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The efficacy of the River Nile State bee honey against Fungi:*Aspergillusniger*

تأثير عسل نحل ولاية نـهر النيل على فطر الاسبرجلس نيجر

B.Sc (Honurs) Graduation Research Project in Plant Protection

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الآية

بسم الله الرحمن الرحيم

وَأَوحى رَبُّكَ إِلَى النَّحلِ أَنِ اتَّخِذي مِنَ الجِبالِ بُيوتًا وَمِنَ الشَّجَرِ وَمِمّا
يَعرِشونَ ﴿٦٨﴾ ثُمَّ كُلي مِن كُلِّ الثَّمَراتِ فَاسلُكي سُبُلَ رَبِّكِ ذُلُلًا يَخرُجُ مِن بُطونِها
شَرابٌ مُختَلِفٌ أَلوانُهُ فيهِ شِفاءٌ لِلنّاسِ إِنَّ في ذلِكَ لَآيَةً لِقَومٍ يَتَفَكَّرونَ ﴿٦٩﴾

سورة النحل الآية(68-69)

DeDica tion

To my father and mother,

To my brothers, sisters and my family.

To my supervisors Dr. Abdel BagiElsayed Ali.

To all member who help me to protect this work.

To my teachers in plant protection, Sudan University of Sciences and Technology.

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Firstly, my deep thanks for Allah for giving me ability and health to finish this work. I would like to express my gratitude and appreciation to my supervisor Dr Abdel BagiElsayed Ali for his guidance and help to complete this work.

Thanks to teacher Moda for her help, thanks due to my friend Mubarak for analysis my experiment.

Finally, my great thanks to all member and my friend Mogahed for their continuous help and support.

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ملخص البحث

اختبرت هذه الدراسة إمكانية استخدام المنتج الطبيعي من عسل النحل لمكافحة الفطريات. تم استخدام عينتان من العسل الطبيعيالتيأحضرت من ولاية نهر النيل (الدامر وشندى) وتمت معاملة الفول السوداني بهذا العسل ، أعطت هذه الدراسة نتائج ممتازة جدا حيث أثبتتأن العسل الطبيعي له مقدرة عالية جدا فى مكافحة نمو الفطريات في الفول السوداني .

Abstract

This research aim to study the efficacy of two samples of River Nile State bee honey against fungi (*Aspergillusniger*), sample one is pure bee honey from (Aldamer) and sample two is pure honey from (Shandi).

We put all samples in media of fungi (*Aspergillusniger*). After 2day, 3day, 4day and 5day inhibition zone seen around the bee honey,the control showed very good growth. This effect shows that honey has a high ability to fight fungi growth.

CHAPTER:ONE

1-1:Introduction:

Honey is defined as a naturally sweet mixture produced by bees from nectar of flower , from secretion of the living plants or excretion of plant suking insect on the living part of plant that the honey bee collect , transform and combine with specific substance of their own (such as enzyme) , deposit , dehydrate , store and leave in the bee wax honey combs to ripen and mature(OJEC,1974),Honey gets its sweetness from the monosaccharides fructose and glucose, and has about the same relative sweetness as granulated sugar. It has attractive chemical properties for baking and a distinctive flavor when used as a sweetener. Most microorganisms do not grow in honey, so sealed honey does not spoil, even after thousands of years.

The historical background and culture of bees honey, the use and production has a long and varied history. In many cultures honey has associations that go beyond its use as a food. It is frequently used as a talisman and symbol of sweetness. Honey collection is an ancient activity, humans apparently began hunting for honey at least 8000 years ago, an evidenced by a cavepainting is a Mesolithic rock painting, showing to honey hunters collecting honey and honey-comb from a wild bee nest. The figures are depicted carrying baskets or gourds, and using a ladder or series of ropes to reach the wild nest .The bee honey mentioned in the Koran and other holy books, even the chines and Indian. It was used as a cure for baldnees and to prevent pregency and the Germans were using it to treat wounds, burns, fistulas and healing with fish oil and were using it as an ointment soothing added yolk egg with flour. In the man Stone Age eight thousand year ago dealt with in his food honey was used as a treatment and this mangda in the photographs, manuscripts, papyri to the ancient Egyptions and the Sumerians in Iraq and Syria. The ancient Egyption use it in moumyawat.

Honey contains a wide variety of vitamins, minerals, amino acids and antioxidants. The vitamins found in honey include niacin, riboflavin and pantothenic acid; minerals present include calcium, copper, iron, magnesium, manganese, phosphorus, potassium and zinc. In addition honey contains a variety of flavonoids and phenolic acids which act as antioxidants, scavenging and eliminating free radicals.

1-2: Objective:

2- To analysis the moisture of two natural bee honey samples from River Nile State.

1-To study the efficacy of two natural bee honey samples from River Nile State against the fungi: Aspergillusniger.

CHAPTER: TWO

Literature Review

2-1: Formation of honey:

Honey is produced by bees collecting nectar for use as sugars consumed to support metabolism of muscle activity during foraging or to be stored as a long-term food supply. During foraging, bees access part of the nectar collected to support metabolic activity of flight muscles, with the majority of collected nectar destined for regurgitation, digestion, and storage as honey. In cold weather or when other food sources are scarce, adult and larval bees use stored honey as food (Suarez RK,*etal.*,1996). By contriving for bee swarms to nest in human-made hives, people have been able to semi-domesticate the insects and harvest excess honey. In the hive or in a wild nest, the three types of bees are:

- A single female queen bee.
- A seasonally variable number of male drone bees to fertilize new queens.
- 20,000 to 40,000 female worker bees.

Leaving the hive, foraging bees collect sugar-rich flower nectar and return to the hive where they use their "honey stomachs" to ingest and regurgitate the nectar repeatedly until it is partially digested. Bee digestive enzymes – invertase, amylase, and diastase – along with gastric acid hydrolyze sucrose to a mixture of glucose and fructose.The bees work together as a group with the regurgitation and digestion for as long as 20 minutes until the product reaches storage quality. It is then placed in honeycomb cells left unsealed while still high in water content (about 20%) and natural yeasts, which, unchecked, would cause the sugars in the newly formed honey to ferment. The process continues as hive bees flutter their wings constantly to circulate air and evaporate water from the honey to a content around 18%, raising the sugar concentration, and preventing fermentation. The bees then cap the cells with wax to seal them. As removed from the hive by a beekeeper, honey has a long shelf life and will not ferment if properly sealed (Binkley., 2014).

2-2: Physical and chemical properties:

The physical properties of honey vary, depending on water content, the type of flora used to produce it (pasturage), temperature, and the proportion of the specific sugars it contains. Fresh honey is a supersaturated liquid, containing more sugar than the water can typically dissolve at ambient temperatures. At room temperature, honey is a supercooled liquid, in which the glucose will precipitate into solid granules. This forms a semisolid solution of precipitated glucose crystals in a solution of fructose and other ingredients, at the temperature of 20 °C, density of honey typically ranges between 1.38 and 1.45 kg/l (PiotrTomasik., 2003)

2-2-1:Phase transitions:

The melting point of crystallized honey is between 40 and 50 °C (104 and 122 °F), depending on its composition. Below this temperature, honey can be either in a metastable state, meaning that it will not crystallize until a seed crystal is added, or, more often, it is in a "labile" state, being saturated with enough sugars to crystallize spontaneously. The rate of crystallization is affected by many factors, but the primary factor is the ratio of the main sugars: fructose to glucose. Honeys that are supersaturated with a very high percentage of glucose, such as brassica honey, crystallize almost immediately after harvesting, while honeys with a low percentage of glucose, such as chestnut or tupelo honey, do not crystallize. Some types of honey may produce very large but few crystals, while others produce many small crystals (Tomasik., 2004)

Crystallization is also affected by water content, because a high percentage of water inhibits crystallization, as does a high dextrin content. Temperature also affects the rate of crystallization, with the fastest growth occurring between 13 and 17 °C (55 and 63 °F). Crystal nuclei (seeds) tend to form more readily if the honey is disturbed, by stirring, shaking, or agitating, rather than if left at rest. However, the nucleation of microscopic seed-crystals is greatest between 5 and 8 °C (41 and 46 °F). Therefore, larger but fewer crystals tend to form at higher temperatures, while smaller but more-numerous crystals usually form at lower temperatures. Below 5 °C, the honey will not crystallize, thus the original texture and flavor can be preserved indefinitely.

Since honey normally exists below its melting point, it is a supercooled liquid. At very low temperatures, honey does not freeze solid. Instead, as the temperatures become lower, the viscosity of honey increases. Like most viscous liquids, the honey becomes thick and sluggish with decreasing temperature. At −20 °C (−4 °F), honey may appear or even feel solid, but it continues to flow at very low rates. Honey has a glass transition between −42 and −51 °C (−44 and −60 °F). Below this temperature, honey enters a glassy state and becomes an amorphous solid (noncrystalline) (Kántor Z,*et al.*, 1999).

2-2-2: Viscosity:

The viscosity of honey is affected greatly by both temperature and water content. The higher the water percentage, the more easily honey flows. Above its melting point, however, water has little effect on viscosity. Aside from water content, the composition of honey also has little effect on viscosity, with the exception of a few types. At 25 °C (77 °F), honey with 14% water content generally has a viscosity around 400 poise, while a honey containing 20% water has a viscosity around 20 poise. Viscosity increase due to temperature occurs very slowly at first. A honeycontaining 16% water, at 70 °C (158 °F), has a viscosity around 2 poise, while at 30 °C (86 °F), the viscosity is around 70 poise. As cooling progresses, honey becomes more viscous at an increasingly rapid rate, reaching 600 poise around 14 °C (57 °F). However, while honey is very viscous, it has rather low surface tension (Bogdanov.,2009).

A few types of honey have unusual viscous properties. Honeys from heather or manuka display thixotropic properties. These types of honey enter a gel-like state when motionless, but then liquify when stirred.

2-2-3:Electrical and optical properties:

Because honey contains electrolytes, in the form of acids and minerals, it exhibits varying degrees of electrical conductivity. Measurements of the electrical conductivity are used to determine the quality of honey in terms of ash content.

The effect honey has on light is useful for determining the type and quality. Variations in the water content alter the refractive index of honey. Water content can easily be measured with a refractometer. Typically, the refractive index for honey ranges from 1.504 at 13% water content to 1.474 at 25%. Honey also has an effect on polarized light, in that it rotates the polarization plane. The fructose gives a negative rotation, while the glucose gives a positive one. The overall rotation can be used to measure the ratio of the mixture. Honey may vary in color between pale yellow and dark brown, but other bright colors may occasionally be found, depending on the source of the sugar harvested by the bees.

2-2-4:Hygroscopy and fermentation:

Honey has the ability to absorb moisture directly from the air, a phenomenon called Hygroscopy. The amount of water the honey absorbs is dependent on the relative humidity of the air. Because honey contains yeast, this hygroscopic nature requires that honey be stored in sealed containers to prevent fermentation, which usually begins if the honey's water content rises much above 25%. Honey tends to absorb more water in this manner than the individual sugars allow on their own, which may be due to other ingredients it contains.

Fermentation of honey usually occurs after crystallization, because without the glucose, the liquid portion of the honey primarily consists of a concentrated mixture of fructose, acids, and water, providing the yeast with enough of an increase in the water percentage for growth. Honey that is to be stored at room temperature for long periods of time is often pasteurized, to kill any yeast, by heating it above 70 °C (158 °F)

2-2-5:Thermal characteristics:

Like all sugar compounds, honey caramelizes if heated sufficiently, becoming darker in color, and eventually burns. However, honey contains fructose, which caramelizes at lower temperatures than glucose. The temperature at which caramelization begins varies, depending on the composition, but is typically between 70 and 110 °C (158 and 230 °F). Honey also contains acids, which act as catalysts, decreasing the caramelization temperature even more (ZdzislawE., 2007) of these acids, the amino acids, which occur in very small amounts, play an important role in the darkening of honey. The amino acids form darkened compounds called melanoidins, during a Maillard reaction. The

Maillardreaction occurs slowly at room temperature, taking from a few to several months to show visible darkening, but speeds up dramatically with increasing temperatures. However, the reaction can also be slowed by storing the honey at colder temperatures.

Unlike many other liquids, honey has very poor thermal conductivity, taking a long time to reach thermal equilibrium. Melting crystallized honey can easily result in localized caramelization if the heat source is too hot, or if it is not evenly distributed. However, honey takes substantially longer to liquify when just above the melting point than at elevated temperatures. Melting 20 kg of crystallized honey, at 40 °C (104 °F), can take up to 24 hours, while 50 kg may take twice as long. These times can be cut nearly in half by heating at 50 °C (122 °F). However, many of the minor substances in honey can be affected greatly by heating, changing the flavor, aroma, or other properties, so heating is usually done at the lowest temperature possible for the shortest amount of time.

2-2-6:Acid content and flavor effects:

The average pH of honey is 3.9, but can range from 3.4 to 6.1. Honey contains many kinds of acids, both organic and amino. However, the different types and their amounts vary considerably, depending on the type of honey. These acids may be aromatic or aliphatic (nonaromatic). The aliphatic acids contribute greatly to the flavor of honey by interacting with the flavors of other ingredients.

Organic acids comprise most of the acids in honey, accounting for 0.17–1.17% of the mixture, with gluconic acid formed by the actions of an enzyme called glucose oxidase as the most prevalent. Other organic acids are minor, consisting of formic, acetic, butyric, citric, lactic, malic, pyroglutamic, propionic, valeric, capronic, palmitic, and succinic, among many others.

2-3: History and culture of honey:

Honey use and production has a long and varied history. In many cultures, honey has associations that go beyond its use as a food (Ruber., 2015). It is frequently used as a talisman and symbol of sweetness.The greater honeyguide bird guides humans to wild bee hivesand this behavior may have evolved with early hominids.The oldest known honey remains were found in the country of Georgia. Archaeologists found honey remains on the inner surface of clay vessels unearthed in an ancient tomb, dating back some 4,700–5,500 years.In ancient Georgia, several types of honey were buried with a person for their journey into the afterlife, including linden, berry, and meadow-flower varieties.

In ancient Egypt, honey was used to sweeten cakes and biscuits, and was used in many other dishes. Ancient Egyptian and Middle Eastern peoples also used honey for embalming the dead. The fertility god of Egypt, Min, was offered honey.In ancient Greece, honey was produced from the Archaic to the Hellenistic periods. In 594 BC, beekeeping around Athens was so widespread that Solon passed a law about it: "He who sets up hives of bees must put them 300 feet (91 meters) away from those already installed by another".Greek archaeological excavations of pottery located ancient hives. According to Columella, Greek beekeepers of the Hellenistic period did not hesitate to move their hives over rather long distances to maximise production, taking advantage of the different vegetative cycles in different regions (Bresson., 2015).

In the absence of sugar, honey was an integral sweetening ingredient in Greek and Roman cuisine. During Roman times, honey was part of many recipes and it is mentioned in the work of many authors, such as Virgil, Pliny, Cicero, and others.

The spiritual and therapeutic use of honey in ancient India is documented in both the Vedas and the Ayurveda texts, which were both composed at least 4,000 years ago (PećanacM,et al., 2013). Beekeeping in ancient China has existed since ancient times and appears to be untraceable to its origin. In the book *Golden Rules of Business Success* written by Fan Li (or Tao Zhu Gong) during the spring and autumn period, some parts mention the art of beekeeping and the importance of the quality of the wooden box for beekeeping that can affect the quality of its honey.

Honey was also cultivated in ancient Mesoamerica. The Maya used honey from the stingless bee for culinary purposes, and continue to do so today. The Maya also regard the bee as sacred (see Mayan stingless bees of Central America).Some cultures believed honey had many practical health uses. It was used as an ointment for rashes and burns, and to help soothe the sore throats when no other practices were available.

2-4:Adulteration of bees honey:

Adulteration usually refers to mixing other matter (substance) of an inferior and sometimes harmful quality with food or drink intended to be sold. With companies concerned about the bottom line, the temptation to cheat is considerable, and unfortunately, the adulteration of honey is a serious economic and regulatory problem. As usual, the losers are the consumers and the processor or re-processor seeking to provide a wholesome product that meets regulatory standards. From an economic point of view, food product adulteration can destabilize the market by bringing in unfair competition (OJEC.,1974).

Honey adulteration appeared on the world market in the 1970 when high fructose can syrup was introduced by the industry. As the sugars (60.7-77.8%) are the major components of honey and the most dominant are the mono-saccharides fructose and glucose (accounting for 85-95%), the actual proportion of glucose to fructose in any particular honey depends largely on the source of nectar.

2-5: The means fraud of honey:

- Immaturity honey of any sort before maturity.
- Adding sucrose or invert sugar.
- Fraud sugar glucose.
- Fraud high fructose corn syrup.
- Term storage and thermal treatment of honey.
- Mixing natural honey with industrial honey.
- Add water to honey(www.na7la.com)

2-6: The global standard of honey analysis:

- The water content ratio of moisture
- Virtual reducing sugars content.
- Sucrose content.
- Solid content and insoluble in water.
- Ash minerals ratio.
- Acidity.
- Diastase activity.
- Hydroxyl methyl furfural.

2-7: Fungi:

A **fungus** (plural: **fungi** or **funguses)** is any member of the group of eukaryotic organisms that includes microorganisms such as yeasts and molds, as well as the more familiar mushrooms. These organisms are classified as a kingdom, **Fungi**, which is separate from the other eukaryotic life kingdoms of plants and animals.

A characteristic that places fungi in a different kingdom from plants, bacteria, and some protists is chitin in their cell walls. Similar to animals, fungi are heterotrophs; they acquire their food by absorbing dissolved molecules, typically by secreting digestive enzymes into their environment. Fungi do not photosynthesis. Growth is their means of mobility, except for spores (a few of which are flagellated), which may travel through the air or water. Fungi are the principal decomposers in ecological systems. These and other differences place fungi in a single group of related organisms, named the Eumycota (true fungi or Eumycetes), which share a common ancestor (form a monophyletic group), an interpretation that is also strongly supported by molecular phylogenetics. This fungal group is distinct from the structurally similar myxomycetes (slime molds) and oomycetes (water molds). The discipline of biology devoted to the study of fungi is known as mycology (from the Greek μύκης*mykes*, meaning "fungus"). In the past, mycology was regarded as a branch of botany, although it is now known fungi are genetically more closely related to animals than to plants.

2-7-1:Entomology:

The English word *fungus* is directly adopted from the Latinfungus (mushroom), used in the writings of Horace and Pliny This in turn is derived from the Greek word sphongos(σφογγος "sponge"), which refers to the macroscopic structures and morphology of mushrooms and moldsthe root is also used in other languages, such as the German Schwamm ("sponge") and Schimmel ("mold")|(Mitzka.,1960) . The use of the word mycology, which is derived from the Greek mykes(μύκης "mushroom") and logos (λόγος "discourse"), to denote the scientific study of fungi is thought to have originated in 1836 with English naturalist Miles Joseph Berkeley's publication The English Flora of Sir James Edward Smith, Vol. 5 A group of all the fungi present in a particular area or geographic region is known as mycobiota(plural noun, no singular), e.g., "the mycobiota of Ireland"

2-7-2:Diversity:

Fungi have a worldwide distribution, and grow in a wide range of habitats, including extreme environments such as deserts or areas with high salt concentrations(VaupoticT,et al.,2008) or ionizing radiation, as well as in deep sea sediments. Some can survive the intense.UVand cosmic radiation encountered during space travel. Most grow in terrestrial environments, though several species live partly or solely in aquatic habitats, such as the chytrid fungus *Batrachochytriumdendrobatidis*, a parasite that has been responsible for a worldwide decline in amphibian populations. This organism spends part of its life cycle as a motile zoospore, enabling it to propel itself through water and enter its amphibian host. Other examples of aquatic fungi include those living in hydrothermal areas of the ocean (Le Calvez T and Burgaud G., 2009).

Around 120,000 species of fungi have been described by taxonomists, but the global biodiversity of the fungus kingdom is not fully understood. A 2017 estimate suggests there may be between 2.2 and 3.8 million species. In mycology, species have historically been distinguished by a variety of methods and concepts. Classification based on morphological characteristics, such as the size and shape of spores or fruiting structures, has traditionally dominated fungal taxonomy. Species may also be distinguished by their biochemical and physiological characteristics, such as their ability to metabolize certain biochemicals, or their reaction to chemical tests. The biological species concept discriminates species based on their ability to mate. The application of molecular tools, such as DNA sequencing and phylogenetic analysis, to study diversity has greatly enhanced the resolution and added robustness to estimates of genetic diversity within various taxonomic groups.

2-7-3:Growth and physiology:

The growth of fungi as hyphae on or in solid substrates or as single cells in aquatic environments is adapted for the efficient extraction of nutrients, because these growth forms have high surface area to volume ratios (Moss ST.,1986). Hyphae are specifically adapted for growth on solid surfaces, and to invade substrates and tissues (Penalva MA. and ArstHN., 2002). They can exert large penetrative mechanical forces; for example, many plant pathogens, including *Magnaporthegrisea*, form a structure called an appressorium that evolved to puncture plant tissues. The pressure generated by the appressorium, directed against the plant epidermis, can exceed 8 megapascals(1,200 psi).The filamentous fungus *Paecilomyceslilacinus*uses a similar structure to penetrate the eggs of nematodes.

The mechanical pressure exerted by the appressorium is generated from physiological processes that increase intracellular turgor by producing osmolytes such as glycerol.Adaptations such as these are complemented by hydrolytic enzymes secreted into the environment to digest large organic molecules such as polysaccharides, proteins, and lipids into smaller molecules that may then be absorbed as nutrients. The vast majority of filamentous fungi grow in a polar fashion (extending in one direction) by elongation at the tip (apex) of the hypha . Other forms of fungal growth include intercalary extension (longitudinal expansion of hyphal compartments that are below the apex) as in the case of some endophyticfungi or growth by volume expansion during the development of mushroom stipes and other large organs (Money NP., 2002). Growth of fungi as multicellular structures consisting of somatic and reproductive cells a feature independently evolved in animals and plantshas several functions, including the development of fruit bodies for dissemination of sexual spores and biofilms for substrate colonization and intercellular communication.

2-7-4:Mycology:

Mycology is the branch of biology concerned with the systematic study of fungi, including their genetic and biochemical properties, their taxonomy, and their use to humans as a source of medicine, food, and psychotropic substances consumed for religious purposes, as well as their dangers, such as poisoning or infection. The field of phytopathology, the study of plant diseases, is closely related because many plant pathogens are fungi (Struck C.,2006).

The use of fungi by humans dates back to prehistory; Ötzi the Iceman, a well-preserved mummy of a 5,300-year-old Neolithic man found frozen in the Austrian Alps, carried two species of polypore mushrooms that may have been used as tinder (*Fomesfomentarius*), or for medicinal purposes (*Piptoporusbetulinus*). Ancient peoples have used fungi as food sources–often unknowingly–for millennia, in the preparation of leavened bread and fermented juices. Some of the oldest written records contain references to the destruction of crops that were probably caused by pathogenic fungi.

2-7-5:Reproduction:

Fungal reproduction is complex, reflecting the differences in lifestyles and genetic makeup within this diverse kingdom of organisms. It is estimated that a third of all fungi reproduce using more than one method of propagation; for example, reproduction may occur in two well-differentiated stages within the life cycle of a species, the teleomorph and the anamorph. Environmental conditions trigger genetically determined developmental states that lead to the creation of specialized structures for sexual or asexual reproduction. These structures aid reproduction by efficiently dispersing spores or spore-containing propagules.

2-7-6:Taxonomy:

Although commonly included in botany curricula and textbooks, fungi are more closely related to animals than to plants and are placed with the animals in the monophyletic group of opisthokonts (Shalchian-TabriziK,etal.,2008).Analyses using molecular phylogenetics support a monophyletic origin of the Fungi. The taxonomy of the Fungi is in a state of constant flux, especially due to recent research based on DNA comparisons. These current phylogenetic analyses often overturn classifications based on older and sometimes less discriminative methods based on morphological features and biological species concepts obtained from experimental matings.

2-7-7:Ecology:

Although often inconspicuous, fungi occur in every environment on earth and play very important roles in most ecosystems. Along with bacteria, fungi are the major decomposers in most terrestrial (and some aquatic) ecosystems, and therefore play a critical role in biogeochemical cycles and in many food webs. As decomposers, they play an essential role in nutrient cycling, especially as saprotrophs and symbionts, degrading organic matter to inorganic molecules, which can then re-enter anabolic metabolic pathways in plants or other organisms.

2-7-8:Mycotoxins:

Many fungi produce biologically active compounds, several of which are toxic to animals or plants and are therefore called mycotoxins. Of particular relevance to humans are mycotoxins produced by molds causing food spoilage, and poisonous mushrooms (see above).

Particularly infamous are the lethal amatoxins in some *Amanita* mushrooms, and ergot alkaloids, which have a long history of causing serious epidemics of ergotism (St Anthony's Fire) in people consuming rye or related cereals contaminated with sclerotia of the ergot fungus, *Clavicepspurpurea*. Other notable mycotoxins include the aflatoxins, which are insidious liver toxins and highly carcinogenic metabolites produced by certain *Aspergillus* species often growing in or on grains and nuts consumed by humans, ochratoxins, patulin, and trichothecenes (e.g., T-2 mycotoxin) and fumonisins, which have significant impact on human food supplies or animal livestock (van Egmond HP,etal.,2007).

2-7-9:Human use:

The human use of fungi for food preparation or preservation and other purposes is extensive and has a long history. Mushroom farming and mushroom gathering are large industries in many countries. The study of the historical uses and sociological impact of fungi is known as ethnomycology. Because of the capacity of this group to produce an enormous range of natural products with antimicrobial or other biological activities, many species have long been used or are being developed for industrial production of antibiotics, vitamins, and anti-cancer and cholesterol-lowering drugs. More recently, methods have been developed for genetic engineering of fungi, enabling metabolic engineering of fungal species. For example, genetic modification of yeast specieswhich are easy to grow at fast rates in large fermentation vessels has opened up ways of pharmaceutical production that are potentially more efficient than production by the original source organisms (Huang B,etal .,2008).

2-7-10: Pathogenic mechanisms:

Ustilagomaydis is a pathogenic plant fungus that causes smut disease in maize and teosinte. Plants have evolved efficient defense systems against pathogenic microbes such as *U. maydis*. A rapid defense reaction after pathogen attack is the oxidative burst where the plant produces reactive oxygen species at the site of the attempted invasion. *U. maydis* can respond to the oxidative burst with an oxidative stress response, regulated by the gene *YAP1*. The response protects *U. maydis* from the host defense, and is necessary for the pathogen's virulence. Furthermore, *U. maydis* has a well-established recombinationalDNA repair system which acts during mitosis and meiosis. The system may assist the pathogen in surviving DNA damage arising from the host plant's oxidative defensive response to infection.

Cryptococcus neoformans is an encapsulated yeast that can live in both plants and animals. *C. neoformans* usually infects the lungs, where it is phagocytosed by alveolar macrophages. Some *C. neoformans* can survive inside macrophages, which appears to be the basis for latency, disseminated disease, and resistance to antifungal agents. One mechanism by which *C. neoformans* survives the hostile macrophage environment is by up-regulating the expression of genes involved in the oxidative stress response.Another mechanism involves meiosis. The majority of *C. neoformans* are mating "type a". Filaments of mating "type a" ordinarily have haploid nuclei, but they can become diploid (perhaps by endoduplication or by stimulated nuclear fusion) to form blastospores. The diploid nuclei of blastospores can undergo meiosis, including recombination, to form haploid basidiospores that can be dispersed. This process is referred to as monokaryotic fruiting. This process requires a gene called DMC1, which is a conserved homologue of genes recA in bacteria and RAD51in eukaryotes that mediates homologous chromosome pairing during meiosis and repair of DNA double-strand breaks. Thus, *C. neoformans* can undergo a meiosis, monokaryotic fruiting, that promotes recombinational repair in the oxidative, DNA damaging environment of the host macrophage, and the repair capability may contribute to its virulence (MichodRE,et al.,2008).

2-8:*Aspergillus niger:*

Aspergillusniger is a fungus and one of the most common species of the genus *Aspergillus*.

It causes a disease called **black mould** on certain fruits and vegetables such as grapes, apricots, onions, and peanuts, and is a common contaminant of food. It is ubiquitous in soil and is commonly reported from indoor environments, where its black colonies can be confused with those of *Stachybotrys*(species of which have also been called "black mould") (Samson RA,etal., 2001).

2-8-1:Scientific classification

Kingdom:	Fungi
Division:	Ascomycota
Class:	Eurotiomycetes
Order:	Eurotiales
Family:	Trichocomaceae
Genus:	*Aspergillus*
Species:	***A. niger***

2-8-2: Pathogenicity:

2-8-2-1: Plant disease:

A. niger causes black mold of onions and ornamental plants. Infection of onion seedlings by *A. niger* can become systemic, manifesting only when conditions are conducive. *A. niger* causes a common postharvest disease of onions, in which the black conidia can be observed between the scales of the bulb. The fungus also causes disease in peanuts and in grapes.

2-8-2-2: Human and animal disease:

A. niger is less likely to cause human disease than some other *Aspergillus* species. In extremely rare instances, humans may become ill, but this is due to a serious lung disease, aspergillosis , that can occur. Aspergillosis is, in particular, frequent among horticultural workers who inhale peat dust, which can be rich in *Aspergillus* spores. It has been found in the mummies of ancient Egyptian tombs and can be inhaled when they are disturbed (Handwerk and Brian., 2005)

A. niger is one of the most common causes of otomycosis (fungal ear infections), which can cause pain, temporary hearing loss, and, in severe cases, damage to the ear canal and tympanic membrane.

2-8-3: Industrial uses:

A. niger is cultured for the industrial production of many substances. Various strains of *A. niger* are used in the industrial preparation of citric acid (E330) and gluconic acid (E574) and have been assessed as acceptable for daily intake by the World Health Organisation. *A. niger*fermentation is "generally recognized as safe" (GRAS) by the

United States Food and Drug Administration under the Federal Food, Drug, and Cosmetic Act.

Many useful enzymes are produced using industrial fermentation of *A. niger*. For example, *A. niger*glucoamylase is used in the production of high-fructose corn syrup, and pectinases are used in cider and wine clarification. Alpha-galactosidase, an enzyme that breaks down certain complex sugars, is a component of Beano and other products that decrease flatulence. Another use for *A. niger* within the biotechnology industry is in the production of magnetic isotope-containing variants of biological macromolecules for NMR analysis.

A. niger growing from gold-mining solution contained cyano-metal complexes, such as gold, silver, copper, iron, and zinc. The fungus also plays a role in the solubilization of heavy-metal sulfides (Samson RA,etal .,2001) Alkali-treated *A. niger* binds to silver to 10% of dry weight. Silver biosorbtion occurs by stoichiometric exchange with Ca (II) and Mg (II) of the sorbent.

2-9: Pea nut:

The **peanut,** also known as the **groundnut** and the **goober**and taxonomically classified as ***Arachishypogaea,*** is a legume crop grown mainly for its edible seeds. It is widely grown in the tropics and subtropics, being important to both small and large commercial producers. It is classified as both a grain legume(www.hort.purdue.edu.) and, because of its high oil content, an oil crop. World annual production of shelled peanuts was 42 million tonnes in 2014. Atypically among crop plants, peanut pods develop underground rather than aboveground. It is this characteristic that the botanist Linnaeus used to assign the specific name *hypogaea*, which means "under the earth."

As a legume, the peanut belongs to the botanical family Fabaceae; this is also known as Leguminosae, and commonly known as the *bean*, or *pea*, family. Like most other legumes, peanuts harbor symbiotic nitrogen-fixing bacteria in root nodules (www.kew.org.) . This capacity to fix nitrogen means peanuts require less nitrogen-containing fertilizer and improve soil fertility, making them valuable in crop rotations.

Peanuts are similar in taste and nutritional profile to tree nuts such as walnuts and almonds, and are often served in similar ways in Western cuisines. The botanical definition of a "nut" is a fruit whose ovary wall becomes very hard at maturity. Using this criterion, the peanut is not a true nut, but rather a legume. However, for culinary purposes and in common English language usage, peanuts are usually referred to as nuts.

2-9-1: Scientific classification:

Kingdom:	Plantae
phylum:	Magnoliophyta
class:	Magnoliopsida
Order:	Fabales
Family:	Fabaceae
Subfamily:	Faboideae
Tribe:	Dalbergieae
Genus:	*Arachis*
Species:	***A. hypogaea***

2-9-2:History:

Cultivated peanut (*A. hypogaea*) arose from a hybrid between two wild species of peanut, thought to be *A. duranensis* and *A. ipaensis*. The initial hybrid would have been sterile, but spontaneous chromosome doubling restored its fertility, forming what is termed an amphidiploid or allotetraploid. Genetic analysis suggests the hybridization event probably occurred only once and gave rise to *A. monticola*, a wild form of peanut that occurs in a few restricted locations in northwestern Argentina, and by artificial selection to *A. hypogaea* (Husted and Ladley.,1936). The process of domestication through artificial selection made *A. hypogaea* dramatically different from its wild relatives. The domesticated plants are bushier and more compact, and have a different pod structure and larger seeds. The initial domestication may have taken place in northwestern Argentina, or in southeastern Bolivia, where the peanut landraces with the most wild-like features are grown today. From this primary center of origin, cultivation spread and formed secondary and tertiary centers of diversity in Peru, Ecuador, Brazil, Paraguay, and Uruguay. Over time, thousands of peanut landraces evolved; these are classified into six botanical varieties and two subspecies (as listed in the peanut scientific classification table). Subspecies *A. h. fastigiata* types are more upright in their growth habit and have shorter crop cycles. Subspecies *A. h. hypogaea* types spread more on the ground and have longer crop cycles.

The oldest known archeological remains of pods have been dated at about 7,600 years old. These may be pods from a wild species that was in cultivation, or *A. hypogaea* in the early phase of domestication. They were found in Peru, where dry climatic conditions are favorable to the preservation of organic material. Almost certainly, peanut cultivation antedated this at the center of origin where the climate is moister. Many pre-Columbian cultures, such as the Moche, depicted peanuts in their art. Cultivation was well established in Mesoamerica before the Spanish arrived. There, the conquistadors found the *tlalcacahuatl* (the plant's Nahuatl name, whence Mexican Spanish *cacahuate*, Castillian Spanish *cacahuete*, and French *cacahuète*) being offered for sale in the marketplace of Tenochtitlan. The peanut was later spread worldwide by European traders, and cultivation is now very widespread in tropical and subtropical regions. In West Africa, it substantially replaced a crop plant from the same family, the Bambara groundnut, whose seed pods also develop underground. In Asia, it became an agricultural mainstay and this region is now the largest producer in the world.

In the English-speaking world, peanut growing is most important in the United States. Although it was mainly a garden crop for much of the colonial period, it was mostly used as animal feed stock until the 1930s (Putnam D.H .,etal,1991) .The US Department of Agriculture initiated a program to encourage agricultural production and human consumption of peanuts in the late 19th and early 20th centuries. George Washington Carver developed hundreds of recipes for peanuts during his tenure in the program.

2-9-3: Botany:

Peanut is an annual herbaceous plant growing 30 to 50 cm (1.0 to 1.6 ft) tall. As a legume, it belongs to the botanical family Fabaceae (also known as Leguminosae, and commonly known as the bean or pea family). Like most other legumes, peanuts harbor symbiotic nitrogen-fixing bacteria in their root nodules. The leaves are opposite and pinnate with four leaflets (two opposite pairs; no terminal leaflet); each leaflet is 1 to 7 cm (⅜ to

2¾ in) long and 1 to 3 cm (⅜ to 1 in) across. Like many other legumes, the leaves are nyctinastic, that is, they have "sleep" movements, closing at night.

Peanut pods develop underground, an unusual feature known as geocarpy (Smith and Ben W., 1950). The flowers are 1.0 to 1.5 cm (0.4 to 0.6 in) across, and yellowish orange with reddish veining. They are borne in axillary clusters on the stems above ground and last for just one day. The ovary is located at the base of what appears to be the flower stem but is actually a highly elongated floral cup. After fertilization, a short stalk at the base of the ovary (termed a pedicel) elongates to form a thread-like structure known as a "peg". This peg grows down into the soil, and the tip, which contains the ovary, develops into a mature peanut pod. Pods are 3 to 7 cm (1.2 to 2.8 in) long, normally containing one to four seeds.

CHAPTER:THREE

Materials and method

3-1: Site Location:

The experiment was conducted in the laboratory of the Pathology and JICA laboratory the Sudan University of Science and Technology,College of Agricultural Studies.

3-2: Theexperimental materials:

3-2-1: Peanuts:

Peanut (*Arachishypogaea*) is an herbaceous annual plant in the family Fabaceae grown for its oil and edible nuts. Peanut plants are small, usually erect.

3-2-2: Two samples of natural honey from River Nile State:

3-2-2-1: Honey:

Honey is naturally sweet mixture produced by bees from nectar of flowers, from secretion of the living plants or excretion of plant sucking insect on the living part of plant that the honey bee collect.

3-2-2-2:River Nile State:

Located to the north of Khartoum state, which is the capital of the Sudan,between latitudes 16-22 north latitude and east of 30-32 and has an area of 124 thousand square kilometers, equivalent to 29.5 million acres .The climate in it semi-desert and rain ranging from 150mm to 25mm in the south to the north in the year and temperatures ranging from 47 degrees in the summer to a maximum of 8 degrees minimum in winter.

3-2-3:*Aspergillusniger:*

Aspergillusniger is a fungus and one of the most common species of the genus Aspergillus. It causes a disease called **black mould** on certain fruits and vegetables such as grapes, apricots, onions, and peanuts, and is a common contaminant of food.

Plate (1):Experiment material

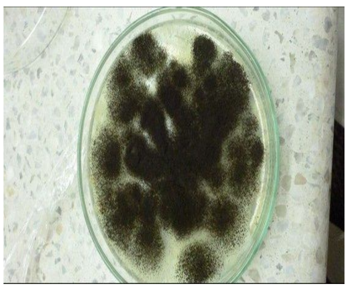


Plate (A): Aspergillusniger**Plate (B):** Peanut

Plate (C): Honey

Plate (2): Laboratory Equipments


Plate(A): sensitive balance**Plate (B):**Petri dish

Plate (C): Filter paper**Plate (D):** Gloves

Plate (E): Forceps**Plate (F):** Laminar flow

Plate (G): Oven

3-3: Methodology:

Theexperimental design was completely randomized, with two replications of (10 seeds). The treatment consisted of treating the seeds with natural honey from Al-Damer and natural honey from Shandi.

I use nine petri-dishes in my experimental, in any petri dish I put ten seeds of peanuts.The first three petri dish treated with Al-Damer honey (2gm), other three treated with Shandi honey (2gm) and the last three petri dish considered the control.

And then immediately subjected to the following tests: growth the fungi, length of growth and type of fungi are growing.

3-4: Statistical analyses:

The obtained data was statistically analyses by mustatc microcomputer program according to analysis of variance (ANOVA) -Duncan's Multiple Range Test was used for mean separation.

CHAPTER: FOUR

Results

4-1: The moisture of River Nile State bee honey (Aldamer and Shandi):

The analysis was conducted in JICA laboratory in collage of agriculture studies, Sudan University of Sciences and technology. The equipment used for analysis sensitive balance, oven and refractometer. The moisture of Shandi honey is 21.4% and Aldamer is 18.9%.

4-2:The efficacy of River Nile State bee honey against fungi:*Aspergillusniger*during (13-April to 20- April):

The table(1) indicates to the signification different on germination of fungi in all experimental.

Note that there are no fungal growth during the experiment week in Shandi honey and Aldamer honey and remained as it is until the end of the experiment, which indicates that this bee honey is not adulterated, according to the results are good bee honey, indicating that this bee honey is of high quality.

However, the treatments considerd as control indicate that there were too many macrophages of the fungus and those fungi that accompanied the experiment is *Aspergillusflavus* , *Aspergillusniger, Penicilliumdigitatum and Rhizopusnigricans* , where the injury rate of *Aspergillusniger* was up to 75%.

Table (1):The efficacy of River Nile State bee honey against fungi: *Aspergillusniger*during (13-April to 20- April).

TREM	D1	D2	D3	D4
Control	62.500 A	60.000 A	50.000 A	93 A
Shandi honey	0.0000 B	0.0000 B	0.0000 B	0 B
Aldamer honey	0.0000 B	0.0000 B	0.0000 B	0 B
CV	4	4.4	4.5	21.4
LSD	27.5	26.1	23.8	13.3
SE	12.1	11.5	10.5	5.4

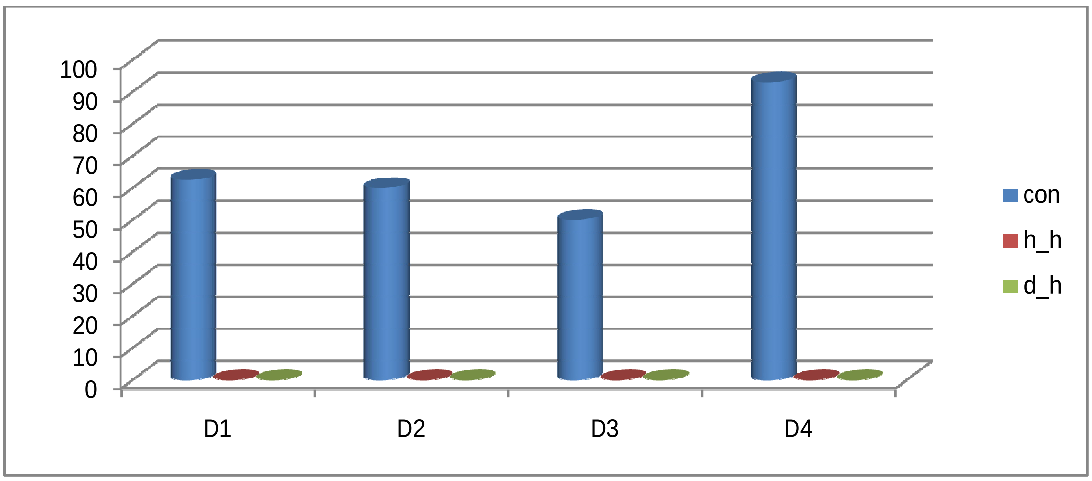

Figure (1): Effect of treatments on germination

CHAPTER: FIVE

5-1: Discussion

The results of this study for the fungi Aspergillusniger as showed in table (1) for sample one (pure bee honey from Shandi) and sample two (pure bee honey from Aldamer) indicated positive reaction on the growth of fungi Aspergillusniger.

In six petri dishes thatnote that there are no fungal growth during the experiment week in Shandi honey and Aldamer honey and remained as it is until the end of the experiment, which indicates that this bee honey is not adulterated, according to the results are good bee honey, indicating that this bee honey is of high quality.

However, the treatments considerd as control indicate that there were too many macrophages of the fungus.

5-2:Conclusions:

Controlobtained a higher growth of fungi rate than other treatments and the River Nile State bee honey there is no any growth of fungi.

5-3:Recommendation:

The natural bee honey is good to protect the food, seeds and other nutrient materials from fungi, bacteria and other micro-organisms , and I recommended to use the bee honey to fight fungi and other micro-organisms.

5-4:References:

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Appendages (1):