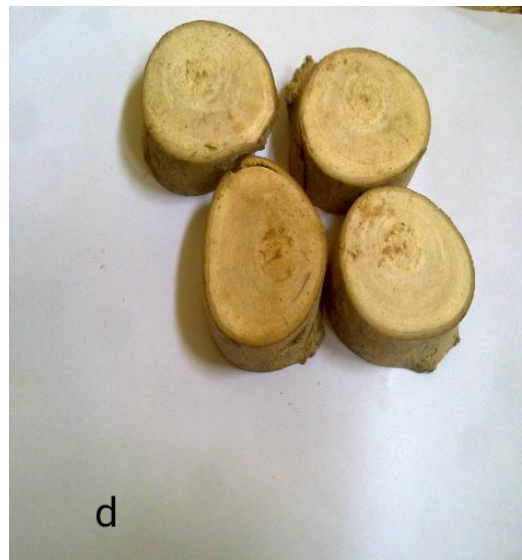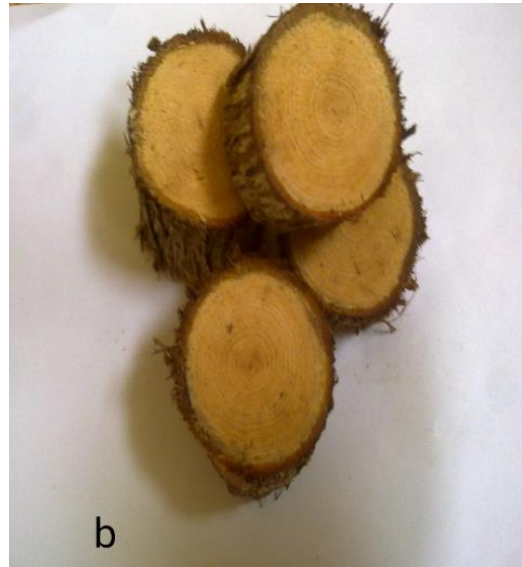
(Figure 3.5) Shows cutting discs
from five different trees ;
a - *P. halepensis* .
b - *C. sempervirens* .
c - *C. siliqua* .
d - *E. macroryncha* .
e - *P. atlantica*.

(Figure 3.6) Prepared wood blocks with diameter (2x1.5x1.5cm3) 20 mm x 15 mm x 1.5 mm , in the longitudinal directions (L) ,radial (R) and tangential (T) .

(Figure 3.7) Conical flasks contain a sterilized PDA before inoculation of isolated wood – decay fungi .
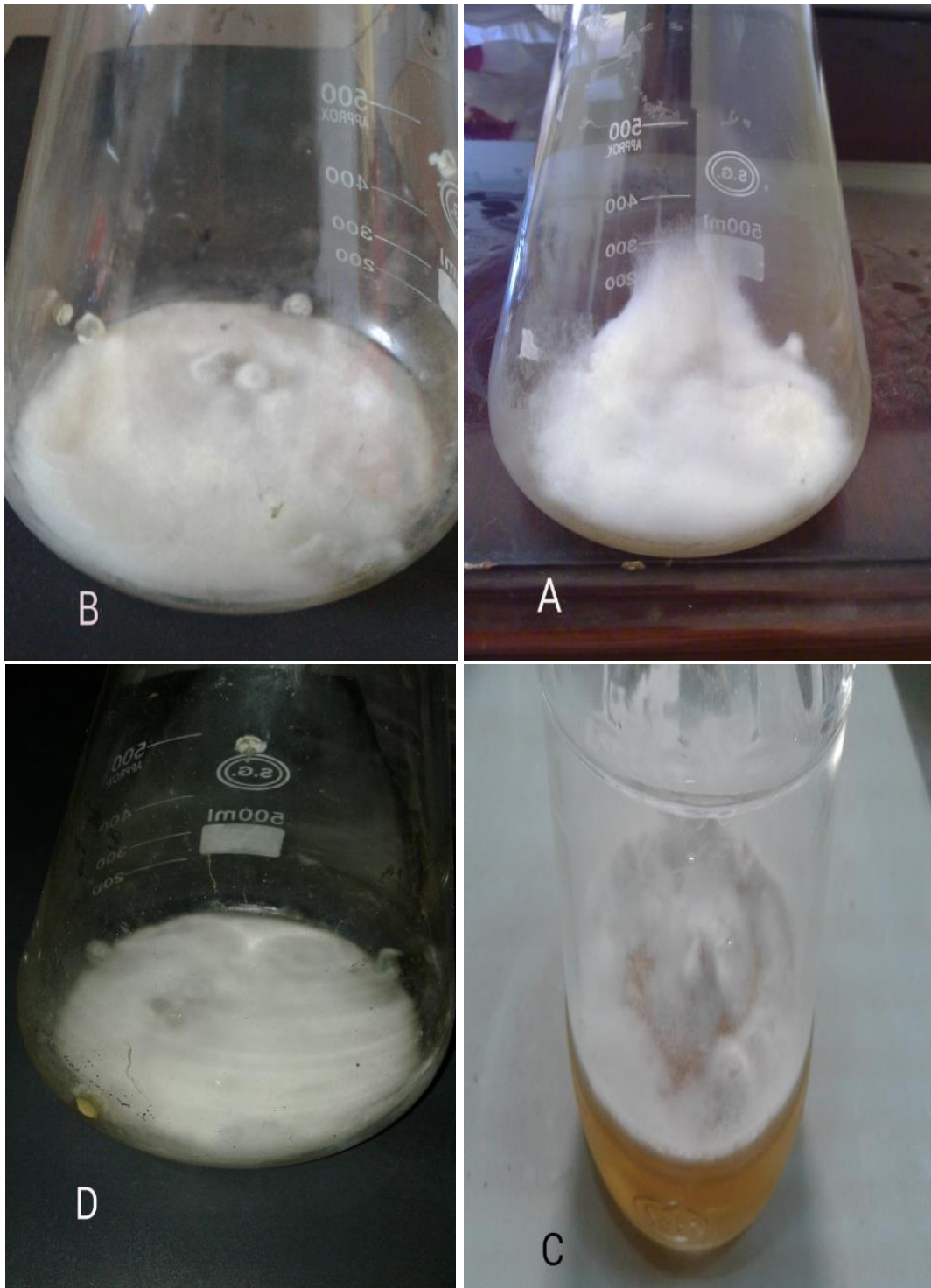
(Figure 3.8) Shows the media in conical flask were colonized by the four tested wood decay fungi: A- *Stereum hirsutum* , B- *Inonotus hispidus* , C- *Hypholoma fasciculare* and D- *Auricularia polytricha* .

(Figure 3.9) The treated blocks were removed and their surfaces were scraped free of mycelium and nutrient medium by using lancet .

(Figure 3.10) Shows the infected parts and the frouting bodies on the forest trees before isolation and using in experment ; A- *Stereum hirsutum* , B- *Inonotus hispidus* , C- *Hypholoma fasciculare* and D- *Auricularia polytricha* .
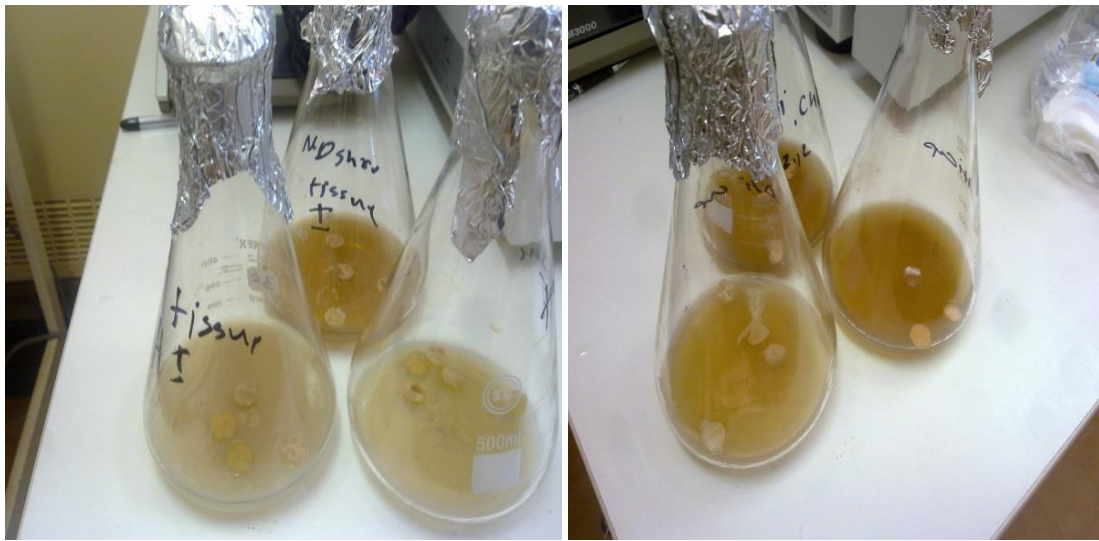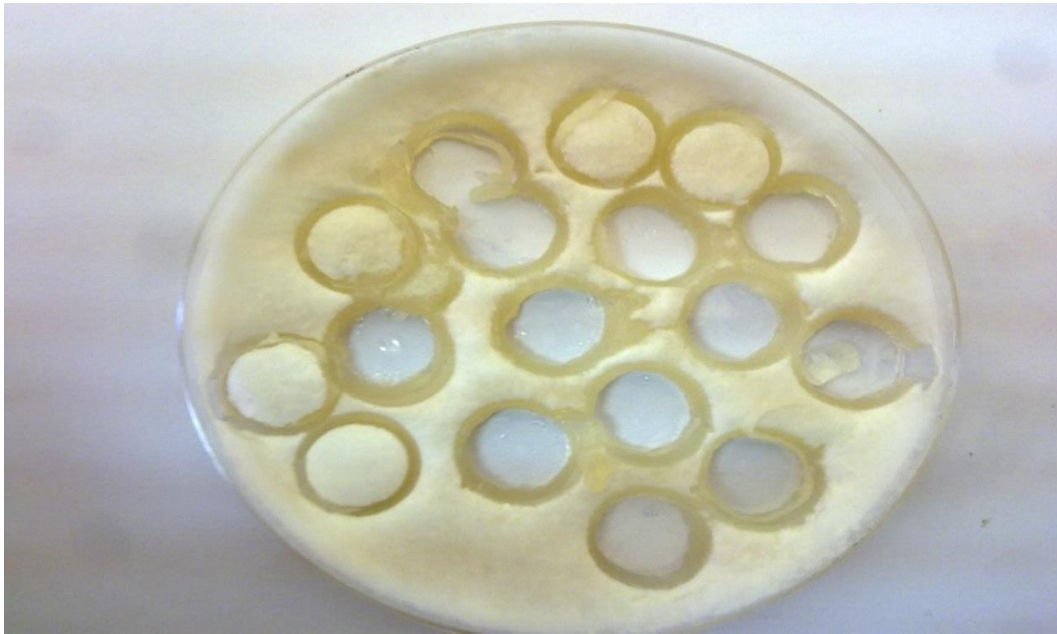
(Figure 3.11) Transfer the pure fungus species by using a cork porar instrument , to a necked conical flask .

Chapter Four

Results

4.1-The results of the presence of endophayta in the wooden samples

The results gained from the tests on the wood branches of *P. halepensis* , *C . siliqua C . sempervirens* , *E . macroryncha* and *P . atlantica* showed no presence of the endophaytic fungi within the wood tissue for these plants . worth mentioning that the test was run frequently that to make sure for the absence or presence of the endophayta . Because the presence of these fungi causes the consuming of the nutrients within the wood tissues such as cellulose , hemicellulose , lignin and pectin , which are the basic components of the wood tissues . These basic components are most consumed by the wood decay fungi used in this study . For this reason , the wood samples should be clear of the endophayta (Rodriguez *et al .* , 2008) .

4.2- Identification of isolated wood-decay fungi from forest trees

Four types of wood inhabiting fungi were isolated from dead wood of the same tested trees species that predominant in the study area. While , cultured on PDA media in petri dishes and sent to Assiut University Mycological Center , Egypt (AUMC). for identification . Whereas, the results of identification confirmed that the all fungus test were the saprophytic fungus. These pure fungus are 1- *Stereum hirsutum* (Willd. ex. Fr.) , 2- *Inonotus hispidus* (Bull.) P. Karst , 3- *Hypholoma fasciculare* (Huds . ex .Fr.) P.Kumm and 4- *Auricularia polytricha* sacc .(mont) , Subsequently (Fig. 4.1).

4.3 - The results of wood blocks decaying in , vitro

After keeping the studied wood blocks in the prepared flasks with PDA and the invasive fungi, the decay process started . Within the first two weeks, the hyphae start growing , ramify on the blocks that were originally kept on the surface of mycelium . After a month, the fungi colonized the whole wood blocks . Then the fungi were left to carry on the decay process ,which persisted 2-6 months and over a five- period time. Each period results from exposure of blocks to individual fungi showed clearly differences in the decay rates caused to the wood blocks , for every plant type . The

results demonstrated that for every fungal species there is a weight loss rate differ from a stage to another.

4.4 - Weight Loss Rates

Weight loss collected data from the incubation process for the tree species showed important differences in the ability of the fungal species in the biodegradation process. These data can be used to determine which of the used fungi has the most biodegradation effect on the trees (wood blocks), comparable to other species. Furthermore , to find out to what extend the tree species can be effected by the those fungi . Consequently, determining the tree that has the most resistance and durability when they are being affected by the used fungi , and compare them with other trees being tested in the study, on the other hand . The results were seen since the first incubation period (after 2 months). Where the signs appeared on the cubes for all the tree species that treated with the fungal strains used . To evaluate the percentage of weight loss rates an empirical formulae was used as follow ;

$$\% Mass.loss(g) = \frac{initial\,,weight - final\,,weight}{initial\,,weight} \times 100$$

4.4.1 -Weight Loss Rate Percentage in *Pinus halepensis*

As mentioned in (Table 4.1), the data collected during five periods of incubation, and tabulated. These data illustrate the main and principle differences in the weight loss rate of *Pinus halepensis* blocks when were treated by the fungi species on used in the study.

1-After two months of incubation ,the results showed that the fungus *S.hirsutum* gave the highest weight loss rate which was 1.34% , in the time the fungus *I. hispidus* caused only 0.36% of weight loss . While the fungi *H . fasciculare* and *A.polytricha* produced weight loss in the rate of 0.65% , 0.19% respectively (Fig. 4.2) .

2- While the incubation process was in progress there was an increase in the decay rate for the fungal species . In this stage the fungus *S. hirsutum* still the dominant within the other fungi . It produced 6.76% of weight loss, while the result was 4.48%

for the fungus *I. hispidus* , which was less than 4.52% for the fungus *H . fasciculare*. in the last order was the fungus *A. polytricha* , as usual .

3- The same order as in the previous period with the continuity in decay process. The results after four months showed that the fungi *S. hirsutum* , *I. hispidus* , *H.fasciculare* and *A. polytricha* produced 13.77% , 11.03% , 11.32 and 5.34% respectively of weight loss .

4-After five months of incubation, the results showed that the fungi *S. hirsutum* still have their activity and caused weight loss percentage with different rates. The highest percentage of weight loss was 20.94% , while the fungus *I. hispidus* produced only 15.97% and in the third order was the fungus *H . fasciculare* which caused 17.14% , whereas the fungus *A . polytricha* caused only 8.16% of weight loss.

5- With end the periods of incubation . The results showed that the fungus *S.hirsutum* was the most active fungus among the invasive fungi for this species of plants . It occurred 27.29% of weight loss . Secondly came the fungus *H . fasciculare* which produced 23.29% , then the fungus *I. hispidus* with weight loss rate of 21.98%, and finally came the fungus *A.polytricha* that was less activity in decaying the blocks whereas caused just only 12.16% (Fig. 4.3) .

By considering the mean average. and the standard deviation for the weight loss rates for the wood blocks of *Pinus halepensis* that being treated by the fungi used in the study ,the results showed that the average for the fungus *S. hirsutum* was 14.02 at SD of 10.45. This explains that this fungus is the most active one, in the decay process, comparable to the other fungi. While the average for the fungus *I . hispidus* was 10.76 at SD of 8.67, and for the fungus *H . fasciculare* was 11.38 at SD9.18. the weaker in the decay process was the fungus *A . polytricha* with average of 5.52 and SD of 4.83 . (Table 4.2) .

(Table 4.1) Percentage weight loss (g) of *Pinus halepensis* wood blocks (2x1.5x1.5cm) for five intervals after inoculated with 4 wood decay fungi .

Tested fungi	Percentage weight loss (g)% after five time intervals				
	After 2 Months	After 3 Months	After 4 Months	After 5 Months	After 6 Months
S. hirsutum	1.34	6.76	13.77	20.94	27.29
I. hispidus	0.36	4.48	11.03	15.97	21.98
H . fasciculare	0.65	4.52	11.32	17.14	23.29
A. polytricha	0.19	1.79	5.34	8.16	12.16

(Table 4.2) Mean weight loss (g) , STDEV of percentage weight loss of *Pinus halepensis* wood decayed for five time intervals of inoculation .

Tested fungi	percentage weight loss% (g)	MEAN	ST DEV
S. hirsutum	1.34 -27.29	14.02	10.45863
I. hispidus	0.36-21.98	10.764	8.670405
H . fasciculare	0.65-23.29	11.384	9.181799
A .polytricha	0.19-12.16	5.528	4.832522

4.4.2 -Weight Loss Rate Percentage in *Cupressus sempervirens*

It can be seen that from the outcomes listed in (Table 4.3) there are differences in the weight loss percentages for *Cupressus sempervirens* incubated and experienced the effect of the used fungi. Also it is noted that as the incubation continued the decay rate persisted, as explained below:

1- The evidences collected from this period of incubation demonstrated that the fungus *S.hirsutum* caused 0.29% of weight loss, while the fungus , in its starting activity *I. hispidus* caused 2.02% of losing the weight, which is a higher percentage compared to the other rates. Where the fungi *H . fasciculare* and *A. polytricha* produced 0.36% and 0.15% respectively (Fig. 4.4) .

2-Incubation the wood blocks for three months with the used fungi caused the fungus *S.hirsutum* to give 3.41% of weight loss ,and the fungus *I. hispidus* to give 6.85% . in the middle was the fungus *H . fasciculare* which produced 4.29% of weight loss, and lastly the fungus *A. polytricha* causing 2.14% of weight loss.

3- Listing the weight loss percentages from the highest, we can see that the fungus *I.hispidus* takes the first place producing 10.13% , then the fungus *S.hirsutum* giving 9.52% then the fungus *H . fasciculare* 7.69% of weight loss. Whereas the fungus *A.polytricha* takes the last position and produced only 5.9% of losing the weight for the blocks .

4-Keeping the blocks of *Cupressus sempervirens* incubated for five months gives the support to the idea that the fungus *I. hispidus* still in the first order and it gives 16.96% of weight loss . A tiny difference between what produced from the fungus *S. hirsutum* and the fungus *H . fasciculare*, where the first caused 14.89% and the other caused 14.38% of weight loss. Among these fungi the fungus *A. polytricha* took the last order and gave only 8.64% of weight loss.

5- In this stage the blocks were incubated with the used fungi for six months. The results illustrate that the fungus *S. hirsutum* produced 21.79% and the fungus *I.hispidus* 22.83% ,and the fungus *H . fasciculare* 20.52% of weight loss. While the fungus *A.polytricha* ,which was less active, produced only 9.37% (Fig. 4.5) .

As can be seen from the (Table 4.4) the averages for the weight loss rates show that the fungus *S. hirsutum* has the mean average of 9.98 at SD of 8.67, while the average for the fungus *I. hispidus* is 11.73 at SD 8.19(this fungus gave the highest rate of wood decay, as previously mentioned). Furthermore, the average of the fungus *H.fasciculare* is 9.44 at standard deviation of 8.04 , and that for the fungus *A.polytricha* is 5.25 at SD of 4.017, which is the lowest among the fungal group .

(Table 4.3) Percentage weight loss (g) of *Cupressus sempervirens* wood blocks (2x1.5x1.5cm) for five intervals after inoculated with 4 wood decay fungi .

Tested fungi	Percentage weight loss (g)% after five time intervals				
	After 2 Months	After 3 Months	After 4 Months	After 5 Months	After 6 Months
S. hirsutum	0.29	3.41	9.52	14.89	21.79
I. hispidus	2.02	6.85	10.13	16.96	22.83
H . fasciculare	0.36	4.29	7.69	14.38	20.52
A. polytricha	0.15	2.14	5.9	8.64	9.37

(Table 4.4) Mean weight loss (g) , STDEV of percentage weight loss of *Cupressus sempervirens* wood decayed for five time intervals of inoculation .

Test fungus	percentage weight loss (g)%	MEAN	ST DEV
S. hirsutum	0.29-21.79	9.98	8.672612
I. hispidus	2.02-22.83	11.732	8.190731
H . fasciculare	0.36-20.52	9.448	8.049861
A. polytricha	0.15-12.37	5.24	4.017992

4.4.3 - Weight Loss Rate Percentage in *Pistacia atlantica*

Table (4.5) shows the differences in weight loss percentage for the blocks being treated with the fungi for five period. And these difference are listed as follow :

1- By the end of this period, the results showed that fungus *S. hirsutum* produced 2.16% of weight loss , whereas the fungus *I. hispidus* was the most dominant where it produced 2.93% of weight loss . On the other hand, the fungus *H . fasciculare* caused only 1.98% of weight loss , and the fungus *A. polytricha* was the weaker one among the used fungi and caused only 0.63% of weight loss, in this period of incubation , (that is continue to 2 months) (Fig. 4.6) .

2- Still in stage the fungus *I. hispidus* is the most dominant and produced 10.81% of weight loss , while the fungus *S. hirsutum* was in the second order and produced 8.92% of weight loss. What was noted is that the activity of the fungus *H. fasciculare* had increased , and it became more active , comparable to the first period , causing 11.99% of weight loss . While the fungus *A. polytricha* still the weaker and produced only 3.93% of weight loss .

3- This stage lasted for four months , and its results clarified the continuity of the fungi in biodegradation process , depending on the weight loss rate. Where the fungus *S. hirsutum* gave 19.57% and the fungus *I. hispidus* gave 23.01% of weight loss . The fungus *H . fasciculare* came in the second order in this period and produced 22.48% of weight loss, while the fungus *A.polytricha* in the last and gave weight loss of the percentage 6.83% .

4-The biodegradation activity of the fungi still in progress . But generally talking the fungus *I. hispidus* remained in the first order and produced the highest weight loss rate 37.87% . In the second place was the fungus *H. fasciculare* which produced 32.46% , and in the third place was the fungus *S. hirsutum* causing 30.01% of weight loss . While the fungus *A. polytricha* produced only 12.72% of weight loss rate .

5- This was the last period , (6 months of incubation) at which the incubation process ended . The results of this stage illustrated that the fungus *S. hirsutum* continued in its biodegradation action and hence the weight loss rate increased by 44.79% , and the fungus *I. hispidus* which was the most active one produced the highest weight loss rate with 48.55% .While the fungus *H . fasciculare* gave 39.12% , the fungus *A.polytricha* caused only 20.64% of weight loss (Fig. 4.7) .

The mean average and standard deviation calculations of the weight loss rate for *Pistacia atlantica* blocks being treated by the fungi used in this study showed that the mean average for the fungus *I. hispidus* was 24.63 at SD of 18.78, which indicates that this fungus was the most active in the biodegradation process among the fungi used in the study . While the mean average for the fungus *A. polytricha* was 8.95 at SD of 7.90 which represents the decline in its biodegradation action . In the meanwhile, the mean average of the fungus *S. hirsutum* was 21.08 and the standard deviation was 16.95, and they are 21.60 and 15.02 for the fungus *H. fasciculare* (Table 4.6) .

(Table 4.5) Percentage weight loss (g) of *Pistacia atlantica* wood blocks (2x1.5x1.5cm) for five intervals after inoculated with 4 wood decay fungi .

Tested fungi	Percentage weight loss (g)% after five time intervals				
	After 2 Months	After 3 Months	After 4 Months	After 5 Months	After 6 Months
S. hirsutum	2.16	8.92	19.57	30 .01	44.79
I. hispidus	2.93	10.81	23.01	37.87	48.55
H . fasciculare	1.98	11 .99	22.48	32.46	39.12
A. polytricha	0.63	3.93	6.83	12.72	20.64

(Table 4.6) Mean weight loss (g) , STDEV of percentage weight loss of *Pistacia atlantica* wood decayed for five time intervals of inoculation .

Tested fungi	percentage weight loss (g)%	MEAN	ST DEV
S. hirsutum	2.16 - 44.79	21.088	16.95466
I. hispidus	2.93 - 48.55	24.634	18.7881
H . fasciculare	1.98 - 39.12	21.608	15.02646
A. polytricha	0.63-20.64	8.95	7.902313

4.4.4 -Weight Loss Rate Percentage in *Ceratonia siliqua*

The data mentioned in (Table 4.7) show the main differences within the fungi used in the study when they attack *Ceratonia siliqua* blocks . The differences are listed below according to the periods of incubation .

1-Incubating the blocks for two months caused them to be decayed and lost their weight with different rates . Hence , the fungus *S. hirsutum* used caused a weight loss of the percentage of 4.7% , whereas the fungus *I. hispidus* caused more weight loss with the rate of 6.38% . On the other hand , the fungus *H . fasciculare* showed more unusual activity and caused 12.44% of weight loss , which is the highest . Lastly the fungus *A. polytricha* was the lowest in activity and caused a decay rate of 2.63% (Fig. 4.8) .

2- This interval of incubation resulted in wood decay rate with different percentages. So, the fungus *S. hirsutum* produced 13.7% of weight loss, and the fungus *I. hispidus* caused 11.21% of *Ceratonia siliqua* to be decayed . While the fungus *H. fasciculare* which was the most active fungus among affected the wood cubes, produced 25.55% of weight loss . But the fungus *A. polytricha* caused only 5.61% .

3-It is seen that as the incubation period prolonged , the decay rate increases . This is obviously noted where the fungus *S. hirsutum* caused 24.42% of weight loss, and the fungus *I. hispidus* caused 21.38% . Also the activity of the fungus *H. fasciculare* increased , and it caused 30.86% of losing the wood blocks their weight . However, the activity of the fungus *A. polytricha* increased , it caused *Ceratonia siliqua* to loss 19.04% of its weight .

4- The data collected from this stage showed that the decay rate the fungi *S. hirsutum* and *I. hispidus* were produced 29.33%, 32.97% respectively, while fungu*s H.fasciculare* caused higher decayed in blocks , whereas gave 41.37% its high weight loss and *A. polytricha* fungi that gave 26.01% of weight loss .

5-By the end of this period the weight loss rates were as follow : the fungus *S.hirsutum* 34.33% , the fungus *I. hispidus* 43.98% , the fungus *H. fasciculare* 49.5% and the fungus *A. polytricha* caused 29.19% of weight loss (Fig. 4.9) .

By calculating the standard deviation and the mean averages for the percentages of weight loss rates of *Ceratonia siliqua* blocks , (Table 4.8) it is clear that the mean average for the fungus *S. hirsutum* is 21.29 at SD of 12.01, and for the fungus *I.hispidus* is 23.18 at SD of 15.469 . While the average for the fungus *H. fasciculare* , who gave the highest weight loss rate , is 31.94 at SD of 14.31, whilst the mean for the fungus *A. polytricha* is 16.91 at SD of 11.48, which is the lowest among this group.

(Table 4.7) Percentage weight loss (g) of *Ceratonia siliqua* wood blocks (2x1.5x1.5cm) for five intervals after inoculated with 4 wood decay fungi .

Tested fungi	Percentage weight loss (g)% after five time intervals				
	After 2 Months	After 3 Months	After 4 Months	After 5 Months	After 6 Months
S. hirsutum	4.7	13.7	24.42	29.33	34.33
I. hispidus	6.38	11.21	21.38	32.97	43.98
H . fasciculare	12.44	25.55	30.86	41.37	49.5
A.polytricha	2.63	7.61	19.04	26.01	29.19

(Table 4.8) Mean weight loss (g) , STDEV of percentage weight loss of *Ceratonia siliqua* wood decayed for five time intervals of inoculation.

Tested fungi	percentage weight loss (g) %	MEAN	ST DEV
S. hirsutum	4.7-34.33	21.296	12.0137
I. hispidus	6.38-43.98	23.184	15.46969
H . fasciculare	12.44-49.5	31.944	14.31369
A.polytricha	2.63-29.19	16.914	11.4897

4.4.5 -Weight Loss Rate Percentage in *Eucalyptus macroryncha*

The wood decay-activity test, for the four fungal species that were used in the study with blocks of *Eucalyptus macroryncha* , showed apparent differences in the weight loss rates all over the incubation periods, as noted on the (Table 4.9) .

1-The results of this period showed that the fungus *S. hirsutum* was the most active one and produced 2.16% of weight loss , while the fungus *I. hispidus* was less active and produced only 0.67% . in the middle were the other two fungi *H. fasciculare* , and *A.polytricha* with weight loss rates of 1.7% and 1.72% respectively (Fig. 4.10) .

2-By the end of this period , the results demonstrated that the fungus *S. hirsutum* still in the front by causing 7.7% of weight loss rate , and the fungus *I. hispidus* caused 4.12% , which was close to the activity of the fungus *A. polytricha* who produced 4.08% of weight loss . In between the higher and lower active fungi was the fungus *H. fasciculare* who caused *E. macroryncha* cubes to lose 5.44% of their weight .

3-Continuity of incubation caused the decay rate to increase and consequently increasing the weight loss rate . The results explained that the fungus *S. hirsutum* produced the highest weight loss rate , in the range 18.61% , while the fungus *I. hispidus* caused 12.13%. The fungi *H . fasciculare* and *A. polytricha* caused 9.6% and 8.62% of weight loss.

4- By keeping the blocks of *E . macroryncha* incubated for five months , the results showed that the fungus *S. hirsutum* still in the first order and it gives 25.81% of weight loss while the fungus *I. hispidus* 21.67% and the fungus *H . fasciculare* , where caused 13.58% and a tiny difference between what produced from the fungus *A . polytricha* was produced 13.47 % of weight loss .

5-After six months of incubation , the results showed that the fungus *S. hirsutum* was the higher activity in process of wood decay it caused 37.52% of weight loss, and fungus *I.hispidus* caused 29.64% , while that the fungus *A. polytricha* was more active it produced 19.14% . But the fungus *H . fasciculare* was gave the less active in this process it caused only 16.45% (Fig. 4.11) .

From data that noted in (Table 4.10) , by considering the mean average and the standard deviation for the weight loss rates for the wood blocks of *Eucalyptus macroryncha* that being treated by the fungi used in the study , the results showed that the average for the fungus *S . hirsutum* was 18.36 at SD of 14.13 . This explains that this fungus is the most active one , in the decay process , comparable to the other fungi . While the average for the fungus *I. hispidus* was 13.042 at SD of 10.52 , and for the fungus *A. polytricha* was 9.406 at 7.059 SD. The weaker in the decay process was the fungus *H . fasciculare* with average of 9.35 and SD of 5.96 .

(Table 4.9) Percentage weight loss (g) of *Eucalyptus macroryncha* wood blocks (2x1.5x1.5cm) for five intervals after inoculated with 4 wood decay fungi .

Tested fungi	percentage weight loss (g)% after five time intervals				
	After 2 Months	After 3 Months	After 4 Months	After 5 Months	After 6 Months
S. hirsutum	2.16	7.7	18.61	25.81	37.52
I. hispidus	1.65	4.12	12.13	21.67	29.64
H . fasciculare	1.7	5.44	9.6	13.58	16.45
A.polytricha	1.72	4.08	8.62	13.47	19.14

(Table 4.10) Mean weight loss (g) , STDEV of percentage weight loss of *Eucalyptus macroryncha* wood decayed for five time intervals of inoculation .

Test fungus	percentage weight loss (g) %	MEAN	ST DEV
S. hirsutum	2.16-37.52	18.36	14.13105
I . hispidus	1.65-25.64	13.042	10.5281
H . fasciculare	1.7-16.45	9.354	5.961877
A . polytricha	1.72-19.14	9.406	7.059135

(Figure 4.1) Shows the isolation of test fungi in pure culture, identification in (AUMC) clear that A- *S. hirsutum* , B- *I. hispidus* ,   C- *H . fasciculare* and E – *A . polytricha* .
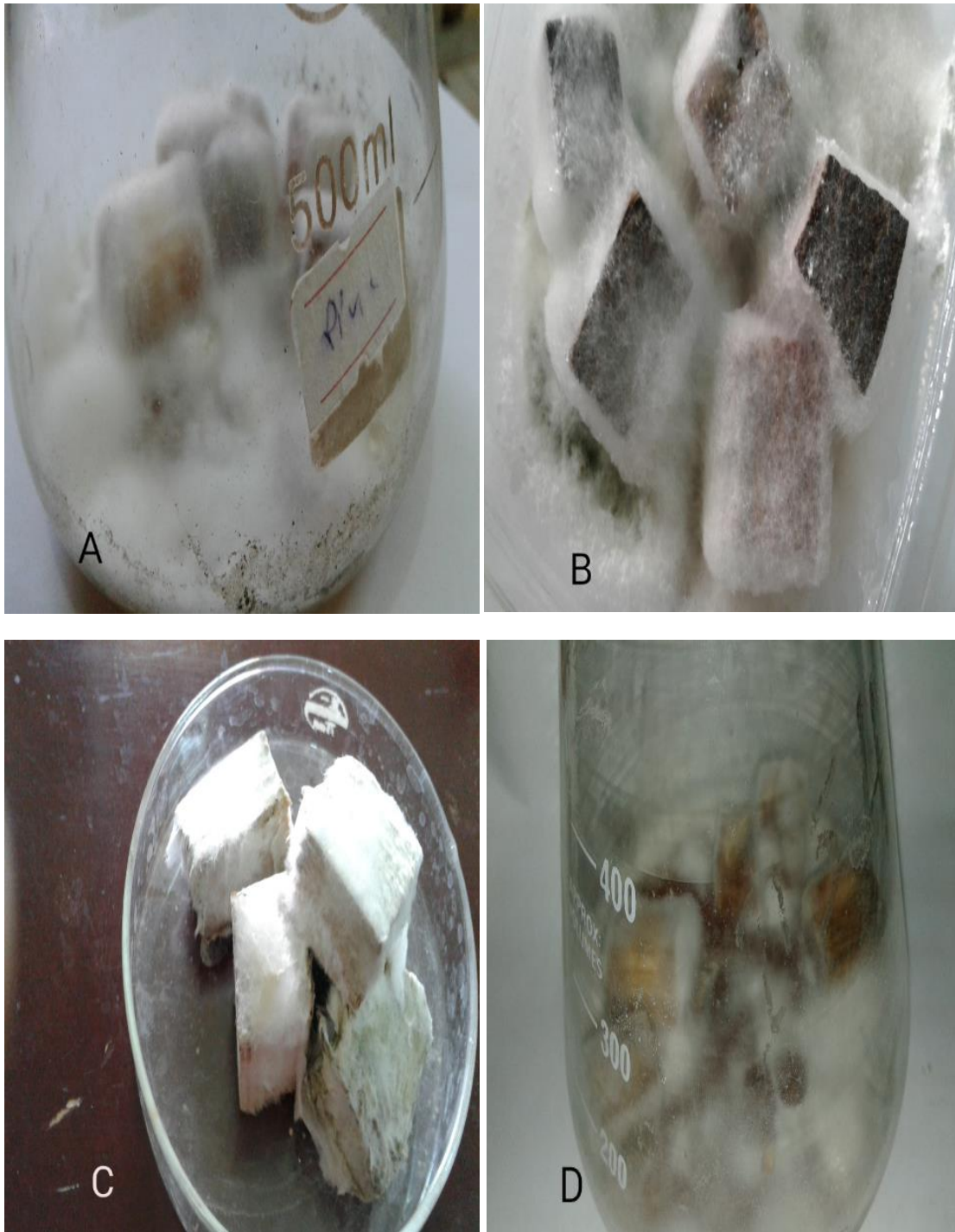
(Figure 4.2) Shows growth of A- *S. hirsutum* , B- *I. hispidus* , C- *H . fasciculare* and D- *A. polytricha* after the first period of incubation 2 months on wood blocks of *P. halepensis* .
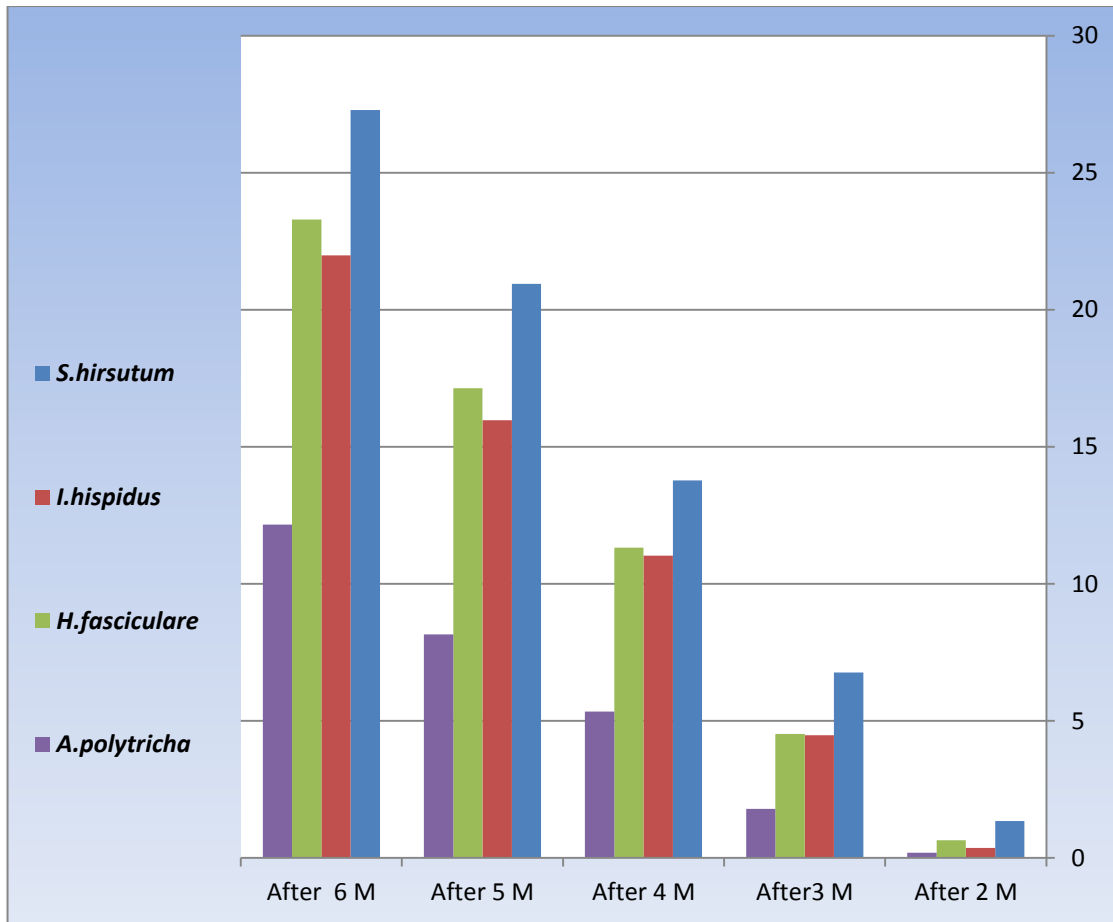
Five time intervals of incubation

(Figure 4.3) Mass loss of *P. halepensis* wood blocks after incubation for 5 period (2,3,4,5and 6 months) with (*S . hirsutum* , *I. hispidus* , *H . fasciculare* and *A . polytricha*) fungi .
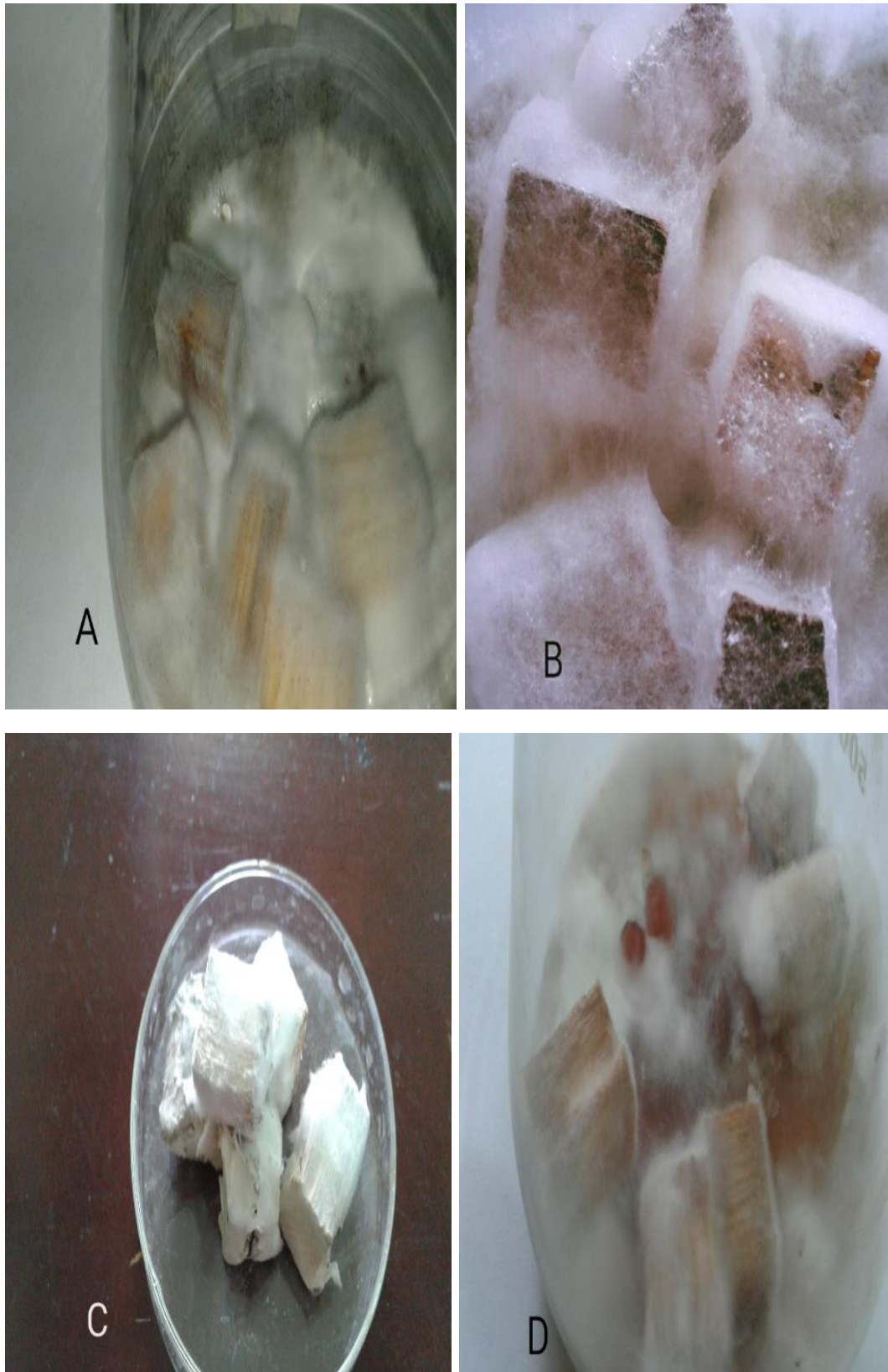
(Figure 4.4) Shows growth of A- *S. hirsutum* , B- *I. hispidus* ,C- *H. fasciculare* and D- *A. polytricha* after the first period of incubation 2 months on wood blocks of *C. sempervirens* .

Five time intervals of incubation

(Figure 4.5) Mass loss of *C. sempervirens* wood blocks after incubation for 5 period (2,3,4,5and 6 months) with (*S . hirsutum* , *I. hispidus* , *H . fasciculare* and *A . polytricha*) fungi .
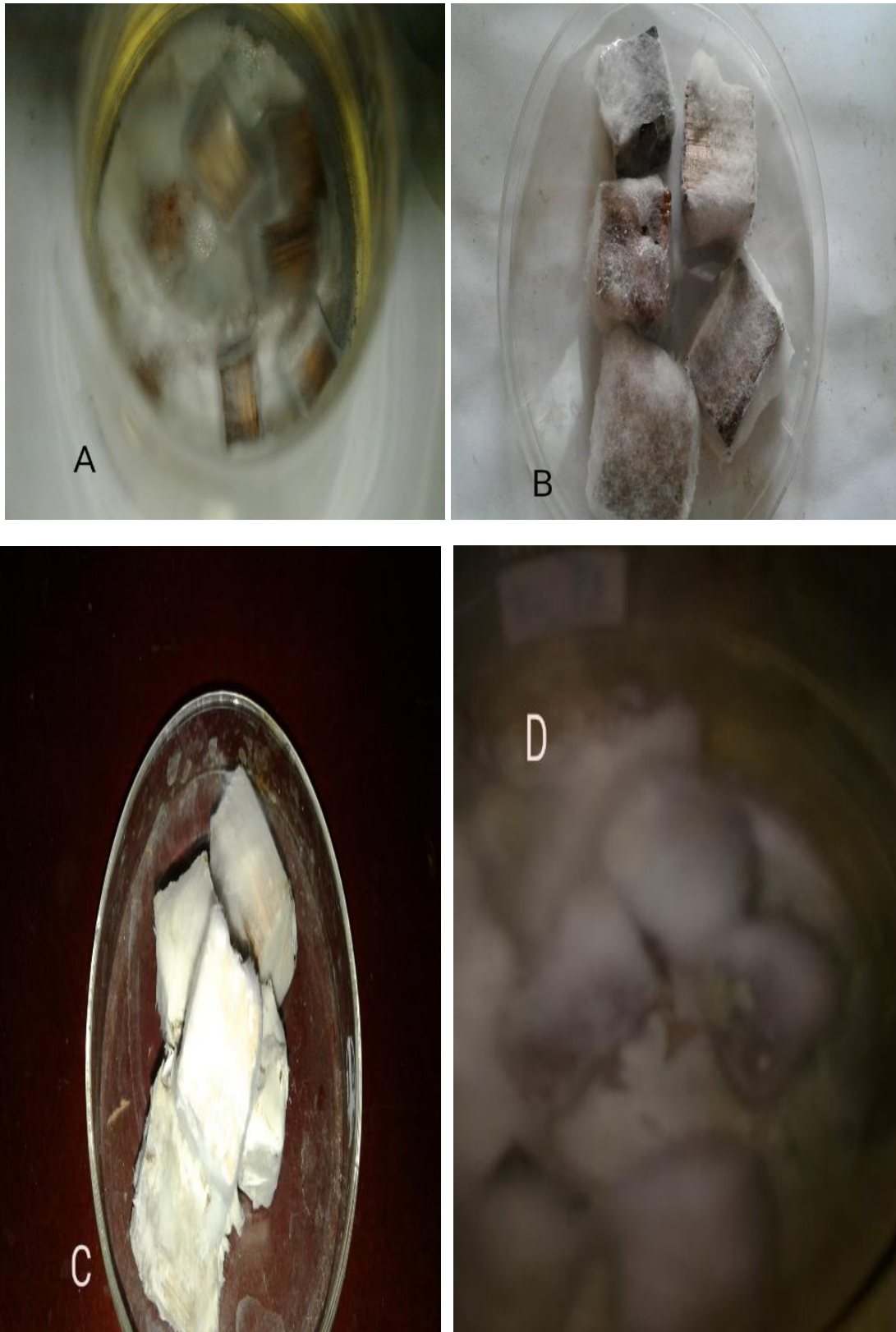
(Figure 4.6) Showing growth of A- *S. hirsutum* , B- *I. hispidus* ,C- *H. fasciculare* , D- *A.polytricha* after the first period of incubation 2 months on wood blocks of *P. atlantica* .
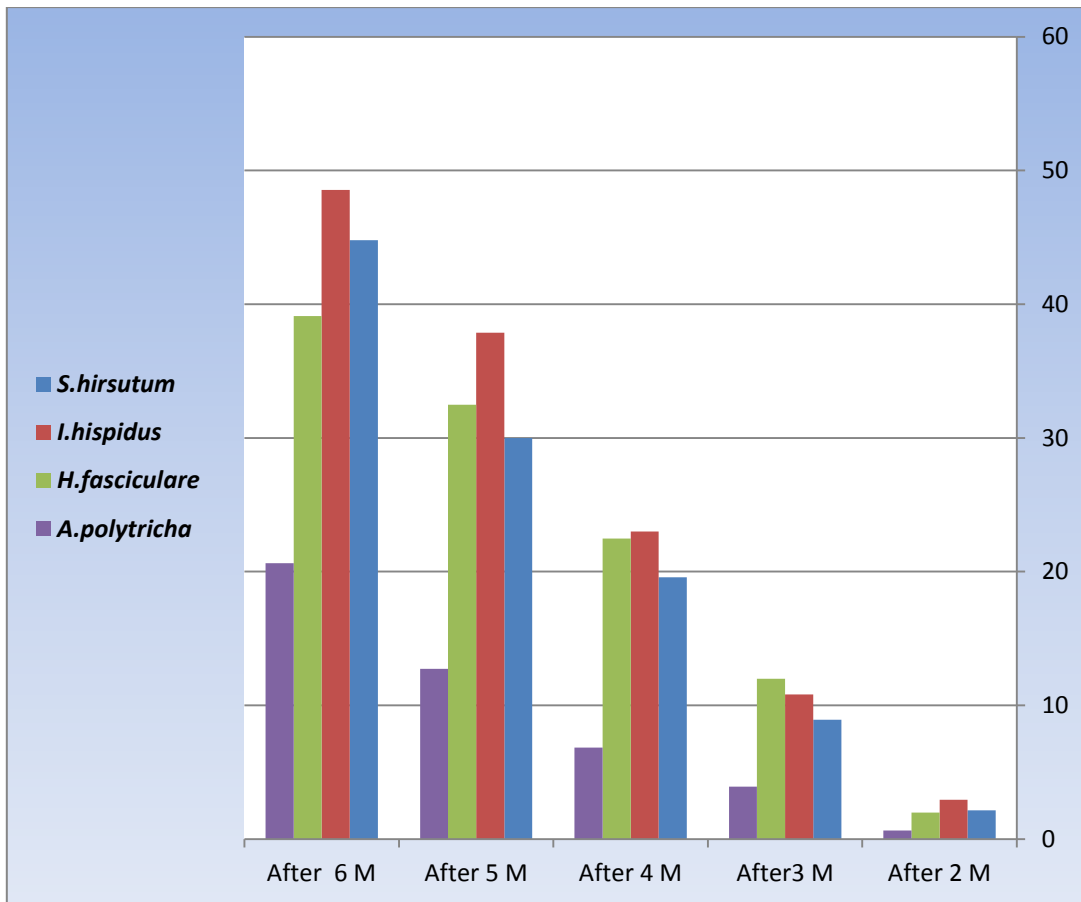
Five time intervals of incubation

(Figure 4.7) Mass loss of *P. atlantica* wood blocks after incubation for 5 period (2,3,4,5and 6 months) with (*S . hirsutum , I . hispidus , H . fasciculare* and *A . polytricha*) fungi .
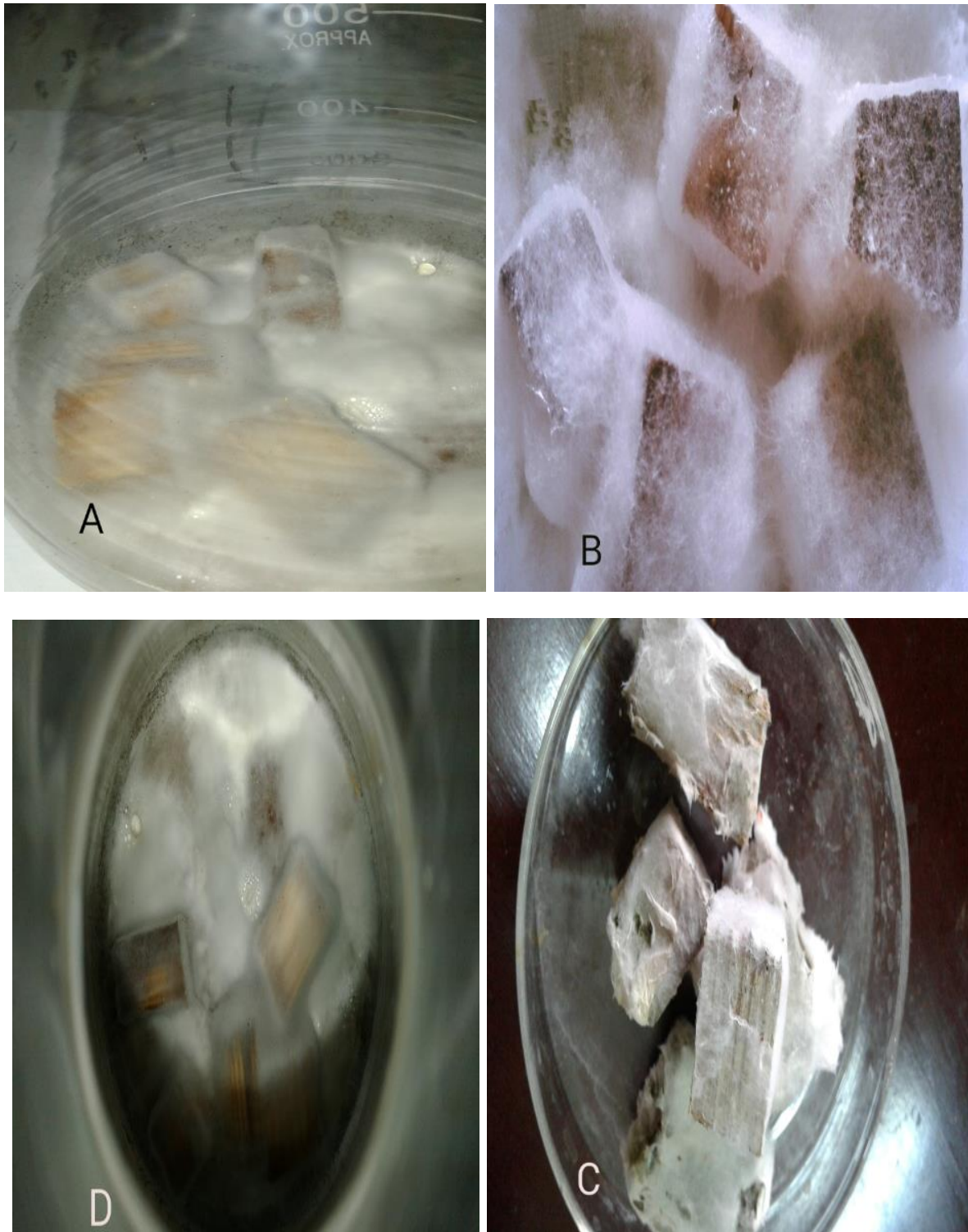
(Figure 4.8) Shows growth of A- *S* . *hirsutum* , B- *I* . *hispidus* , C - *H* . *fasciculare* and
 D- *A* . *polytricha* after the first period of incubation 2 months on wood blocks of
C. siliqua .
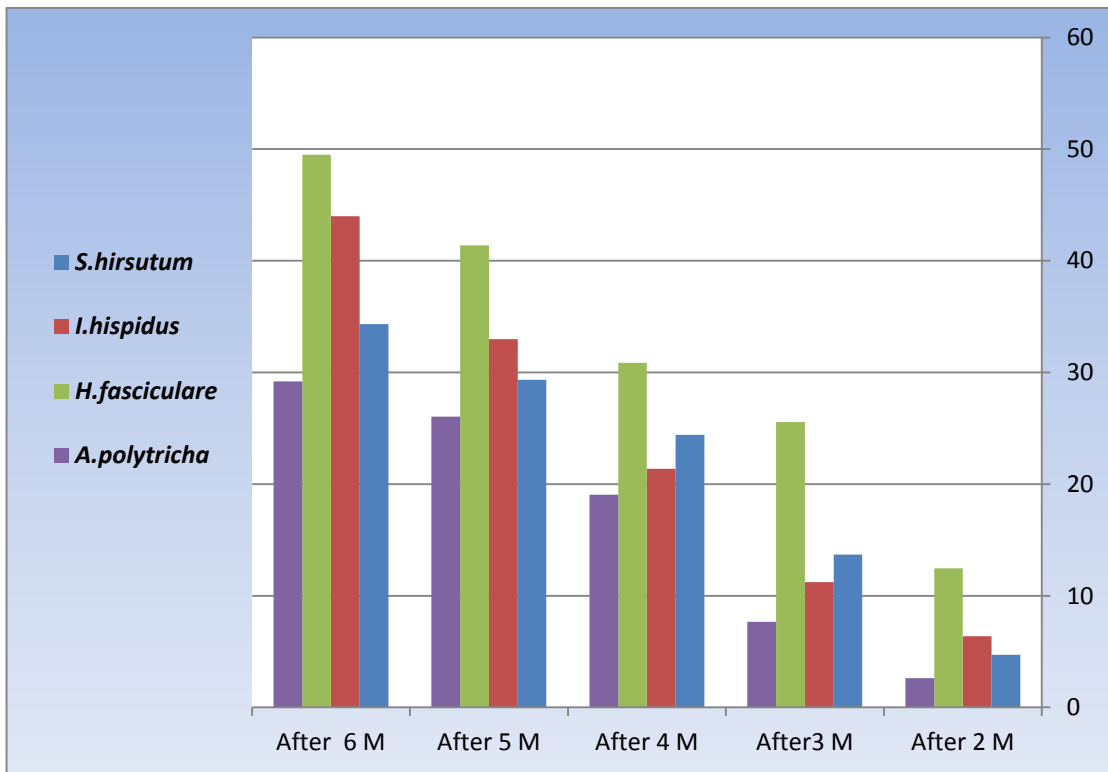
Five time intervals of incubation

(Figure 4.9) Mass loss of *C . siliqua* wood blocks after incubation for 5 period (2,3,4,5and 6 months) with (*S . hirsutum* , *I . hispidus* , *H . fasciculare* and *A . polytricha*) fungi .

(Figure 4.10) Shows growth of A- *S . hirsutum* , B- *I . hispidus* , C - *H . fasciculare* and
D- *A . polytricha* after the first period of incubation 2 months on wood blocks of
 E. macroryncha .
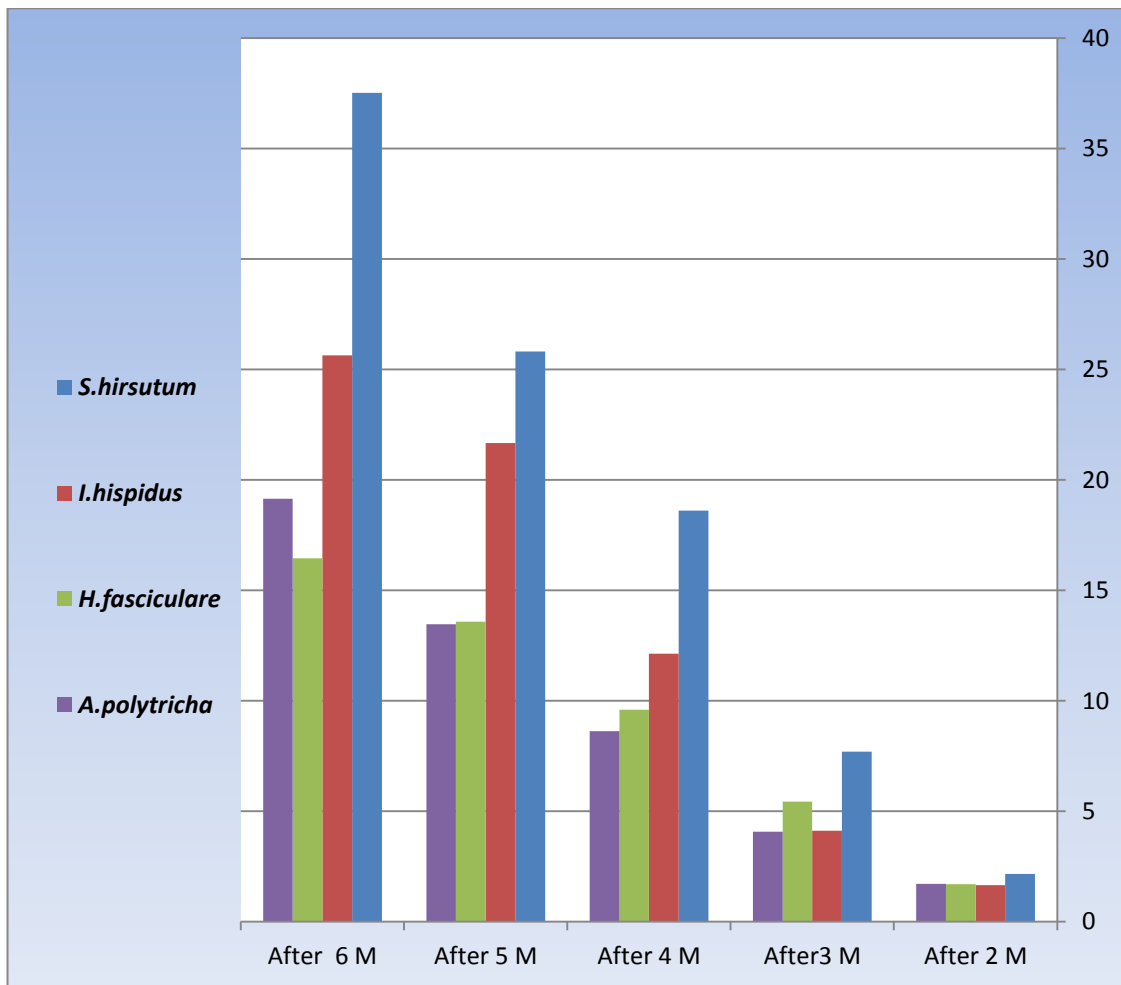
Five time intervals of incubation

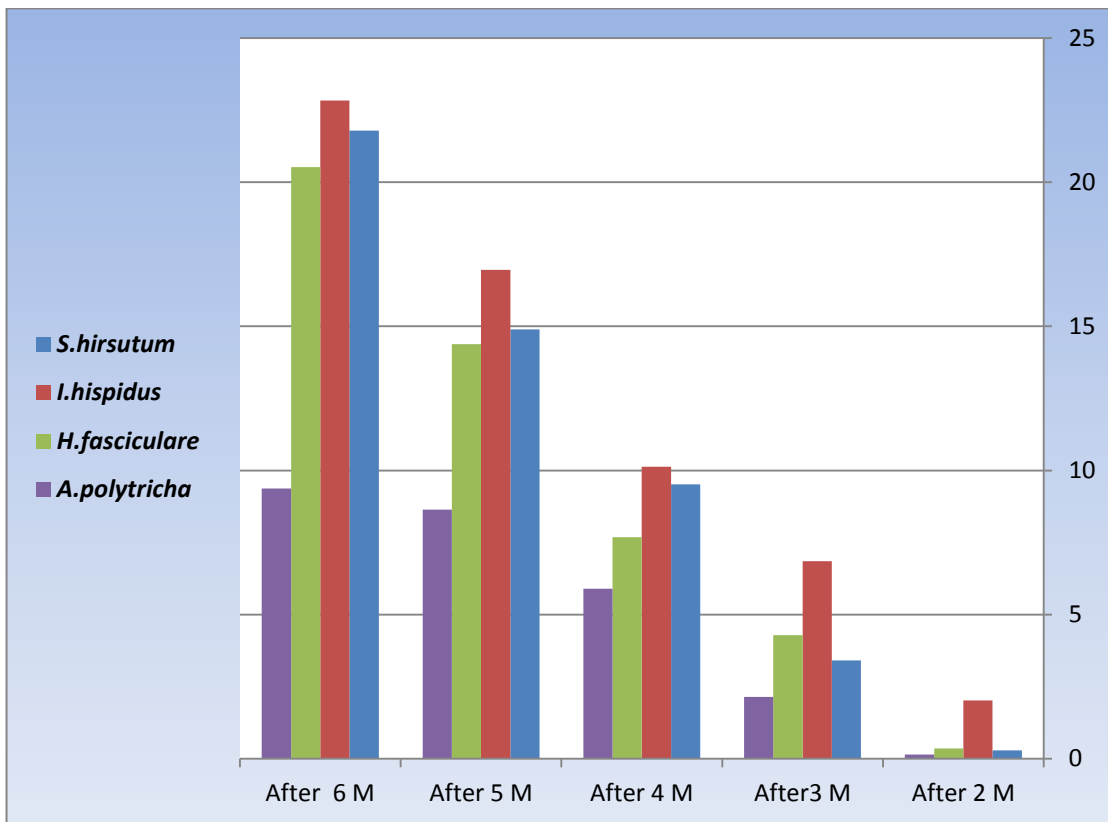(Figure 4.11) Mass loss of *E. macroryncha* wood blocks after incubation for 5 period (2,3,4,5and 6 months) with (*S* . *hirsutum* , *I* . *hispidus* , *H* . *fasciculare* and *A* . *polytricha*) fungi .

4.5 - A comparison between the used fungi to determine the most active and effective fungus in the wood decay process

Using the data contrast analysis the results showed that there were obvious differences between the fungal species in the concept of weight loss , during the decay process . This analysis method enabled us to determine which type of the fungi is the most effective than the others .

4.5.1- Determination of the most effective fungus in *P. halepensis* cubes decay and compare it with the others

The contrast analysis for the weight loss mean averages caused by all fungal species on the *P. halepensis* showed an important difference at P-value= 0.003≤ 0.05. This value confirms the presence of differences between the mean averages.

In order to find out the difference we consider the less significant difference (LSD) as shown in (Table 4.11) . It is appeared that the fungus *S. hirsutum* is generally the most effective and active type in the wood decay process , when compared to the other used fungi in the study. The mean difference between it and the fungus *I.hispidus* achieved 3.256* ,and to the fungus *H . fasciculare* attained 2.636*. while it reached 8.493* between the fungus *S. hirsutum* and the fungus *A . polytricha* .

Moreover, it is clear from the (Table 4.11) that the fungus *A . polytricha* is the lesser active in the wood decay among the group. The mean difference between it and the fungus *S . hirsutum* is -8.493*, and -5.236* to the fungus , *I. hispidus* , and -5.856* to the fungus *H . fasciculare* .

However, the fungus *I. hispidus* indicated low effectiveness comparing to the fungus *S. hirsutum*, and an activity slightly close to the activity of the fungus *H.fasciculare* at a mean difference of 0.62* . While its activity is much better than that of the fungus *A . polytricha* at 5.236* mean difference .

4.5.2- Determination of the most effective fungus in *C. sempervirens* cubes decay and compare it with the others

The contrast analysis for the weight loss mean averages caused by all fungal species on the *C . sempervirens* showed important differences at P-value= 0.021≤ 0.05**.** this value confirms the presence of differences between the mean averages .

Taking the LSD for the data presented on the (Table 4.12) to determine these difference, it is apparent that the fungus *I. hispidus* is the preeminent in the wood decay process compared to the other used fungi . It gave a mean difference of 1.754* relative to the fungus *S. hirsutum* , and gave a mean difference of 2.284* compared to the fungus   *H . fasciculare*. whereas ,the mean difference between it and the fungus *A. polytricha* reached 6.492*.

Moreover , it is obvious that the fungus *A. polytricha* showed the less effectiveness in the wood decay process when evaluated against the other fungi . It provided a difference rate of -4.742* relative to the fungus *S. hirsutum* , and a mean difference of -6.492* to the fungus *I. hispidus* , and the mean difference between it and the fungus *H . fasciculare* reached -4.205* .

The results on the (Table 4.12) showed that the fungus *S. hirsutum* has the less activity when compared to the fungus *I. hispidus* , where the mean difference reached -1.754*. however , it presented an activity that is nearer to that of the fungus *H.fasciculare* , as the mean difference attained only 0.532* , its activity was much better than that of the fungus *H . fasciculare* with a mean difference of 4.741*.

By considering the activity of the fungus *H . fasciculare* it showed a mean difference of -0.532* to the fungus *S. hirsutum* , and -2.284* compared to the fungus *I. hispidus* while it presented high activity compared to the fungus   *A . polytricha* where the mean difference reached 4.208* .

4.5.3- Determination of the most effective fungus in *P. atlantica* cubes decay and compare it with the others

The contrast analysis for the weight loss mean averages caused by all fungal species on the *P. atlantica* showed an important difference at P-value= 0.002≤ 0.05. this value confirms the presence of differences between the mean averages.

Table(4.13) shows the less significant difference in comparing the fungal activity. From this table we could determine the fungal species that is most effective in wood decay process. The results illustrated that the fungus *I. hispidus* is the most active effective in wood decay process, at mean differences reached 3.5460^* compared to the fungus *S. hirsutum* ,and with mean differences reached 3.0260^* when compared to the fungus *H . fasciculare* . Also the mean differences between the fungus *I. hispidus* and the fungus *A. polytricha* reached 15.6840^*.

It is clear that the fungus *A. polytricha* is the lesser effective species among the group, as it caused the lower weight loss rate when compared to the other fungi . Furthermore , the fungus *S. hirsutum* was more active and effective matched up to the fungus *A. polytricha* at a mean difference of 12.1380*, and has no significant mean difference compared to the fungus *H . fasciculare* (-0.5200*) . On the other hand , the fungus *H . fasciculare* appeared much more effectiveness than the fungus *A.polytricha* at mean difference of 12.6580* .

4.5.4- Determination of the most effective fungus in *C. siliqua* cubes decay and compare it with the others

The contrast analysis(ANOVA) for the weight loss mean averages caused by all fungal species on the *C. siliqua* showed significant differences at P-value= 0.000≤ 0.05. This value confirms the presence of differences between the mean averages, as shown on (Table 4.14) .

In the same time LSD shows that the fungus *H. fasciculare* is the most active and effective fungus among the rest . It showed activity more over that of the fungus *S.hirsutum* with a mean difference of 10.6482* , and that of the fungus *I. hispidus* which reached 8.8782* , and much higher than that of the fungus *A . polytricha* at

15.0378*. Conversely, the fungus *A. polytricha* showed less wood decay effectiveness than the other fungi . It gave a difference of -4.382* relative to the fungus *S . hirsutum* and 6.273* with the fungus *I. hispidus* , and a considerable difference to the fungus *H . fasciculare* which reached -15.0378*. In addition , the results showed that the fungus *S. hirsutum* is less active compared the fungus *I. hispidus* with a mean difference of 1.888* , and with a huge difference relative to the fungus *H.fasciculare* at -10.648* , and moderate effective compared to the fungus *A. polytricha* at -4.382* mean difference.

4.5.5 Determination of the most effective fungus in *E. macroryncha* cubes decay and compare it with the others

Using the ANOVA for the weight loss mean differences for all the used fungi that colonized *E. macroryncha* cubes showed significant differences at P-value= 0.0034 ≤ 0.05, which corresponding to the presence of variations among the mean averages .

To determine the differences we consider the low significant differences, as presented on (Table 4.15) . It is clear that the fungus *S. hirsutum* was more active and effective than the other fungi . The mean differences between it and the *I. hispidus* , *H . fasciculare* and *A . polytricha* fungi were 5.318*, 9.006* and 8.954* respectively .

Also the (Table 4.15) shows the fungus *H . fasciculare* is the less affected and has lower effectiveness in biodegradation process than the other fungi. The mean difference to the fungus *S. hirsutum* reached as high as -9.006* , and is -3.688* relative to the fungus *I. hispidus* . While its activity is similar to that of the fungus *A.polytricha* with a mean difference of the order -0.052* . in the time the fungus *I.hispidus* gave higher effectiveness with a difference attained 3.636* compared to the fungus *A. polytricha* .

4.6- A Comparison Between The Plant Species to Determine The Lower resistant and the More Affected Species

Using the ANOVA it obvious that there are important and significant differences in the weight loss rates during the wood decay process , among the whole plant species being studied . These differences consequently determine the wood durability of some

plant species that are less decayed compared that are more affected by the activity the biodegrading fungi. additionally , these differences give an idea about the fungal selectivity in the degradation process of the plant components.

4.6.1- Test the activity of fungus *S. hirsutum* on the five plant species and compare it with the others

 The evidences on (Table 4.16) showed that the wood decay rate of *C. siliqua*, caused by the fungus *S. hirsutum* , was the most (high weight loss) when compared to the other species . The decay rate of *C . siliqua* is 7.276* relative to that of *P.halepensis* , and is 11.316* compared to that of *C . sempervirens* ,and it reached 0.208* relative to that of *P . atlantica* , and 7.136* compared to that caused to *E.macroryncha*.

 Furthermore, it is apparent that *C. sempervirens* wood decay rate was the lower compared to that of the other plant species cubes. It reached -11.108* compared to decay rate of *P. atlantica* cubes , and attained only -4.04* relative to *P. halepensis* , and reached as high as -11.316* relative to *C . siliqua* cubes , and with mean difference of -4.18* compared to the decay rate of *E . macroryncha* cubes .

 As well the table shows that the decay rate of *P. atlantica* cubes was more than that of *P. halepensis* with a mean difference of 7.068*, hence it is more weight losing species. Also it more degrading than *E . macroryncha* with a mean difference of 6.928*. The results also showed that the decay rate of *P. halepensis* cubes was less and near to that of *E . macroryncha* cubes with a mean difference of -0.1405*.

4.6.2- Test the activity of fungus *I. hispidus* on the five plant species and compare it with the others

 The results from (Table 4.17) illustrate LSD between the weight loss mean averages. The *P. atlantica* wood decay rate was the higher compared to the other plant cubes ,therefore has the most weight loss rate, due to the fungus *I. hispidus* decay activity. Its mean difference is 13.870* compared to *P. halepensis* decay rate, and with 12.902* compared to *C. sempervirens*, and with 1.45* relative to *C. siliqua* decay rate , and also 7.392* compared to *E. macroryncha* .

In addition , the results showed that the *P. halepensis* cubes decay rate was the lower compared to the other plant species, with a difference of -13.870* to *P.atlantica* , and with near figure to the decay rate of *C . sempervirens* at a difference of -0.968* ,and at -12.42* compared to *C . siliqua* , and -6.478* compared to *E.macroryncha* decay rate .

Also the decay caused to the *C . sempervirens* cubes was more and near to that of *P. halepensis* at a difference of 0.968* , but it is less than that of *P. atlantica* at a difference of -12.902* , and as well less than *C. siliqua* decay rate at a difference of -11.452* , and less than the decay rate of *E . macroryncha* at -5.51*.

4.6.3- Test the activity of fungus *H . fasciculare* on the five plant species and compare it with the others

The results of LSD test on activity range of the fungus *H . fasciculare* ,that shown on (Table 4.18) , that the *C. siliqua* cubes decay rate was the most compared to the other species , by a difference of 10.336* relative to *P. atlantica* and with 20.56* relative to *P. halepensis*, while it was 22.496* relative to *C. sempervirens*,and with a difference of 22.59* compared to *E. macroryncha* cubes decay rate .

The results also showed that the decay rate of *E . macroryncha* cubes was the less compared to the other plant species with a difference of -12.254*relative to *P.atlantica*, and with a difference of -2.3* compared to *P. halepensis* ,and near to that of *C. sempervirens* cubes at -0.094* , and with high difference at -22.59* relative to *C. siliqua* .

4.6.4- Test the activity of fungus *A . polytricha* on the five plant species and compare it with the others

Taking the LSD for the data on activity of the fungus *A . polytricha* mentioned in (Table 4.19) we see that the *C . siliqua* cubes decay rate was the most dominant and the higher compared to the other plant species. The mean difference compared to *P.atlantica* is 7.964* ,while it was 11.389* relative to *P. halepensis* ,and with 11.674* compared to *C . sempervirens* ,and with 7.508* compared to *E. macroryncha* .

Furthermore , the table show that the decay of *C. sempervirens* was the lowest compared to the other plants , with a mean difference of -3.71* balanced to *P.atlantica*, and with 0.288* compared to *P. halepensis*, and with a mean difference of -11.674* compared to *C . siliqua* ,and with a difference of -7.508* relative to *E.macroryncha* .

Moreover , the decay rate of *P. atlantica* cubes was near to that of *E. macroryncha* with a difference of -0.456*.

Chapter Five

Discussion

This is first study in eastern part of Libya specially in green mountain region , in resituating and focusing on wood decay fungi in five forest trees ; *P. halepensis* , *C. sempervirens* , *C . siliqua*   , *E. macroryncha* and *P. atlantica* .

The part of research have less attention for long time ago .Now a days due to huge loss of wood yields due to attack and infection of fallen woods by different causative agents .The main causal agent are wood decay fungi either basidiomycwtes or ascomycetes . After period of time all wood will be decomposed by mode of action of saprophytic fungi feeding on cellulose , hemicellulose and lignin .

In this study five wood decay fungi have been isolated and identified based on shape and size of fruiting body , colony character , morphology of mycelium and spores .

Wood rot diseases , caused by a wide variety of wood saprophytic colonizing fungi can attack trunk , branches and roots of all woody plants . decay usually start from arrival of spores , or part of mycelium entering through woods or mechanical injuries . Decay usually develops slowly over a period of many months .

Five types of different forest trees were selected in this study named ; *Pinus halepensis* , *Cupressus sempervirens* , *Pistacia atlantica* , *Ceratonia siliqua*   and *Eucalyptus macroryncha* .

The purpose and the objective to select this particular type of trees that are very common and wide spread at ALBAKOR region . These five trees show a Variety of differences in growth patterns , wood structure and environmental adaptation . Wood quality as hard wood , semi hard wood or sapwood . will be surely determine the Fungal specification to infect and feed on components e.g lignin , cellulose , carbohydrates   and hemicellulose .The quality and quantity of the previous compounds will make , parasitic fungi survive long time of colonization and utilization such as kind of substances . water potential or water activities of each tree organ e.g small branches , logs or trunk , hare drastic effect for fungal species

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invasion to spread very fast during summary and drought seasons . other bitotic and abiotic factors contribute directly or indirectly on wood decay processes , mainly insect infection like wood borers , wood beetles, antes , will creak canals , wounds and penetration sites of saprophytic wood fungi to enter into wood in quick ways and go more deep to wood center . The wood structure of each single tree will differ from other tree of the same species , this differences in chemical and physical structure is very significantly important and play a major roles of enzymatic potential of the fungi .The amount of enzymes production , which degrade and decompose the wood substance are the key assume to enhance the digestion processes of wood component , leading to increase more inoculum potential of spores and reproductive structures .

The investigated trees in this work are shown a great variation and differences in the anatomical structure of their wood , but also down to structural differences of individual tissues , cell contents and cell wall layers . All possess differing altercation mode for fungal enzymes to break them down in a limit time , when macro- and micro environmental conditions are favorable during establishment of fungus infection . This physiological and biochemical differences in wood composition , being manifested and directed by the diverse patterns and behavior of wood decay observed. Beyond the purely and clearly visual changes and alterations , of wood quality, has far- reaching consequences for the mechanical properties of the fungus infected wood such as the stiffness or strength of xylem tissues .

In the laboratory, varying responses have been reported of exploratory wood decay fungi systems of *Stereum hirsutum , Inonotus hispidus , hypholoma fasciculare* and *Auricularia polytricha* . to different sizes of wood blocks baits .Mycelial growth from *S. hirsutum* toward to sterilized wood blocks base 2x1.5x1.5cm^3 , exhibited clear colonization of this type of in a short period 7-14 days after inoculation , this results are conformable and congruously with Dowson , (Rayner and Boddy , 1986) .

The vitro experiments has made to study the colonization abilities of isolated wood decay fungi of freshly cutted wood blocks of the same infected tree on floor of artificial media . (PDA) , revealed that colonization occurred rapidly throughout the enter site , with more than 80-85% of incubated wood blocks becoming extensively colonized by *S. hirsutum* within two months .

The predominant of the previous five isolated display a range of combative abilities with respect to each other and toward other wood-inhabiting fungi ability , such types of fungi compete directly for space and nutrients . (Thompson , 1982). Wood-inhabiting fungi shown and provides some indication of the relative combative ability of these fungi .

In nature the outcome of interaction between different wood-inhabiting fungi occupy the same wood resource the final results is either " deadlock " (where neither fungus replaces the other , with the formation of a coloured contact zone) or replacement (with the growth of one fungus into or over another). (Chapela ,1987, Rayner and Webber, 1984 and Dawson *et al* . ,1988) .

Interactions between fungi may be regarded at the individual hyphal level or at the level of the whole mycelium (Rayner and Boddy , 1988) . The effect of antibiotics may play some role in the dynamics of mycelial interactions . During the interaction , hypha may affect each other from a distance by the production of volatile antibiotic compounds . It has become obvious that growth inhibition often may no occur prior to actual contact between hyphae or mycelia this reported conclusion support our study that during inspection and investigation of tested decayed trees . Particular fungal wood-decaying fungi were found , while other does not exist .

The results obtained from weight loss and percentage weight loss of wood inocula in laboratory experiment , shows significant differences between tested wood-decayed fungi . However , the decay process have been started within 10 days of placed autoclaved wood blocks . After the first two weeks , the hyphae are completely coverage the wood blocks . After a month from inoculation , the inoculated fungi colonized the whole wood blocks and fungi continue on decaying process , which may take long time over 2- 3 months depending on wood block quality and quantity ($2x1.5x1.5cm^{3}$) . (Abdalla , 1995) . The period of exposure of wood blocks to individual inoculated fungi showed clearly differences in the decay rates caused by to the wood blocks , for each tree type . The results demonstrated that for every fugal species there is a constant level of weight loss rate which differ from one stage to anther stage of growth and colonization .

The percentage weight loss of *P. halepensis* wood blocks (2x1.5x1.5cm^3) for Five time intervals 2,3,4,5 and 6 months after inoculated with four wood decay fungi named as ; *Stereum hirsutum* , *Inonotus hispidus* , *Hypholoma fasciculare* and *Auricularia polytricha* from table (1) the percentage of weight loss of pine wood blocks by (g) after inoculation with four wood decay fungi . There is a significant increase of wood weight loss by time increasing the highest figure is (27.29 g) after 6 months interval time of inoculation by *S. hirsutum* . This indicate that this previous fungus have great biodegradation ability , compared with others , *I. hispidus* , *H.fasciculare* and *A . polytricha* respectively . This results are agreed with (Abdalla and Boddy ,1996) . in table (3) the highest weight loss of *C. sempervirens* was (22.83 g) after 6 months of inoculation by *I . hispidus* . This indicate that this previous fungus have great biodegradation ability , compared with others , *S. hirsutum* , *H.fasciculare* and *A . polytricha* respectively . While the highest weight loss of *P.atlantica* was (48.55 g) after 6 months by *I . hispidus* .

Table (5) . About the highest weight loss of *C.siliqua* was (49.5 g) after 6 months by *H. fasciculare* . Also in *E. macroryncha* the weight loss was (37.52 g) after 6 months caused by *S . hirsutum* . This indicate that this previous fungus have great biodegradation ability , compared with others , *I . hispidus* , *H . fasciculare* and *A.polytricha* respectively .

The variation of biodegradation between the previous mentioned fungi indicate the difference of enzymes production of each single wood-decay fungi . Wood structures and availability of nutrients of wood tissues and cell walls composition .

Future research direction ; One of the most important avenues for further research will be the survey of all forest tree species in the Aljabl Alkedar region and study the all wood-inhabiting fungi to safe the new forest generation and plantation .

Conclusion

This recent study in eastern part of Libya was highlighted to one branch of plant pathology (forest pathology) which including wood- inhabiting fungi or wood decay fungi . This interesting science which have been neglected from Libya , plant pathology.

Wood and timber are one of largest natural resource of most world – wide countries. We most disinterest and caring the forest trees from infection and attacks by pests and microbiota in particular Fungi infection .The study of ecology of saprophytic basidio-mycetes have open , huge data and information about the behavior and strategies and foraging fungi on fallen logs , branches and leaves on forest ecosystem .

This research provide and add four new recorded fungi to Libyan Fungal flora names as : *Stereum hirsutum , Inonotus hispidus* , *Hypholoma fasciculare* and *Auricularia polytricha* .

These four new wood – decaying fungi have been isolation from four dead forest trees in ALBAKOR region . The results of laboratory or in vitro experiments shows very significant parameters to calculate and measure wood – decay loss and percentage of weight loss of artificially inoculated sterilized wood blocks sterilized flasks containing PDA media , wood block will leaved to encountered by wood – decay fungi for five time intervals 2,3,4,5 and 6 months . The highest figure of weight loss was 31.94g caused by *H . fasciculare* when incubated with *Ceratonia siliqua* blocks . Whereas , the *S. hirsutum* fungi caused the highest weight loss in both , *P. halepensis* and *E. macroryncha* (14.02g , 18.36g) . About *I. hispidus* fungi who caused the highest weight loss in *C. sempervirens* and *P. atlantica* blocks it gave (11.73 g , 24.63g) . On the other hand , *A. polytricha* fungi who gave the lowest figure of weight loss in all wood decay processes with different types of forest trees under this study .

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Appendix

1- A comparison between the used fungi to determine the most active and effective fungus in the wood decay process

(Table 4.11) Show the most effective fungus in *P. halepensis*
cubes decay and compare it with the others

Tested Fungus	Tested Fungus	Mean Difference	Std .Error	Sig.
S .hirsutum	*I. hispidus*	3.256*	2.02284	0.216
	H . fasciculare	2.6365*	2.02284	0.156
	A . polytricha	8.4930*	2.02284	0.000
I. hispidus	*S .hirsutum*	-3.256*	2.02284	0.216
	H . fasciculare	0.625*	2.02284	0.839
	A . polytricha	5.236*	2.02284	0.004
H . fasciculare	*S .hirsutum*	-2.6365*	2.02284	0.156
	I. hispidus	-0.625*	2.02284	0.839
	A . polytricha	5.8565*	2.02284	0.006
A . polytricha	*S .hirsutum*	-8.4930*	2.02284	0.000
	I. hispidus	-5.236*	2.02284	0.004
	H . fasciculare	-5.856*	2.02284	0.006

significant level at (P-value< 0.05)

(Table4.12) Show the most effective fungus in *C. sempervirens* cubes decay and compare it with the others

Tested Fungus	Tested Fungus	Mean Difference	Std .Error	Sig.
S .hirsutum	I. hispidus	-1.7546*	1.43104	0.238
	H . fasciculare	0.532*	1.43104	0.929
	A . polytricha	4.741*	1.43104	0.031
I. hispidus	S .hirsutum	1.7546*	1.43104	0.238
	H . fasciculare	2.284*	1.43104	0.208
	A . polytricha	6.492*	1.43104	0.003
H . fasciculare	S .hirsutum	-0.532*	1.43104	0.929
	I. hispidus	-2.284*	1.43104	0.208
	A . polytricha	4.208*	1.43104	0.036
A . polytricha	S .hirsutum	-4.742*	1.43104	0.031
	I. hispidus	-6.492*	1.43104	0.003
	H . fasciculare	-4.208*	1.43104	0.036

significant level at (P-value< 0.05)

(Table 4.13) show the most effective fungus in *P. atlantica* cubes decay and compare it with the others

Tested Fungus	Tested Fungus	Mean Difference	Std .Error	Sig.
S .hirsutum	I. hispidus	-3.5460*	4.01238	0.290
	H . fasciculare	-0.5200*	4.01238	0.874
	A . polytricha	12.1380*	4.01238	0.003
I. hispidus	S .hirsutum	3.5460*	4.01238	0.290
	H . fasciculare	3.0260	4.01238	0.364
	A . polytricha	15.6840*	4.01238	0.000
H . fasciculare	S .hirsutum	0.5200*	4.01238	0.874
	I. hispidus	-3.0260*	4.01238	0.364
	A . polytricha	12.6580*	4.01238	0.002
A . polytricha	S .hirsutum	-12.1380*	4.01238	0.003
	I. hispidus	-15.6840*	4.01238	0.000
	H . fasciculare	-12.6580*	4.01238	0.002

significant level at (P-value< 0.05)

(Table 4.14) Show the most effective fungus in *C. siliqua* cubes decay and compare it with the others

Tested Fungus	Tested Fungus	Mean Difference	Std .Error	Sig.
S .hirsutum	I. hispidus	-1.8884*	2.05997	0.328
	H . fasciculare	-10.6482*	2.05997	0.000
	A . polytricha	4.382*	2.05997	0.024
I. hispidus	S .hirsutum	1.8884*	2.05997	0.328
	H . fasciculare	-8.8782*	2.05997	0.000
	A . polytricha	6.273*	2.05997	0.004
H . fasciculare	S .hirsutum	10.6482*	2.05997	0.000
	I. hispidus	8.8782*	2.05997	0.000
	A . polytricha	15.0378*	2.05997	0.000
A . polytricha	S .hirsutum	-4.382*	2.05997	0.024
	I. hispidus	-6.273*	2.05997	0.004
	H . fasciculare	-15.0378*	2.05997	0.000

significant level at (P-value< 0.05)

(Table 4.15) Show the most effective fungus in *E. macroryncha* cubes decay and compare it with the others

Tested Fungus	Tested Fungus	Mean Difference	Std .Error	Sig.
S .hirsutum	I. hispidus	5.318*	2.62511	0.279
	H . fasciculare	9.006*	2.62511	0.103
	A . polytricha	8.954*	2.62511	0.106
I. hispidus	S .hirsutum	-5.318*	2.62511	0.279
	H . fasciculare	3.688*	2.62511	0.013
	A . polytricha	3.636*	2.62511	0.014
H . fasciculare	S .hirsutum	-9.006*	2.62511	0.103
	I. hispidus	-3.688*	2.62511	0.013
	A . polytricha	-0.052*	2.62511	0.985
A . polytricha	S .hirsutum	-8.954*	2.62511	0.106
	I. hispidus	-3.636*	2.62511	0.014
	H . fasciculare	0.052*	2.62511	0.985

significant level at (P-value< 0.05)

2- A comparison between the plant species to determine the lower resistant and the more affected species

(Table 4.16) Show the activity of fungus *S. hirsutum* on the five plant species and compare it with the others .

Type of Trees	Trees species	Mean Difference (I-J)	Std .Error	Sig.
C. sempervirens	*P. halepensis*	-7.068*	2.26724	0.018
	P. atlantica	4.041*	2.26724	0.003
	C.siliqua	-7.276*	2.26724	0
	E. macroryncha	-0.1405*	2.26724	0.863
P. atlantica	*P. halepensis*	-11.108*	2.26724	.0.00
	C. sempervirens	-4.041*	2.26724	0.003
	C.siliqua	-11.316*	2.26724	0.00
	E . macroryncha	-4.18*	2.26724	0.004
P. halepensis	*C. sempervirens*	7.068*	2.26724	0.018
	P. atlantica	11.108*	2.26724	0
	C.siliqua	-0.208-*	2.26724	0.984
	E .macroryncha	6.928*	2.26724	0
C.siliqua	*P. halepensis*	0.208*	2.26724	0.984
	C. sempervirens	7.2760*	2.26724	0.0
	P. atlantica	11.316*	2.26724	0.00
	E. macroryncha	7.136*	2.26724	0.00
E. macroryncha	*P. halepensis*	-6.928*	2.26724	0.0
	C. sempervirens	0.1405*	2.26724	0.863
	P. atlantica	4.180*	2.26724	0.004
	C.siliqua	-7.1360*	2.26724	0.00

significant level at (P-value< 0.05)

(Table 4.17) Show the activity of fungus *I. hispidus* on the five plant species and compare it with the others

Tree type	Trees species	Mean Difference (I-J)	Std .Error	Sig.
P. halepensis	*C. sempervirens*	13.870[*]	2.81334	0.0
	P. atlantica	12.902[*]	2.81334	0.0
	C.siliqua	1.450[*]	2.81334	0.02
	E. macroryncha	7.392[*]	2.81334	0.0
C. sempervirens	*P. halepensis*	-13.870[*]	2.81334	0.0
	P. atlantica	-0.968[*]	2.81334	0.63
	C.siliqua	-12.42[*]	2.81334	0.0
	E. macroryncha	-6.478[*]	2.81334	0.0
P. atlantica	*P. halepensis*	-12.902[*]	2.81334	0.0
	C. sempervirens	0.968[*]	2.81334	0.64
	C.siliqua	-11.452[*]	2.81334	0.00
	E. macroryncha	-5.51[*]	2.81334	0.0
C.siliqua	*P. halepensis*	-1.450[*]	2.81334	0.02
	C. sempervirens	12.420[*]	2.81334	0.0
	P. atlantica	11.452[*]	2.81334	0.00
	E. macroryncha	5.942[*]	2.81334	0.00
E. macroryncha	*P. halepensis*	-7.392[*]	2.81334	0.0
	C. sempervirens	6.478[*]	2.81334	0.0
	P. atlantica	5.510[*]	2.81334	0.0
	C.siliqua	-5.942[*]	2.81334	0.00

significant level at (P-value< 0.05)

(Table 4.18) Show the activity of fungus *H . fasciculare* on the five plant species and compare it with the others

Tree type	Trees species	Mean Difference (I-J)	Std .Error	Sig.
P. halepensis	C. sempervirens	10.224*	2.60885	0.015
	P. atlantica	12.16*	2.60885	0.0
	C.siliqua	-10.336*	2.60885	0.0
	E. macroryncha	12.254*	2.60885	0.0
C. sempervirens	P. halepensis	-10.224*	2.60885	0.015
	P. atlantica	1.936*	2.60885	0.04
	C.siliqua	-20.56*	2.60885	0.0
	E. macroryncha	2.04*	2.60885	0.0
P. atlantica	P. halepensis	-12.160*	2.60885	0.0
	C. sempervirens	-1.936*	2.60885	0.04
	C.siliqua	-22.496*	2.60885	0.012
	E .macroryncha	0.094*	2.60885	0.98
C.siliqua	P. halepensis	-10.336*	2.60885	0.0
	C. sempervirens	20.56*	2.60885	0.0
	P. atlantica	22.496*	2.60885	0.012
	E. macroryncha	22.59*	2.60885	0.031
E. macroryncha	P. halepensis	-12.254*	2.60885	0.0
	C. sempervirens	-2.254*	2.60885	0.0
	P. atlantica	-0.094*	2.60885	0.98
	C.siliqua	-22.59*	2.60885	0.031

significant level at (P-value< 0.05)

(Table 4.19) Show the activity of fungus *A . polytricha* on the five plant species
and compare it with the others

Tree type	Trees species	Mean Difference (I-J)	Std .Error	Sig.
P. halepensis	*C. sempervirens*	3.422*	2.11	0.0179
	P. atlantica	3.710*	2.11	0.002
	C.siliqua	-7.964*	2.11	0.0
	E macroryncha	-0.456*	2.11	0.845
C. sempervirens	*P. halepensis*	-3.422*	2.11	0.0179
	P. atlantica	0.288*	2.11	0.0978
	C.siliqua	-11.386*	2.11	0.0
	E. macroryncha	-3.878*	2.11	0.00
P. atlantica	*P. halepensis*	-3.710*	2.11	0.002
	C. sempervirens	-0.288*	2.11	0.0987
	C.siliqua	-11.674*	2.11	0.0
	E. macroryncha	-4.166*	2.11	0.0
C.siliqua	*P. halepensis*	7.964*	2.11	0,0
	C. sempervirens	11.386*	2.11	0,0
	P. atlantica	11.674*	2.11	0.00
	E. macroryncha	7.508*	2.11	0.00
E. macroryncha	*P. halepensis*	0.456*	2.11	0.845
	C. sempervirens	3.878*	2.11	0.0
	P. atlantica	4.166*	2.11	0.0
	C.siliqua	-7.508*	2.11	0.0

significant level at (P-value< 0.05)

فحص وعزل وتعريف بعض انواع من الفطريات المحللة للخشب على خمس انواع من اشجار الغابة

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الخلاصة

الهدف من هذه الدراسة هو التحقق من تحلل الخشب ، مع تحديد نسبة هذا التحلل في خمسة أنواع من أشجار الغابة وهي : *C. sempervirens*, *P. atlantica* , *P. halepensis* , و *E. macroryncha* , *C.siliqua* وذلك عندما عرضت و حضنت مع أربع أنواع من الفطريات المحللة للخشب *S. hirsutum* ، *I. hispidus* ، *H . fasciculare* وفطر *A. polytricha* ، أما عملية تقدير نسبة ومعدل الفقد في الوزن أجريت طبقا للطريقة التي وصفت في (*ASTM D 143-94*)، حيث اجريت التجربة في هذه الدراسة استادا على خمس خطوات وهي: (قطع العينات النباتية لاختبارها فيما بعد مع فطريات الاختبار، تحضير الوسط الغذائي المناسب، تجفيف العينات النباتية ، توزيع العينات الخشبية وتعريضها لفطريات الاختبار ، و فحص العينات المعرضة) . فقد الوزن اصبح ملاحظ بعد اول فترة من عملية التحضين واصبح يتزايد مع استمرار عملية التحضين . حيث اظهرت النتائج المتحصل عليها اختلافات في مقدرة فطريات الاختبار على التحلل والتي من خلالها تبين ما مدى مقاومة خشب هذه الانواع النباتية

، حيث أنه وبشكل مثير بينت النتائج ان خشب نبات *C. sempervirens* كان الاكثر مقاومة ضد جميع فطريات الاختبار ، في حين ان النبات *C. siliqua* كان اكثر قابلية للتحلل (الاقل مقاومة للفطريات المحللة للخشب) و من بين فطريات الاختبار عرض لها هو *H . fasciculare* و الذي اظهر اعلى فاعلية في عملية التحلل حيث تسبب في معدل فقد في الوزن حوالي 31.94g والذي يمثل اعلى معدل فقد في الوزن خلال هذه التجربة . ومن ناحية اخرى فقد كان الفطر المحلل *A . polytricha* الاقل نشاطا في عملية التحلل حيث اعطى معدل فقد في الوزن تراوح بين (g 5.528− 16.91g).

واستنادا على نتائج هذه الدراسة فإن هنالك اختلافات واضحة في قابلية المكعبات الخشبية للتحلل وذلك باختلاف الانواع الفطرية المحللة للخشب المستعملة في هذه الدراسة .

فحص وعزل وتعريف بعض انواع من الفطريات المحللة للخشب على خمس انواع من اشجار الغابة

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قدمت هذه الرسالة استكمالاً لمتطلبات الحصول على درجة الماجستير في

علم الاحياء الدقيقة

جامعة بنغازي

كلية العلوم

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