Aim of the study

The aim of this Study to evaluate antifungal properties of some Egyptian wild and medicinal plants extracts and essential oils to many phytopathogenic fungal isolates, a causal agents of destructive diseases for economical crops, vegetables and fruits

In addition, to preliminary screening and analyze of phytoconstituents of wild and medicinal plants oils and extracts for detecting most biologically active compounds which environmental safe controlling the investigated fungal isolates

Specific objectives

The following specific objectives were achieved:

1- To collect and botanical identify of wiled and medicinal plants.
2- To preparation of volatile oils and methanolic and aqueous extracts of the selected plants
3- To isolation and Molecular identification of phytopathogenic fungal species
4- To measure the minimum inhibitory concentration (MIC) of the selected plant extracts against the tested isolates
5- To found a functional natural plant extract for controlling fungal diseases in agricultural crops

II. REVIEW OF LITERATURE

II.1. Fungal Plant Pathogens and diseases

Fungi are eukaryotic, normally filamentous, microscopic organisms that lack chlorophyll and often reproduce via sexual (or asexual) spores able to spread via air or water. However, many members of a large group of fungi. These fungi spread by hyphal growth and fragments. Like plants, fungi have cell walls but the wall is built from chit in and glucan elements, sugars and glycoproteins. Most of the known fungi (over 100 000 species) are strict saprophytes, that live on dead organic material, but over 10 000 fungal species are known to cause diseases on plants [51]. The majority of plant pathogenic fungi spend at least part of their life outside host, but in biotrophs, only inactive, ungerminated spores are able to survive on soil.

Many airborne pathogens are biotrophic. These pathogens are spread by wind-carried asexual spores, conidia, which infect above ground parts of the plant. Production of many important crop plants is annually jeopardised due to the infection pressure of foliar biotrophic pathogens causing rusts, and powdery and downy mildews [52, 53].

Throughout history, one of the most devastating airborne plant disease outbreaks in Europe was potato famine caused by late blight (oomycete Phytophthora infestans) in the mid 19th century [54]. Besides appropriate cultivation practices, fungicide sprays and computer-based modelling of the infection pressure [55], intensive conventional and molecular breeding programmes have been employed in order to improve crop resistances against various obligate and non-obligate parasitic fungi, with variable degrees of success [56].

The host range of soilborne necrotrophic fungi is considerably wider than that of airborne bio- or hemi-biotrophs, which are more dependent for proliferation on a suitable host plant [57]. For example, soilborne necrotrophic Sclerotinia sclerotiorum can infect hundreds of hosts [58]. Fortunately, specific host parasitism also occurs among necrotrophs, and then the population levels of the necrotrophic fungus tend to decline in the absence of the proper hosts [59, 5 1].

In order to infect a host plant, a pathogenic fungus needs to grow into close proximity of the host and enter the plant tissue to feed. Some fungi grow only on the surface of the host, whereas some enter the plant via natural openings [51]. Depending on the fungus, its feeding structures, haustoria, can grow inter- or intracellularly after breakdown of the cell wall [60]. To obtain nutrients, the fungus has to pass through the rigid cell wall and this is accomplished by producing hydrolytic enzymes [61] or by using mechanical force (biotrophs and hemi-biotrophs) [62]. In general, fungi that need a living host rarely produce enzymes, in order to prevent cell death, whereas for necrotrophs, the fate of the host is unimportant [51]. Fungi are one of the most important groups of plant pathogens. To date, there are about 10,000 species of fungi, that can cause diseases in plants. All plants are attacked by some kinds of fungi, and each of the parasitic fungi can attack one or many kinds of plants [63, 64]. Some species of fungi, the mycorrhizae, live symbiotically on or in the roots of many plants. This relationship is basically parasitic but in many situations is probably beneficial to both the plant and the fungus. The growth of the plant ispromoted by the improved uptake of some mineral nutrients whiles the fungus gains access to organic nutrients and shelter [64].

The majority of phytopathogenic fungi belong to the Ascomycetes and the Basidiomycetes. Fungi are reproduced both sexually and asexually by production of spores that may be spread long distances by air or water or may be soil borne. Fungi are common in soil, in air (mainly as spores) and on plant surfaces throughout the world [64].

II.1.1. Soil-Borne plant diseases

Soil-borne pathogenic fungi cause diseases on roots and other underground plant organs such as stolons, tubers and basal parts of the stems. Compared to airborne pathogenic fungi, soil-borne pathogens have wider host range and they persist longer without suitable hosts. Typical symptoms of the soil-borne diseases are damping-off of seedlings, wilting of adult plants, and death of the aboveground plant parts due to the decay of the root system and breakdown of the flow of water and nutrients [51]. The

incidence of plant infection with soil-borne pathogens increases during relatively cool and wet weather that, at the same time, slows infection by many airborne pathogens that spread better during dry conditions. Control of soil-borne diseases is relatively more laborious than that of airborne ones, since the pathogen starts to do the damage while hidden in the soil and the disease outbreak may become visible too late for effective plant protection. For example, Pythium spp., Fusarium spp., Sclerotinia *sclerotiorum* and *Rhizoctonia solani* cause significant losses on crop quantity and quality of many crop species annually [65-69, 58].

Many soil-borne fungal pathogens are known to cause disease on a large number of crop plants; soil borne fungi represent causal agents of legume diseases of increasing economic importance such as root rots, seedling damping-off and vascular wilts. In comparison to plant responses to foliar pathogens relatively little is known about responses to root infecting pathogens, primarily due to the difficulty in observing the early stages of the interaction and attaining synchronous infection for gene expression studies [70]. Soil-borne fungi survive in the soil and in residues on the soil surface; they attack plant penetrating through lesions into the roots or collets. Thus the soil is a reservoir of inoculums of these pathogens, the majority of which are widely distributed in agricultural soils [64].

These diseases are difficult to control because they are caused by pathogens which can survive for long periods in the absence of the normal crop host and often have a wide host range including weeds; chemical control often does not work well or is not practical or too expensive and it is difficult to develop resistant varieties of plants. These diseases are often very difficult to diagnose accurately. Damages to root and crown tissues is hidden in the soil. Thus, these diseases may not be noticed until the above-ground (foliar) parts of the plant are affected severely showing symptoms such as stunting, wilting, chlorosis and death [64].

II.1.1.1.Genus *Fusarium*

Fusarium is a large genus of filamentous fungi with over 1000 species being recognized by various taxonomists during the past 100 years depending on the species concept employed [71, 72]. The fungus can be soil-borne, airborne, or carried in plant residue. Most species are harmless saprobes and are relatively abundant members of the soil microbial community, but the genus also includes a number of economically important plant pathogenic species. *Fusarium oxysporum* causes *Fusarium* wilt in more than 100 plant species. It does so by colonizing the water-conducting vessels (xylem) of the plant, leading to leaf wilting, yellowing and eventually plant death. [73].

Fusarium species may produce three types of spores called macroconidia, microconidia, and chlamydospores [71, 70]. Some species produce all three types of spores, while other species do not.

The morphology of the macroconidia and the conidiophores bearing the microconidia as well as the presence or absence of microconidia and chlamydospores are primary characters used to separate species in *Fusarium* taxonomy [70].

The International Maize and Wheat Improvement Center have appointed *Fusarium* Head Blight (FHB) as one of the major diseases threatening harvests and human health worldwide. This dual threat is due in part to serious crop losses in different cereals, and otherwise to the plethora of mycotoxins produced by the *Fusarium* species associated with FHB. To date, at least 18 species and sibling species complexes in *Fusarium* have been found to be able to cause FHB [58]. Morphologically the Fusaria are difficult to distinguish from each other, strainswith similar morphology representing different biological groups composed of saprobes, endophytes and plant pathogens [59]. Closely similar species may vary in fungicide sensitivity and mycotoxin production [60]

II.1.1.1.1. *Fusarium oxysporum*

The most ubiquitous; prevalent and adaptable soil-borne pathogen *Fusarium oxysporum* causes serious losses in protected agricultural production areas all over the world. Since control of the pathogen is not possible or is very difficult using chemicals

and cultural methods, it is imperative to find an effective control method. Infectious diseases have long been a major threat to food security directly relevant to a world population that has been growing at an annual rate of 1.2%, i.e. 77 million people per year [74].

Fusarium oxysporum is the causal agent of *Fusarium* wilt. Compared to other soil borne fungal pathogens, the wilt pathogens have a more specialized host range (classified as forma speciales) and are adapted to grow in the vascular system of their host. The *Fusarium* vascular wilt is a disease of many agricultural and horticultural crops, including banana, tomato, cotton, chickpea, lupin, among others. [75].

In solid media culture, such as potato dextrose agar (PDA), the different special forms of *F. oxysporum* can have varying appearances. In general, the aerial mycelium first appears white, and then may change to a variety of colors - ranging from violet to dark purple according to the strain (or special form) of *F. oxysporum* and The fungus is known to produce sparse to abundant aerial mycelium [76] .The fungus is known to produce white, pink, salmon and purple pigmentation on the reverse side of the colony in culture [77, 78].

II.1.1.1.2. *Fusarium solani*

The root-rot diseases caused by soil-borne fungi are one of the most common diseases of cucumbers *(Cucumis sativus L.*) grown under both field and greenhouse conditions **[79]**. Diseases caused by *Fusarium solani* are a limiting factor in plant production, and yield quantity. *F. solani* causes the death of young and adult plants, with consequent economic losses **[80]**.*Fusarium solani*, the causal agent of storage rot of potato tuber, generally occurs as dry powdery white colony superficially on the infected parts. The fungus gains entry through eye spots or, wounds or, through lesions caused by other fungi or bacteria. The disease develops more rapidly under the conditions of high humidity and at temperature around 200C. In culture, the colonies are whitish brown with numerous micro conidia and macro conidia. Conidiophores are short and hyaline which bear conidia. Micro conidia are oval with somewhat thicker walls, hyaline measuring 8-

16×2-4 μ and may be aseptate or single septate. Macro conidia develop after 4-7 days which bear tapering ends with widest centre [81].

II.1.1.1.3. *Fusarium brachygibbosum*

A dark brown to black leaf spots were observed on evergreen ornamental oleander leaves (*Nerium oleander*) in the Yazd province, Iran. As disease progressed, leaf spots increased from 10 to 20 mm in diameter and changed from circular to elliptical in shape. Three leaf spots were excised, surface- sterilized in 0.5% sodium hypochlorite and placed on potato dextrose agar (PDA). Colonies were initially white, and then turned from orange-yellow to medium red with abundant aerial mycelium. Macroconidia were rare and scattered with three to four septa; microconidia were slightly curved, ovoid, and fusiform with zero to two septa. Spherical chlamydospores were terminal and intercalary, single or in chains. Based on these characteristics, the fungus was identified as *Fusarium brachygibbosum*. Pathogenicity of the fungal isolate was proven by placing seven-day-old mycelial plugs (5 mm) grown on PDA on oleander leaves. Eight days after inoculation, leaf spots developed on the inoculated plants [82].

II.1.1.2. Genus *Rhizoctonia*

Genus *Rhizoctonia* is a highly heterogeneous group of filamentous fungi that share similarities in their anamorphic, sterile state. For example, they do not produce asexual spores and the teleomorphic, sexual state occurs only rarely. The group contains several economically important and global plant pathogens like *Rhizoctonia solani* [83].

II.1.1.2.1. *Rhizoctonia solani*

Rhizoctonia solani (R. solani) is an important fungal pathogen {84] that live in the soil in the form of sclerotia and do not generate asexual spores. The host range and distribuation of *R. solani* is wide and it causes various diseases on important crop plants of the world including species in the Solanaceae , Fabaceae , Asteraceae Poaceae and Braccicaceae as well as ornamental plants and forest trees [85]. Disease symptoms include leaf blights, leaf spots, damping-off, rots on roots, shoots and fruits, canker lesions on

sprouts and stolons, and sclerotial diseases. However, some *R. solani* strains form symbiotic mycorrhizal relationships with orchid plants [86, 87]

Rhizoctonia solani causes sheath blight in some field crops, such as corn [88-91] *Rhizoctonia* solani is the causal organism of Rhizoctonia disease complex in potato [92] resulting in two different appearances of the disease, namely stem canker and black scurf. These are recognized as necrotic lesions on underground plant parts, and sclerotia covering progeny tubers, respectively [59]. Early in the growing season, the fungus causes necrosis in emerging sprouts, killing the sprout tip and causing stem canker disease symptoms. Later in the season, *R. solani* attacks the developing stolons, preventing them from growing to their full length [66]. In the field, stem canker infection causes late emergence of the potato plants, lowers the stem number, shortens the length of the stolons resulting in misshapen progeny tubers with high size distribution, and lowers the processing quality of the potato [93]. Extensive greening of the tubers is also found since the short stolons do not allow tubers to form deep enough in the soil. Incidence of the disease is higher in cool and wet growing seasons [94]. The second form of the disease can be seen later in the season close to the harvest, when dark sclerotia start to cover maturing daughter tubers [95].

II.1.1.3. Genus *Aspergillus*

Species of the genus *Aspergillus* Section *Nigri* or the Black *Aspergillus* are widely distributed around the world and have a capacity of developing in a vast variety of substrates. Many species are able to cause deterioration of food although some of them are used in fermentation industries to produce organic acids, such as citric and gluconic acids, as well as hydrolytic enzymes like lipases and amylases [96, 97].

Species of *Aspergillus* are important medically and commercially. Some species can cause infection in humans and other animals. Some infections found in animals have been studied for years, while other species found in animals have been described as new and specific to the investigated disease, and others have been known as names already in

use for organisms such as saprophytes. More than 60 *Aspergillus* species are medically relevant pathogens [98].

II.1.1.3.1. *Aspergillus fumigates*

Aspergillus fumigatus is a fungus of the genus *Aspergillus*, and is one of the most common *Aspergillus* species to cause disease in individuals with an immunodeficiency. *A. fumigatus*, a saprotroph widespread in nature, is typically found in soil and decaying organic matter, such as compost heaps, where it plays an essential role in carbon and nitrogen recycling. Colonies of the fungus produce from conidiophores thousands of minute grey-green conidia (2–3 μm) that readily become airborne. For many years, *A. fumigatus* was not thought to only reproduce asexually, as neither mating nor meiosis had ever been observed. In 2008, however, *A. fumigatus* was shown to possess a fully functional sexual reproductive cycle, 145 years after its original description by Fresenius [99].

II.1.2. Food- Borne plant diseases

Post-harvest diseases are posing a major problem to the agriculture industry, where they account to about 50% losses in fruits stored in poor storage conditions [64]. Among of the most important fungi causing post-harvest diseases of plants are Aspergillus spp., Alternaria spp., and *Rhizopus stolonifer* [100]. *Alternaria solani* is the main pathogen causing early blight disease and yield losses in numerous economically important crop plants of family Solanaceae such as potato, tomato and eggplant crops [101, 102]. The early symptoms are in the form of small spots on the leaves which later on enlarge to form concentric rings. Also, this fungus can infect fruits and tubers in severe conditions [103]. Species of Aspergillus fungus are among the major reported fungi having the ability to produce mycotoxins during storage which reduce the quality of food products [104].*A. niger* is a saprophytic filamentous fungus found in soil, forage, organic debris and food product, causing black mould of onion and shallot; boll rot of cotton; spoilage of cashew kernels, dates, figs, vanilla and dried prune [105]. Rhizopus rot, caused

by *Rhizopus stoloniferis* one of the most destructive post-harvest diseases. The spores of *R. stoloniferare* very common in the atmosphere and this infection of fruits occur mainly at wound sites during harvest or packing [106]. *R. stolonifer* is reported to cause food spoilage and decay in fruits, particularly peaches, strawberries, raspberries and grapes [107].

Filamentous Fungi cause serious problems in food by producing mycotoxins and potentially allergenic spores, causing spoilage of food and food products that is costly as well as sometimes dangerous. Aspergillus and Penicillium species are commonly found as contaminants in foods throughout drying and subsequent storage [108, 109]. Filamentous fungi mainly, Aspergillus spp., Penicilliumspp, And Fusarium spp. produced mycotoxins, secondary metabolites, under the appropriate environmental conditions, are toxic [110].

Aflatoxins are carcinogenic and immunosuppressive metabolites produced by *Aspergilllus flavus* and *Aspergilllus parasiticus* when these fungi infect crops before and after harvest thereby contaminating food and feed and threatening both human and animal health [111, 112].These toxins have been incriminated as the cause of high mortality in livestock and some cases of death in human being [113, 114]. Among all classes of aflatoxins, aflatoxin B1is known to be the mostsignificant in terms of animal and human health risk [115] Thus, foods contaminated with these toxigenic fungi and presence of aflatoxins is a major concern which has received worldwide attention due to their deleterious effect on human and animal health as well as their importance in international food trade [116, 117].

Food safety is an increasingly important public health issue. Nearly, 30% people in the world suffer from food borne diseases every year caused by microbes [39, 118]. Most diseases in plants are caused by various pathogens including fungi; nematodes, bacteria, and viruses [119].Fungi are the major pathogens and a source of many diseases of plants. Pathogenic fungi also could decrease the growth of many economically important crops [120]. Moulds or microscopic filamentous fungi are ubiquitous microorganisms with a

great capacity to colonize many kinds of substrates and to proliferate under extreme environmental conditions [121, 122].

II.1.2.1. genus Aspergillus

II.1.2.1.1. A. flavus

Aspergillus flavus causes diseases of agronomically important crops, such as corn and peanuts, is second only to *Aspergillus fumigatus* as the cause of human invasive aspergillosis, and is the *Aspergillus* species most frequently reported to infect insects. It was demonstrated that most *Aspergillus flavus* strains can cause disease in both plants and animals. Many fungi moved from opportunistic forms to specialized pathogens by gaining the ability to produce host-selective toxins that provided the genetic isolation for evolutionary change. Although *Aspergillus flavus* produces a variety of toxins, including aflatoxins, the routine association of *Aspergillus flavus* with various plants and insects in an opportunistic fashion, as these nutritional resources temporarily become available, could explain why populations of *Aspergillus flavus* have not diverged into separate pathogenicity types. It is possible that *Aspergillus flavus* routinely infects both plants and animals with the insect acting as vector. In this scenario, the insect eventually serves as a substrate to create a very large inoculum to exploit insect damage in the plant [123]. The occurrence of *Aspergillus flavus* in field maize was first reported 75 years ago [124]. *Aspergillus flavus* and *Aspergillus parasiticus* are the predominant species responsible for aflatoxin contamination of crops prior to harvest or during storage [125].

II.1.2.1.2. *Aspergillus* oryzae

Aspergillus oryzae is an asexual, ascomycetous fungus used for hundreds of years in the production of soy sauce, miso and sake without recorded incidents. *Aspergillus oryzae* S is a filamentous fungus, which has an ability to secrete large amounts of hydrolytic enzymes. It is widely used in the manufacture of traditional fermented soy sauce in Asia. There are conflicting opinions about whether *A. oryzae* can be isolated in nature. Although the details of the genetic relationship between *A. oryzae* and *Aspergillus flavus* remain unclear, the two species are so closely related that all strains of *Aspergillus*

oryzae are regarded by some as natural variants of *A. flavus* modified through years of selection for fermenting of foods. *A. oryzae* is regarded as not being pathogenic for plants or animals, though there are a handful of reports of isolation of *Aspergillus oryzae* from patients. There are also several reports of products of *Aspergillus oryzae* fermentations, e.g. a-amylase, that seem to be associated with allergic responses in certain occupations with high exposure to those materials. *Aspergillus oryzae* can produce a variety of mycotoxins when fermentation is extended beyond the usual time needed for production of these foods. While wild *Aspergillus flavus* isolates readily produce aflatoxins and other mycotoxins, *Aspergillus oryzae* has not been shown to be capable of aflatoxin production.

Aspergillus oryzae has apparently been an essential part of oriental food production for centuries and is now used in the production of many different oriental foods such as soy sauce, sake and miso. Potential uses under TSCA include fermentations of numerous enzymes, e.g., amylase, protease, B-galactosidase, lipase, and cellulase, and organic compounds such as glutamic acid. While these products have a variety of potential commercial uses, some of them are mostly frequently used in food processing.

The experience of safe commercial use of *Aspergillus oryzae* is extraordinarily well established. As a "koji" mold it has been used safely in the food industry for several hundred years. *Aspergillus oryzae* is also used to produce livestock probiotic feed supplements. Even the commercialization of byproducts of the fermentation was established nearly a century ago. The "koji" mold enzymes were among the first to be isolated and commercialized [126, 127].

The form genus *Aspergillus* represents a taxonomic grouping of a very large number of asexual fungi which are characterized by the production of spores on large black or brown conidia in phialides arranged on a characteristic spherical conidiophore termed the vesicle. This definition leads to inclusion of a complex assortment of organisms within the taxon. To simplify the taxonomy of such a large number of organisms, the genus *Aspergillus* has been divided into sections or groups based on color,

size and roughness of the spore, conidiophore and vesicle as well as the arrangement of phialides and the presence of sclerotia. The separation of individual species into groups is somewhat tenuous and based on distinguishing measured characters with overlapping means. This resulted in the 132 species arranged in 18 groups by Raper and Fennell (1965) due to overlapping morphological or physiological characteristics. However, it is important to remember that taxonomy is "dealing with living variable organisms and that species and group concepts must be reasonably elastic" [128].

II.1.2.2. Genus Penicillium

Contamination of foodborne pathogens and spoilage microorganisms is of great concern in food industries. Fungi, especially *Penicillium* species and *Aspergillus* species, are among the major causes of food spoilage, especially bakery products intermediate-moisture food products, cheese, preserved fruit, and grain. Contamination of wheat bread was mainly Penicillium species (90-100%) and also Aspergillus species [129].

Penicillium is a genus of ascomycetous fungi of major importance in the natural environment as well as food and drug production. Members of the genus produce penicillin, a molecule that is used as an antibiotic, which kills or stops the growth of certain kinds of bacteria inside the body. According to the *Dictionary of the Fungi* [130], the widespread genus contains over 300 species. [109] the ability of these *Penicillium* species to grow on seeds and other stored foods depends on their propensity to thrive in low humidity and to colonize rapidly by aerial dispersion while the seeds are sufficiently moist.[93] Some species have a blue color, commonly growing on old bread and giving it a blue fuzzy texture. Some *Penicillium* species affect the fruits and bulbs of plants, including *P. expansum*, apples and pears; *P. digitatum*, citrus fruits; [131] and *P. allii*, garlic [132].

II.1.2.2.1. *Penicillium chrysogenum (notatum*

Penicillium chrysogenum is a fungus, common in temperate and subtropical regions and can be found on salted food products, [133] but it is mostly found in indoor

environments, especially in damp or waterdamaged buildings [134]. It was previously known as *Penicillium notatum*[135]. It has rarely been reported as a cause of human disease. It is the source of several β-lactam antibiotics, most significantly penicillin. Other secondary metabolites of *P. chrysogenum* include various penicillins, roquefortine C, meleagrin, chrysogine, xanthocillins, secalonic acids, sorrentanone, sorbicillin, and PR-toxin [136].

II.2. Biological control of fungal plant pathogens

II.2.1Chemical or synthetic fungicides

Pesticide use also plays a major role in modern agriculture, and has contributed to dramatic increases in crop yields over the past four decades for most field-grown fruit and vegetable crops. Nevertheless, these chemicals also pose some major risks if used improperly or too frequently. Many pesticides, even when applied legally and according to the label's instructions, may leave residues in or on treated fruits, vegetables, and grains as well as in soil [137]. The development of environmentally friendly alternatives to counter the extensive use of chemical pesticides is one of the biggest ecological challenges facing microbiologists and plant pathologists for combatting crop diseases [138].

II.2.2. Antagonistic Microorganisms (Biocontrol)

Beneficial microorganisms or biopesticides are considered to be one of the most promising methods for more rational and safe crop management practices [138]. There are also a number of plant diseases for which a chemical solution is ineffective or nonexistent. This, together with an increasing demand by consumers and the public for pesticide-free food, has driven the demand for biological control through the use of natural antagonistic microorganisms [139].

Mechanisms which biocontrol microorganisms use to weaken or destroy plant pathogens include their ability to parasitize the pathogens directly, production of antibiotics (toxins), their ability to compete for space and nutrients, production of enzymes that attack cell components of the pathogens, and induction of defense

responses in the plants they surround. Although thousands of microorganisms have been shown to interfere with the growth of plant pathogens and to provide some protection from the diseases, strains of relatively few microorganisms have been registered and are available commercially for use so far [64].

II.2.2.1. Bacterial antagonists to soil-borne diseases

Crown gall of pome, stone, and several small fruits (grapes, raspberries) and ornamentals caused by *Agrobacterium tumefaciens* can be controlled by nonpathogenic *Agrobacterium radiobacter* which produces a specific antibiotic. It is available as a commercial product Galltroll. Treatment of seeds such as cereals, sweet corn, and carrots with *Bacillus subtilis* or *Streptomyces* sp. has protected the plants against root pathogens [64].

Pseudomonas rhizobacteria applied to seeds and roots of plants have resulted in less damping-off and soft rot, and in consistent increases in growth and yield in several crops. They are available as commercial products Kodiak and Dagger G. The most common soilborne diseases controlled by soilborne bacteria are damping-off and root rot diseases caused by the oomycetes *Pythium* and *Phytophthora* and by fungi *Rhizoctonia*, *Fusarium*, and *Gaeumannomyces* [64].

II.2.2.2. Fungal antagonists to of soil-borne diseases

Coniothyrium minitans, *Gliocladium virens (*syn. *Trichoderma virens)*, *Trichoderma harzianum* and *Trichoderma viride* are currently used as fungal biocontrol agents (BCAs) in soil and root microbiomes. *Trichoderma spp*. is the most widely studied biocontrol agents (BCAs) against plant pathogens because of their ability to reduce the population of soil borne plant pathogens [140]. They are soil borne fungi and show significant activity against a wide range of plant pathogenic fungi [141]. Mechanisms used by *Trichoderma spp*. for control of plant pathogen include compet ition, mycoparasitism, antibiosis and induced resistance of the plant host [142, 143]. Moreover, [144]

reported that *Trichoderma viride* could colonize a root of plants and promote plant growth. These mechanisms are useful for *Trichoderma viride* to control pathogens. International Journal of Science, Environment and Technology, Vol. 1, No 4, 2012, 302 - 315 Anita Patil, Aarti Laddha, Ashwin Lunge, Hariprasad Paikrao and Shubhada Mahure SomeTrichoderma strains are known to show acceptable promise for the control plant pathogen in soil, but having low efficiency for competition in the rhizosphere or low ability to produce cell wall lytic enzymes [145, 146] observed that they could excrete an extracellular compound which was named gliotoxin. Many antibiotics and extra cellular enzymes were isolated and characterized later, and the biocontrol mechanisms became clearer [147, 148].

II.2.3.Plant-Derived Products

Natural products seem to be a viable solution to the environmental problems caused by the synthetic pesticides and many researchers are trying to identify the effective natural products to replace the synthetic pesticides [149].

II.2.3.1. Bioactive compounds

Some plant contains components that are toxic to pathogens. When extracted from the plant and applied on infested crops, these components are called botanical pesticides or botanicals [150]. Bioactive compounds or plant secondary metabolites (SMs) consist of low-molecular weight compounds that are regarded as not essential for sustaining life, but as crucial for the survival of the producing organism [151]

II.2.3.2. Major groups of antimicrobial compounds

Plants produce a wide variety of bioactive metabolites which serve as plant defense mechanisms against pests. Some SMs give plants their odors (terpenoides), some are responsible for plant pigments (quinines and tannins) and others (e.g., some of terpenoids) are responsible for plant flavor. These antimicrobial bioactive compounds are

divided by [152] into 5 main classes consisting: Terpenoids and essential oils; phenolics and polyphenols; alkaloids; polypeptides and mixtures (crude extract).

II.2.3.3. Commonly Used Botanicals:-

II.2.3.3.1 Plant extracts:

Neem (*Azadirachta indica, A. Juss*), Garlic (*Allium sativum*, Linn., Eucalyptus (*Eucalyptus globulus*, Labill., Turmeric (*Curcuma Longa*, Linn., Tobacco (*Nicotiana tabacum*, Linn., Ginger (*Zingiber offi-cinale*, Rosc) [151].

I I.2.3.3.2.Essential oils

Essential oils, volatile oils or simply the "oil" of the plant from which they were extracted, such as "oil of lemongrass" are hydrophobic liquids containing volatile aroma compounds extracted from vegetal materials using steam or hydro distillation techniques. Most of these volatile natural products belong to monoterpenoids compounds [153]. The essential oils are important because of their antibacterial, antifungal, antioxidant and anti-carcinogenic properties [154].

Essential oils: Nettle oil (*Urtica spp*.), Thyme oil (*Thymus vulgaris*, Linn.), Eucalyptus oil *Eucalyptus globulus*, Labill. Rue oil (*Ruta graveolens*, Linn.), Lemon grass oil (*Cymbopogon flexuosus* (Steud.) Wats. and Tea tree oil (*Melaleuca alternifolia*). Gel and latex: Aloe vera (*Tourn. Ex Linn*.) [150].

Antifungal activity of volatile components extracted from leaves, stems and flowers of *Lantana camara, Malvaviscus arboreus* and *Hibiscus rosasinensis* were tested against *Alternaria solani*, *Botrytis cinerea, Fusarium solani* f. sp. *cucurbitae*, *Fusarium oxysporum* f. sp. *niveum*, *Pythium ultimum*, *Rhizoctonia solani* and *Verticillium dahlia* [155].

II.3. A historical overview of medicinal plants

Interest in traditional medicine and, in particular, herbal medicines, has increased substantially in both developed and developing countries over the past two decades. Global and national markets for medicinal herbs have been growing rapidly and

significant economic gains are being realized. As a consequence, the safety and quality of herbal medicines have become increasingly important concerns for health authorities and the public alike. The safety and quality of raw medicinal plant materials and finished products depend on factors that may be classified as intrinsic (genetic) or extrinsic (environment, collection methods, cultivation, harvest, post-harvest processing, transport and storage practices). Inadvertent contamination by microbial or chemical agents during any of the production stages can also lead to deterioration in safety and quality. In addition to the crop loses, presence of the fungi in medicinal plants reduces their quality and usefulness [156]. Moreover, under certain conditions, some of the ubiquitous fungal contaminants could secrete the toxic metabolites, mycotoxins, with powerful mutagenic and carcinogenic effect. Mycotoxins are thermo stable and cannot be destroyed by cooking. They have cumulative ability and are eliminated hard from the organism [157].

The usual antimicrobial chemicals used in agriculture for plant disease control (benzimidazoles, aromatic hydrocarbons and sterol biosynthesis inhibitors) are associated with series of problems. Currently, there is a strong debate about the safety aspects of chemical preservatives since they are considered responsible for many carcinogenic and teratogenic attributes as well as residual toxicity [158]. For these reasons, consumers tend to be suspicious of chemical additives and thus the demand for natural and socially more acceptable preservatives has been intensified. The increase of fungal resistance to classical drugs, the treatment costs, and the fact that most available antifungal drugs have only fungistatic activity, justify the search for new strategies [159]. The exploration of naturally occurring antimicrobials for food preservation receives increasing attention due to awareness of natural food products and a growing concern of microbial resistance towards conventional preservatives [160].

In the past decade, due to concerns regarding safety of the synthetic antimicrobial agents, the particular interest has been focused on the potential applications of essential oils as alternative chemical control measures. They have a broad spectrum of anti-fungal

properties [161-164] and they are environmentally friendly (biodegradable, do not leave toxic residues or by-products to contaminate the environment) [165].

Finding healing powers in plants is an ancient idea**.** There are already a large number of known secondary compounds produced by plants, but the recent advances in modern extraction and analysis should enable many more as yet unknown compounds to be found, characterised and utilised. A number of biologically active compounds with varying degrees of action, such as anti-tumor, anti-cancer, anti-microtubule, anti-proliferative, cytotoxic, photo protective, as well as antibiotic and antifouling properties, have been isolated to date from marine sources

The use of medicinal plants has occurred in Egypt since Pharaonic times [166, 167]. This constituted an integral part of the practiced medicine at that time. Nowadays, Egyptians still depend of medicinal plants for treatment. In a recent study, it was found that 23% of the Egyptian use medicinal plants as a remedy; 52% of them are living in urban areas and 48% are living in countryside. It is important to document these uses and perform studies about their pharmacological activities to assure their efficacy and safety [168].

II.3.1. Egyptian wild and medicinal plants used in this study

In (Table 1), in which the plants are arranged in alphabetical synopsis For each species, the following information is provided: botanical name, plant family, local name, part used, method of preparation or administration and ailments treated [169, 170].

Table 1: Medicinal plant parts used and ailments treated by local people of Beni-Sueif, Egypt, Boulos, et al., 1991, Sameh et al., 2011 [169, 170].

plant	Local name	preparation	Traditional uses
Rosmarinus officinalis	rosemary	Infusion, volatile oil	- Carminative - Diuretic - Antiseptic
Zingiber officinale	zinger	Decoction	- Voice problems in

plant	Local name	preparation	Traditional uses
			common cold
Alium sativum	Garlic	Chewing fresh bulbs	- Memory loss - Hypertension - Microbial infections
Alium cepa	onion	Decoction, green parts	- Heart diseases - antiseptic for mouth - infections
Foeniculum vulgare	Fennel	Decoction	- Colic in Gastrointestinal tract
Nigella *Sativa*	Black seed	Powder, entire seeds	- Immuno-stimulant - condiment
Thymus vulgaries	Common, Thyme	Powder	- Condiment
Cassia Senna	senna	Decoction	- Constipation
mentha varidis	Spearmint	Decoction	- Colic in Gastrointestinal tract
Syzygium aromaticum	Syzygium	Fruits	- cough, diabetes, dysentery, - inflammation and ringworm
Anethum graveolens	Dill	*Fruit* Essential oils	- Antimicrobial - Antihyperlipidemic and - Antihypercholesterolemic activities
Eucalyptus globules	Eucalyptus	*leaf essential oils*	- Antibacterial [2], analgesic and anti - Inflammatory effects antitermitic activity - Antioxidative and antiradical activities - Larvicidal and mosquito repellent activities
Lavandula angustifolia	Lavandar	*Lavender* Essential oils	- Aromatherapy in treatments of anxiety - Hypoglycemic effects - Anti-inflammatory - Antioxidant, nervousness, mental stress, insomnia and fatigue
Olea eurpaea	olive	Fruit essential oils	- To support cardiovascular function

plant	Local name	preparation	Traditional uses
			- To enhance the renal excretion of urine - Anti-Inflammatory and Analgesic
ziziphus spina-csisti	Christ,s Thorn	*castor oil*	- Irritability, insomnia and heart palpitations. - Antidiarrhoeal effects

II.3.2.Essential oils antifungal activity

The increasing resistance to antifungal compounds and the reduced number of available drugs led us to search for the new alternatives among aromatic plants and their essential oils, used for their antifungal properties. The antifungal activity can be attributed to the presence of some components such as carvacrol, α-terpinly acetate, cymene, thymol, pinene, linalool which are already known to exhibit antimicrobial activity [171-174]. A number of scientific investigations have highlighted the importance and the contribution of many plant families i.e. Asteraceae, Liliaceae, Apocynaceae, Solanaceae, Caesalpinaceae, Rutaceae, Piperaceae, Sapotaceae, etc., used as medicinal plants [175].

II.3.2.1. Lamiaceae

The family has cosmopolitan distribution. Many members of this family are useful economically for medicinal, culinary, ornamental and various commercial utilizations. Previous studies on the essential oils of many Lamiaceae show that, these plants have a broad range of biological activities, notably their antimicrobial potency [176].

II.3.2.2. Apiaceae

The Apiaceae (or Umbelliferae), commonly known as carrot or parsley family, are a family of mostly aromatic plants with hollow stems. Many plants in this family have been used as a folk medicine. The chemical constituents and antifungal effects of ajwain essential oil, *Trachyspermum ammi* (L.) Sprague was investigated [177].

II.3.2.3. Zingiberaceae

Zingiberaceae is a family of flowering plants consisting of aromatic perennial herbs with creeping horizontal or tuberous rhizomes, distributed throughout tropical Africa, Asia, and America. Many species are important ornamental or medicinal plants. Recently the antifungal activity of essential oils of ginger (*Zingiber officinale* Roscoe), evaluated against mycotoxin producers *Aspergillus flavus* and *A. parasiticus.* The major component is zingiberene, and showed low antifungal activity on the mycelial growth of *A. flavus* [178] and also effective against *F. oxysporum* [[179] .

II.3.2.4. Ranunculaceae

Ranunculaceae is a family of flowering plants, distributed worldwide. Some members of Ranunculaceae are used as herbal medicines because of their presence of alkaloids and glycosides. *Nigella sativa* L. is regarded as a valuable remedy for a number of diseases [180].

II.3.2.5. Theaceae

Theaceae is a family of flowering plants, composed of shrubs and trees. In parts of Asia, other species are used as a beverage. Several species grown widely as ornamentals for their flowers and handsome foliage and includes antifungal essential oil producing *Chamellia sinensis*(L.) Kuntze [181].

II.3.2.6. Myrtaceae

Myrtaceae or Myrtle family includes the species of woody plants with essential oils distributed widely in tropical and warm-temperate regions of the world and are typically common in many of the world's biodiversity hotspots. *Syzygium aromaticum* (L.) Merrill and Perry. oil exhibited strong inhibitory effects with complete inhibition of mycelia growth in *Botrytis cinerea* [182, 183].

This study was undertaken to investigate the *in vitro* antifungal effects of a number of herbal and medicinal plant extracts and essential oils, differing in chemical composition, against some of phytopathogenic and saprophytic fungi isolated from infected crops and fruits for proper utilization of natural and safe sources of herbal plant extracts in biocontrol to phytopathogenic fungi.

III. MATERIALS AND METHO DS

The present study was carried out at the mycology laboratory, Department of Plant Protection and Biomolecular diagnosis, Arid Lands Cultivation Research Institute (ALCRI), City of Scientific Research and Technology Applications, New Borg El-Arab City, Alexandria, Egypt.

III.1. Materials

III.1.1. Raw materials

III.1.1.1. Collection of Plant materials

In this study, fifteen herbal and medicinal plant species classified in 3 families and 19 genera were used. The Lamiaceae (4 species), zingiberaceae, Amaryllidaceae, Apiaceae and mytaceae (2 species). The other families, Ranunculaceae, Theaceae, Fabaceae, Malvaceae, Solanaceae, Lawales, Oleaceae and Rhamnaceae (1 species).

Select the studies plants [184, 185] based upon their ethno-medicinal importance and literature survey and were collected from unrestricted habitat from different Egyptian areas. The plant materials were collected in (March to June 2013) and identified and authenticated by department of Botany, Faculty of Science - Mansoura University (Egypt) Using standard reference. The botanical description of different families, genera and species of the study plants that. May be indicative presence of antifungal compounds listed in (Table 2).

Table2: classification of some herbal and medicinal plants which evaluate their antifungal activity [185, 186]

plant	Local name	family	Genus	species
Rosmarinus officinalis	rosemary	Lamiaceae	*Rosmarinus*	*R. officinalis*
Zingiber officinale	zinger	zingiberaceae	*Zingiber*	*Z. officinale*
Alium sativum	Garlic	Amaryllidaceae	*Allium*	*A. sativum*
Alium cepa	onion	Amaryllidaceae	*Allium*	*A. cepa*
Foeniculum vulgare	Fennel	Apiaceae	*Foeniculum*	*F. vulgare*
Nigella Sativa	Black seed	Ranunculaceae	*Nigella*	*N. Sativa*
Thymus vulgaries	Common, Thyme	Lamiaceae	*Thymus*	*T. vulgaries*
Cassia Angustifolia	senna	Fabaceae	*senna*	*S. Alexandrina*
mentha varidis	Spearmint	Lamiaceae	*Mentha*	*Viridis*
Syzygium aromaticum	Syzygium	mytaceae	*Syzygium*	*S. aromaticum*
Anethum graveolens	Dill	Apiaceae	*Anethum*	*A. graveolens*
Eucalyptus globules	Eucalyptus	mytaceae	*Eucalyptus*	*E. globules*
Lavandula angustifolia	Lavandar	Lamiaceae	*Lavandula*	*L. angustifolia*
Olea eurpaea	olive	Oleaceae	*olea*	*O. eurpaea*
ziziphus spina-csisti	Christ,s Thorn	Rhamnaceae	*Ziziphus*	*Ziziphus jujuba*

III.1.1.2. Fungal Pathogens

Nine isolates of plant pathogenic fungi were used as tested strains.

III.1.2. Culture media and Chemicals

III.1.2.1. Culture medium

III.1.2.1.1. Potato Dextrose Agar (PDA) media

Potato 200g, agar 20g, Glucose 20g and complete with distilled water up to 1L

Adjust pH7

III.1.2.1.2. potato-dextrose broth

Potato 200g, Glucose 20g and complete with distilled water up to 1L

Adjust pH7

III.1.2.1.3. Maintenance culture medium

Yeast extracts, 5; Glucose, 20; Peptone, 20 and Agar, 15 at pH 7. The inoculated slants were incubated at 30oC for one week then stored in a refrigerator with transfers at weekly intervals

III.1.2.2. Chemicals & Buffers

III.1.2.2.1. Chemicls

1- N, N, N', N'- Tetramethylethylen-diamin TEMED (Merck, Germany).

2- Tris-base (Amresco, USA).

3-Ethylene Diamine Tetra Acidic acid EDTA (Amresco, USA).

4- Na_2 EDTA Sodium Ethylene Diamine Tetra Acidic acid EDTA (Amresco, USA).

5- Dimethylsulfoxide, Adweic. Chemicals Co. Egypt.

6- Ninhydrin, Adweic. Chemicals Co. Egypt.

7- glycerine, Adweic. Chemicals Co. Egypt.

8- KOH, Adweic. Chemicals Co. Egypt.

9- Iodine crystal, Adweic. Chemicals Co. Egypt.

10- Feric chloride, Adweic. Chemicals Co. Egypt.

11- Copper sulphate, Adweic. Chemicals Co. Egypt.

12- potassium sodium tartarate, Adweic. Chemicals Co. Egypt.

13- Mercury, Adweic. Chemicals Co. Egypt.

14- Sodium hydroxide pellets, Adweic. Chemicals Co. Egypt.

15- Chloroform, Adweic. Chemicals Co. Egypt.

16- Anhydrous sodium sulphate, Adweic. Chemicals Co. Egypt.

17- Boric acid (Amresco, USA).

18- Ammonium per sulfat APS (Serva,Heidelberg).

19- Sodium chloride NaCl (Adwic, Egypt).

20- Sodium Dodecyl Sulphate SDS (Riedel-dehaen, Germany).

21- Ethanol Absolute (Merck, Germany).

22- Agarose Molecular screening (MS) grade (Boehringer Mannheim, Germany).

23- Ethidium bromide (Amresco, USA).

24- Acrylamid (Fluka, Switzerland).

25- Bis Acrylamid (N,N'- methylene bis acylamide) (Fluka,Switzerland).

26- Isopropyl-β-D-thiogalactopyranoside (IPTG) (Biobasic INC,Canda).

Chemicals employed for the isolation, maintenance and testing of the fungal strains and all molecular assays were of analytical grade, and were obtained from recognized chemical suppliers. Those included:

1- Glucose, Adweic. Chemicals Co. Egypt.

2- Yeast extract, Adweic. Chemicals Co. Egypt.

3- Zinc sulfate, Adweic. Chemicals Co. Egypt.

4- Nystatin, Sigma Chemicals (St. Louis, MO

5- Manganese Sulphate, Adweic. Chemicals Co. Egypt.

6- Potassium dihydrogen phosphate, Adweic. Chemicals Co. Egypt.

7- Ferrous Sulfate, Adweic. Chemicals Co. Egypt.

8- Agar, Adweic. Chemicals Co. Egypt

9- Copper sulfate, Adweic. Chemicals Co. Egypt.

10- Sodium tartrate, Adweic. Chemicals Co. Egypt.

11- Thin layer chromatography plates (TLC), Sigma –Aldrich

12- Silica Gel-G, Sigma-Aldrich

13- Sulphoric acid (96%), Adweic. Chemicals Co. Egypt.

14- Hydrochloric acid(36%), Adweic. Chemicals Co. Egypt.

15- 6x loading dye buffer (Promega, USA).

16- Phenol Equilibrated to pH > 8.0 with Tris buffer (USb, USA).

17- Aluminium, 20* 20 cm, sigma-Aldrich

All are obtained from the laboratory reagents and fine chemicals, El Naser pharmaceutical chemicals co.

III.1.2.2.2. Kits

. Fungus Genomic DNA Extraction Kit (Biospin, China

. Gel Extraction Kit (QIAGEN, USA).

. PCR Clean UP Kit (Maxim biotech INC, USA)

III.1.2.2.3. Enzymes

. Proteinase K: it was obtained from Amresco, USA

10 mg proteinase k dissolved in 1 ml distilled water.

. DreamTaq™ DNA polymerase: it was obtained from Fermentas, Lithuania.

. Hot Start Taq (Thermus aquatiqus) DNA polymerase (Sib- Enzyme, Russia), The enzyme was

Supplied with 10x buffer, and 50 mM $MgCl_2$

III.1.2.2.4. Reagents and Buffer

- **Mayer's reagent was prepared by:**

  Solution A: 1.358 g of Mercuric chloride was dissolved in 60 ml of distilled water.

  Solution B: 2.5g of potassium iodide was dissolved in 10 ml of distilled water.

  Both solution were mixed and the volume was completed to 100 ml with distilled water.

- **Wagner's reagent:**

Tow grams of potassium iodide was dissolved in 5 ml of distilled water ,then 1.27 g of

iodide was added and the volume was completed to 100 ml with distilled water.

- **Fehling reagent**

. Fehling's solution A: copper sulphate (34.66g) is dissolved in distilled water and made up to 500ml using distilled water.

.Fehling's solution B: potassium sodium tartarate (173g) and sodium hydroxide (50g)

I

made up to 500mls dissolved in water and

- **Millon's regent: Mercury** (1g) is dissolved in 9ml of fuming nitric acid. When the reaction is completed equal volume of distilled water is added.

- **10x TBE buffer:**

108g Tris-base, 55g boric acid, and 9.3g EDTA per liter, pH 8.0.

. 3M sodium acetate:

. (40.82g sodium acetate dissolved in 100 ml distilled water, pH 4.5).

. 3M potassium acetate:

(28.98g potassium acetate dissolved in 100 ml distilled water, pH 4.5).

. 10% Ammonium persulfate:

(0.1g ammonium persulfate dissolved in 1 ml distilled water).

. 40% Acrylamid:

(39g acrylamid and 1g bis-acrylamid dissolved in 100 ml distilled water).

. **5x TBE buffer**:

54g Tris-base, 27.5 g boric acid, and 20 ml of 0.5 M EDTA (pH8.0) (Sambrook et al**.** 1989).

. **Lysis Buffer**:

25 mM Tris-HCl (pH 8.0), 20 mM EDTA (pH 8.0), and 10 % SDS

III.1.2.2.5.Molecular primers & ladder

III.1.2.2.5.1.Oligonucleotides (primers)

All primers used in this study were synthesized by metabion international AG, Germany.

III.1.2.2.5.2.DNA molecular weight markers

Molecular weight of DNA ladder (0.25µg/µ1) (supplied by Fermentas, Lithuania) consisted of 11 DNA fragments ranging in size from 1500 to 100 bp (showed as 11 bands on gel) and stored at -20°C.

III.1.3.Apparatus and Instruments

The instruments used for different analyses during the study along with their company

identification

- GC Mass, Shimadzu GCMS and QP2010S
- Thermocycler Gene Amp 9700, (Applied Biosystems (ABI), USA).
- Purified class II biosafety cabinet (Labconco, USA).
- Electrophoresis apparatus with power supply (Biometra, USA).
- U.V. transilluminator (Cole-Parmer, USA).
- Microwave (Micro, Quartz Browner, USA).
- Light microscope (107E), China
- Low pressure UV lamps (Heraeus Noblelight, Germany)
- Digital 0.01 g balance model SBA 51 (Scaltec, Germany).
- Polaroid Camera DS-34 (polariod, USA).
- Microfuge (Eppendorf, Germany).
- Gel documentation system (Alpha-chem Imager, USA).
- ABI 3100 applied biosystem DNA sequencer (ABI, USA).
- -20 vertical deep-freezer (VWR, USA)
- -80 horizontal deep-freezer (Revco, USA)
- Autoclave HEV 25 (Hirayama, Japan).
- Vertical Electrophoresis apparatus (Bio Rad, USA).

- QYC 211 incubator shaker (Beijing , China)
- Rotary vacuum evaporator (N-N Series, Eyela, Rikakikai Co. Ltd., Tokyo, Japan)
- Incubator (Memmert, Germany).
- Water bath (Memmert, Germany).
- Fine pipettes (Labsystem, Finland).
- Sonicatior (Sonics & Materials INC, USA).
- Thin layer chromatography unit, Sigma Aldrich
- Compound microscope, glass slide, cover slip,
- Watch glass, glass tube, painting brush,
- U V Cabinate, Muffle furnace
- Desiccators and other Basic apparatus, instrument were used.

III.1.4. Antifungal standard

- Nystatin (0.1mg/well)

III.2. Methods

III.2. 1.Preparation of herbal & medicinal plant extracts and oils

Previously collected Plant materials (Root, leaf, fruit, bulb, clove, seed, and flower) were washed with tap water, disinfected by immersion in 2% sodium hypochlorite solution for 30 min, rinsed with sterile distilled water to eliminate residual hypochlorite [186].

In this study, air-dried plant materials were homogenized to fine powder in a mill and stored in airtight dark bottles and kept until used. To extract polar and non-polar compounds from selected plant powdered using two different solvents methanolic and aqueous. Water and methanol are the two solvent used for extraction. These two solvent are polar and they can extract the maximum of bioactive compounds.

III.2.1.1.Water reflux

Aqueous extraction performed with some adoption to Parekh (2006) procedure; ground (80-mesh) of air-dried plant sample (20g) was extracted separately with 200 ml of distilled water, (1:10 w/v) and boiled on slow heat for 2 hours. Then it was filtered through 8 layers of muslin cloth and centrifuged at 5000g for 10 min and the supernatant was collected. This procedure was repeated twice; after 6 hours, the supernatant was collected at an interval of 2 hours, pooled together and the solvent was removed under vacuum at 45 °C, using a rotary vacuum evaporator (N-N Series, Eyela, Rikakikai Co. Ltd., Tokyo, Japan). The dried extract stored in refrigerator at -4 ^{0}C for further use [187, 188].

III.2.1.2. Methanol extract

Ground (80-mesh) of air-dried plant sample (20g) was extracted separately with 200 ml of 100% methanol (Elnasr, ADWIC, Egypt), (1:10 w/v) for 8 hours in Soxhlet apparatus and then the extract was filtered and allowed to evaporate in oven at 45 ºC. The solvent was removed under vacuum at 45 °C, using a rotary vacuum. The dried extract stored in refrigerator at -4 ^{0}C for further use [189].

III.2.1.3. Isolation of Essential Oils

III.2.1.3.1. Hydrodistillation Method

The air-dried and finely ground (80 mesh) plant materials (500g) were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus as recommended by British Pharmacopeia (1988). Briefly, the plant was immersed in water and heated to boiling, after which the essential oil was evaporated together with water vapors and finally collected in a condenser. The distillate was isolated (separate using funnels), collected and dried over anhydrous sodium sulfate, filtered and the final product of purified essential oil was then placed in an amber vial, weighed and stored at -80^{o} C until bioassayed [190].

III.2.1.4. Determination of extraction yield

The yield was determined by the ratio of the weight of the dry extract after evaporation on the weight of the plant material used for extraction, multiplied by 100%.

$$Rd\ \% = (m1 \times 100) / m0$$

Where, m1 is the Mass in grams of the dry extract; m0 is the mass in grams of dry plant material; Rd is the yield.

III.2.1.5. Preparation standard concentrations of plant extracts

According to Almola (2010) with slightly modified procedure, One gram of each essential oils, aqueous and methanolic extracts of tested plants pre-prepared (each separately) was taken and the aqueous extract was dissolved in 10 grams sterile distilled water, while alcoholic and oils extracts were dissolved in 10 ml of DiMethyl Sulphoxide (DMSO). Thus 100 mg / ml (10% concentration) of stock was obtained as a standard concentration of essential oils, aqueous and alcoholic extracts. Aqueous extracts were sterilized using 0.22 µm membrane filters while essential oils and alcoholic extracts were pasteurization for 15 minutes at temperature 62 ºC[191].

III.2.2. Isolation and identification of phytopathogenic fungi

III.2.2.1. Culture medium

III.2.2.1. 1.Potato Dextrose Agar media

The potato was peeled and 100g was measured, finely chopped and boiled to a mash in distilled water. The dextrose was measured (12.5g) and placed in a 1L measuring cylinder. Agar was measured (12.5g) and added to the measuring cylinder (with the dextrose).The potato mash was stirred and strained into the cylinder. Hot distilled water was added to make up 500mL.The contents was continuously poured and stirred until consistency was achieved. The content was then poured into a conical flask, plugged with cotton wool, over which aluminum foil was tightly wrapped. The flask was then autoclaved at 121 0C for 24hrs. The pH range was between (6.5-7.0) [192].

III.2.2.1.2. Potato Broth media

The culture medium for fungal isolation was water agar, potato dextrose agar (PD Agar) were used in order to stimulate fungal sporulation [193].

III.2.2.2. Isolation of Fungal pathogens

Pure cultures of phytopathogenic fungal speies were isolated from their respective infected plant hosts, Banana fruits, Potato tubers, Tomato fruits & leaves,

Potato leaves, corn silage, peanut & corn seeds, wheat bran, citrus fruit (orange) and apple fruits. All the pre and post harvest infected host materials were collected from many Egyptian governorates in (September 2012 to august 2013), after having verified their pathogenicity with appropriate tests

Fungi were isolated from different infected samples of crops and fruits using a modified method described by Arnold et al. [194], The material was thoroughly washed in sterile water, surface-disinfected by soaking in 70% ethanol for 30 sec and 0.1% mercuric chloride (HgCl2) solution for 2 min, and rinsed in sterile demineralised water. The plant material was subsequently rinsed in sterile demineralised water. Small pieces of inner tissues and needles were placed on aqueous agar (distilled water and 1.5% agar-agar) supplemented with antibiotic streptomycin (3mg/100mL) in petri plates and incubated at until fungal growth was initiated. The tips of the fungal hyphae were removed from the aqueous agar and placed on mycological medium, that is, Potato Dextrose Agar (PDA: 300g/L diced potatoes, 20g/L dextrose and 20g/L agar) or the Sabouraud agar (SA: 40g/L dextrose, 10g/L peptone, and 20g/L agar). After several days of incubation, the purity of each fungal culture was assessed by examination of colony morphology. After purifying the isolates several times as described above, the final pure cultures were transferred to PDA slant tubes. As controls, uncut, surface-disinfected, and non disinfected pieces were also placed on the same agar to check for contaminated fungi.

III.2.2.3. Identification of phytopathogenic Fungi

III.2.2.3.1. Morphological Examinations

The identification procedure of different isolated fungal species was based on morphology. The nine isolated species were described according to Traditional practices for studies of fungi include their macroscopic features (i.e. the colour, shape and growth of cultured colonies) as well as microscopic characteristics (i.e. the structure of hyphae, conidia and conidiophores) [195, 196].

Colony descriptions were based on observations on PDA under ambient day light conditions. Growth rates at 20, 25, 30, 35, and 40^0C were determined after 72 h following published protocols [197, 198]. Microscopic observations and measurements were made from preparations that were mounted in lactic acid. Conidiophore structure and morphology were described from macronematous conidiophores obtained from the edge of conidiogenous pustules or fascicles during the maturation of conidia, which usually occurred after 4–7 days of incubation [198].

The obtained data were then compared with the descriptions of endophytic and soil-borne fungi species in the literature and matches were recorded. When the morphological investigation fails to reveal the identity of the isolated fungus, the isolated species were sent for molecular confirmation and antifungal activities analysis [199].

III.2.2.3.2. Molecular identification and Phylogenetic Analyses

Molecular techniques have been used as an effective and easy way to detect and identify a variety of fungi based on DNA-based assays. For those poorly sporulating isolates, the internal transcribed spacer (ITS) of ribosomal DNA were amplified and sequenced following the procedure of White et al. (1990). Similar taxon retrieved by Basic Local Alignment Search Tool (BLAST) in GenBank and National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/) was used as reference for further morphological examination and identification [199].

III.2.2.3.2.1. DNA extraction of fungi

A total of 9 representative isolates of various morphological fungi isolated from their infected hosts were selected for molecular identification. For DNA extraction, mycelia were transferred from PDA into 250mL Erlenmeyer's flasks containing potato-dextrose broth without shaking. After 5 days of growth at 28 ± 2 ^{0}C, approximately 100 mg of the mycelial biomass was harvested [198]. Fungal genomic DNA was prepared using Biospin Fungus Genomic DNA Extraction Kit (Bioer Technology Co., Ltd., Hangzhou,

P.R. China). The DNAs were transferred to the new tubes and stored at -20°C respectively. DNA detection concentration was performed by electrophoresis on a 2% (wt/vol) agarose gel stained with ethidium bromide (10mg/mL) and checked for size and purity [200].

III.2.2.3.2.2.Oligonucleotides (primers)

The internal transcribed spacer (ITS) regions of the rDNA repeat from the 3′ end of the 28S and the 5' end of the 28S gene were amplified using the two primers, ITS-1 and ITS-4 which were synthesized by MWG, Germany on the basis of conserved regions of the eukaryotic rRNA gene [201]. The details of the primers are mentioned in (Table3)

Table3. Primers sequences used in ITS rDNA PCR of the selected 9 isolates

primers	Sequence 5'→3'	Amplified product	reference
ITS1 (Forward)	TCTGTAGGTGAACCTGCGG	600 bp	Mohammad Shahid, et al., 2013
ITS4 (Reverse)	TCCTCCGCTTATTGATATGC		Mohammad Shahid, et al., 2013

III.2.2.3.2.3. Amplification of ITS region

Preparation of the amplification reaction of fungal ITS gene (600 bp) was done under the biosafety cabinet in a separate room rather than that in which the isolation were done. In 200 ul Eppindorf tube, the components of the PCR were prepared as a master mix containing the reagents needed to amplify the required number of samples as well as positive and negative control (Table 4) then 4µl (40 ng) of the DNA were added in the PCR tubes and 46 µl of the master mix were dispensed in it, to reach 50µl as a final volume of the reaction.

Table 4: Master Mix PCR reaction components for ITS rRNA gene amplification

Component	Volume (µl)	Final concentration
Sterile Milli Q water	25.8	---
10.0 x PCR reaction buffer	5.0	1.0x
20 mM nucleotide mix	2.0	0.2 mM
50 mM $MgCl_2$	5.0	5mM
Primer F 10.0 pmol/µl	4.0	40.0 pmol/reaction
Primer R 10.0 pmol/µl	4.0	40.0 pmol/reaction
Taq DNA polymerase 5.0 U/µl	0.2	1.0 U
DNA solution 10.0 ng/µl	4.0	100ng
Total	50.0	---

The PCR- amplification carried out in a Thermocycler ABI Gene Amp 9700(Applied Biosystem) and the cycle parameters included an initial denaturation for 5 min at 94°C, followed by 40 cycles; denaturation for 1 min at 94°C, annealing for 2 min at 55°C, extension for 3 min at 72°C, and a final extension for 10 min at 72°C.

Amplified products were separated on 1.2% agarose gel in TAE buffer, post-stained with ethidium bromide (1 µg/ml) and electrophoresis was carried out at 60 V for 3 h in TAE buffer. 1.5 Kb ladder (MBI, Fementas) was used as a marker. The gel was observed in a transilluminator over ultraviolet light. The desired bands were cut and purified from the gel with minimum quantity of gel portion using QIAGEN gel extraction kit using the protocol described in the manufacturer's manual [202].

III.2.2.3.2.3. DNA sequencing of Fungal ITS rDNA gene (600 bp)

A pair of universal ITS primers ITS-1 (forward) and ITS-4 (reverse) was used for sequencing of the amplified 600 bp product in both directions. For this purpose, the Big Dye terminator sequencing kit (Version 3.1, Applied Biosystems) and an ABI PRISMTM 3100 DNA sequencer (Applied Biosystems) were used. All PCRs and sequencing reactions were performed on a GeneAmp PCR System 9700 at Sigma company (Sigma company, Cairo, Egypt) [203].

III.2.2.3.2.4. Sequence analysis and phylogeny

The consensus sequences of 9 representative fungal isolates obtained from both ITS1 and ITS4 primers were first edited and subject to BLAST searches to assign putative identity, designation of operational taxonomic units based on sequence similarity measures, and phylogenetic inference. They were then aligned with other similar sequences downloaded from GenBank using ClustalX [204] and BioEdit [205] Moreover molecular evolutionary genetics analysis *(*MEGA*)* software version 6.0 was used in phylogeny construction [206]. The nucleotide Sequences of the 9 representative pathogenic fungi have been submitted to GenBank to obtained accession numbers.

Sequences obtained were split into different datasets in order to access phylogenetic relationships at the familial and species level. Phylogenetic analyses for maximum parsimony (MP), maximum likelihood (ML) and neighbour joining (NJ) analyses were performed by using MEGA program [206].

A phylogenetic tree was then constructed using the Neighbor Joining method [207]. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4.0 [208].

III.2.3. Antifungal activity of plant extracts

III.2.3.1. Indicator fungal species

The isolated fungal species utilized to access the antifungal activity of medicinal plant materials which isolated from their infected hosts

III.2.3.2. Antagonistic bioassay

III.2.3.2.1. Preparation of the indicator active inoculums (colonies)

The nine fungal cultures grown at 28°C on Potato Dextrose Agar medium, spores of the each fungus was collected from cultures on agar plates after 7 days [209]. PDA broth prepared by transferring a loop full of cells from the stock cultures was diluted with fresh potato dextrose broth. According to standard reported procedure, suspention of spores/conidia are adjusted to $2x10^5$ CFU/mL (colony forming unit) spores by

Haemocytometer cell counting chamber and stored in 0.85% saline solution for testing activity of plant extracts and oils [210, 211].

III.2.3.2.2. Reference Antifungal drug and Control:

The commercial antifungal references (standard) were antibiotic in nature such as Nyastatin as the reference for the investigated fungi. The Control experiment consists of a plate of solidifying agar onto which was inoculated pure solvent with microorganism mixed in a 1:1 portion [191].

III.2.3.2.3. Preparation different concentration of plant extracts and essential oils

A serial concentration of 12.5, 25, 50, 125, 250 and 500 ppm (ug/ml) from the previous standard aqueous and methanolic extracts and essential oils (stocked, 100mg/ml) were obtained using the appropriate solvent, water and DMSO to be tested on the selected indicator isolates

III.2.3.2.4. Antifungal activities of plant extract using antibiotic reference

***In vitro*:** Two effective alternative methods for antifungal comparative study using antibiotic reference by agar diffusion technique

A- <u>Well Diffusion Plate Method (Well diffusion assay)</u>

According to Jagessar (2008) with slightly modified for antifungal comparative study using reference. The nine investigated fungi were inoculated into a test tube containing three of distilled water (medium), using a flamed loop. 50 µl of spore suspension was mixed with the 20 ml warm, melted, autoclaved PDA and poured into separate plates under aseptic conditions. The plates were covered and allowed to cool. As soon as the agar was partly solidified, the plates were inverted and left for 2h. When cooled, 5 similar wells pattern were made in the plate. The wells were made by using a 6 mm cork borer or puncher that was sterilized with alcohol and flame. Concentration of 2o ul (4 mg/disc) from oil,

aqueous and methanolic plant extracts was pipette into three different wells in a sterilized environment at the same plate, using a micro liter syringe. The two solvents water and DMSO at volume 40ul was poured in the 4th well as control whereas dissolved in dichloromethane (Nystatin 0.1mg/well) was used as the reference. The plates were labeled, covered, inverted and placed in a fume hood (no incubator was available) for 48h and incubation, for3–5 days for at 28 °C. The antifungal activity was evaluated by measuring zone of inhibition of fungal growth surrounding the well and the diameters of these zones were measured in millimeters. Each experiment was repeated at least twice [191].

B- Disc diffusion method (Disc diffusion assay)

An inoculum of each of the fungal strains was suspended in 5ml potato dextrose broth and incubated at 37°C for 2 days. The antifungal activity was tested by the disc diffusion assay [215]. For this, the inoculum was spread over PDA medium with sterile glass spreader. Small circular paper discs (5mm diameter) impregnated with known amount 2o ul (4 mg/disc) from oil, aqueous and methanolic plant extracts was placed upon the surface of the inoculated plates separately. The plates were kept at room temperature for absorption of extract in the medium and then incubated at 37°C in the incubator for 24to 48hrs. The antifungal activity was evaluated by measuring the diameter of inhibition zone [212]. Nystatin (0.1 mg/disc) used as positive control and Dimethyl sulfoxide (DMSO) at 40ul was used as negative control. Triplicates were maintained for all experiments.

III.2.3.3. Determination of Minimum Inhibitory concentration using Broth dilution Method

Minimum inhibitory concentration (MICs) was defined as the lowest concentration or highest dilution that exhibits no visible growth. Tube dilution method was used to determine the minimum inhibitory concentration of the

extracts in potato Dextrose Broth as specified by National Committee for Clinical Laboratory Standard [213] with slightly modification. A total of 10ml of each broth was dispensed into separate test tube and was sterilized at 121^{0}C for 15min and then allowed to cool. Two-fold serial dilutions of the extracts in the broth were made from the stock concentration of the extracts (100 mg/ml) to obtain various concentrations; (0.25mg/ml, 0.5mg/ml, 1mg/ml, 2mg/ml and 4mg.ml). About 0.1ml of the standardized inoculums of the microbes was inoculated into the different concentration of the extracts in the broth. The test tubes of the broth were incubated at 30 ^{0}C for 3-5 days. Negative controls were set up as follows: Potato Dextrose broth only; Potato Dextrose broth and sterile plant extract; and finally positive control containing Potato Dextrose broth, and a test organism. The growth of the inoculum in the broth is indicated by turbidity or cloudiness of the broth and the lowest concentration of the extract which inhibited the growth of the test organism were taken as the MIC and the lowest concentration that showed no turbidity in the test tube was recorded as the MIC

III.2.4. PHYTOCHEMICALS ANALYSIS AND TLC FINGERPRINTING

III.2.4.1.Preparation of plant extracts

All the solvents used for extraction, phytochemicals screening and thin layer chromatography (TLC) profiling of plant were of analytical grade, respectively. Silica gel GF-254 (Merck) was used for preparation of TLC plate [214, 215].

sample (30gm) of the air dried materials of all the fifteen herbal and medicinal plants were extracted separately in a soxhlet apparatus with 200ml methanol for 72 hours until extract was obtained. The solvent extracts were concentrated separately under reduced pressure in a rotator evaporator. After complete solvent evaporation, each of these solvent extracts were weighed and subjected to phytochemical Screening and TLC fingerprinting [214, 215].

III.2.4.2. Preliminary Phytochemical analysis

A small portion of the dry extract was used for the phytochemical tests for compounds which include tannins, flavonoids, alkaloids, saponins, and steroids in accordance with the methods in [216, 217] with little modifications.

III.2.4.2.1. Test for carbohydrates

III.2.4.2.1.1. Fehling s test

Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

III.2.4.2.2. Test for proteins

III.2.4.2.2.1. Millon's test

Crude extract when mixed with 2ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein. Ninhydrin test Crude extract when boiled with 2ml of 0.2% solution of Ninhydrin, violet colour appeared suggesting the presence of amino acids and proteins [214, 215].

III.2.4.2.2.2. Iodine test

Crude extract was mixed with 2ml of iodine solution. A dark blue or purple coloration indicated the presence of the carbohydrate.

III.2.4.2.3.Test for phenols and tannins

Crude extract was mixed with 2ml of 2% solution of FeCl3. A blue-green or black coloration indicated the presence of phenols and tannins [214, 215].

III.2.4.2.4.Test for flavonoids

Crude extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet colour appeared after few minutes which indicated the presence of flavonoids [214, 215].

III.2.4.2.5.Alkaline reagent test

Crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids [214, 215].

III.2.4.2.6.Test for saponins

Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

III.2.4.2.7.Test for terpenoids

Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H2SO4 was added and heated for about 2 minutes. A grayish colour indicated the presence of terpenoids [214, 215].

III.2.4.2.8. Test for alkaloids

Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's And Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids

III.2.4.3. Thin Layer Chromatography screening

III.2.4.3.1. TLC plate preparation

The thin layer chromatography (TLC) result confirmed the presence of different bioactive compounds. TLC plates (Sigma-Aldrich) were prepared by using Silica gel on aluminum, 20x20 cm as adsorbent.15gm silica gel-G was mixed with 30ml of distilled water (1:2) to make slurry. The slurry was immediately poured into the plates. Plates were then allowed to air dry for one hour and layer was fixed by drying at 110 0C for one and half hours [214, 215].

III.2.4.3.2. Isolation of active fraction

About 1 mg of each extract was used for chromatography. A solvent system of 4:1 CHCl3: MeOH was prepared and placed in a tank and the lid was replaced. The plant

extract extracts were spotted, each one on separated origin on the pre-coated silica gel plate and Chromatographic separations were carried out, plates was placed carefully into the tank and covered with the lid. After development, the plate was removed and the solvent front was marked with pencil and allowed to dry [194].

III.2.4.3.3. TLC Screening

TLC plates were viewed under UV light at 254 nm for fluorescence quenching spots and at 366nm for fluorescent spots [194].

The chromatograms were observed under visible light and were photographed. The solvent showed different Rf value for the different plant extract and The Rf value was obtained by using the following formula [214, 215].

$$Rf = \frac{\text{Distance travelled by the solute (cm)}}{\text{Distance travelled by the solvent (cm)}}$$

III.2.4.4. Gas chromatography/mass spectrometry (GC-MS) analysis for essential oils

The composition of volatile content was determined at the central lab. Of Research & Technology Application City (Borg El-arab, Egypt) by gas chromatography-mass spectrometry technique. GC-MS analysis were performed on a GC – 2010 Shimadzu capillary gas chromatography directly coupled to the mass spectrometer system (GC-MS – model QP 2010; (Shimadzu) DB – 5ms non polar fused silica capillary column (30m X 0.25mm, 0.25µm film thickness) was used under following conditions: oven temperature program isotherm 2 min at 70ºC, 3ºC/min gradient to 200ºC and final temperature kept for 35 min; injection temperature 200ºC carrier gas is helium with flow rate 1.51ml/min; linear velocity 45.1 cm/sec. The effluent of the GC column was introduced directly into the source of MS and spectra obtained in the EI mode with ionization energy 70eV, in the electronic ionization mode and ion source temperature is

200ºC. The solvent cut time 3 min. The sector mass analyzer was set to scan from 40 to 1000m/z with interface temperature of 240ºC**.**

III.2.4.4.1. Identification of the volatile oils.

The components of the essential oil were identified on the basis of comparison of their relative indices and mass spectra by computer matching with WILEY and National Institute of Standards and Technology (NIST08) libraries provided with the computer controlling GC-MS system [218].

III.3. Statistical analysis

The results are presented as means ± standard deviation (±SD). Data were analyzed using the statistical package for social sciences (SPSS) 16. Data were evaluated by analysis of variance (ANOVA). Statistical differences were considered significant at the $p<0.05$ level

VI. RESULTs

VI.1. Study samples

VI.1.1. Medicinal Plant parts and extracts used

Plant parts used in this study belonging to 15 herbal and medicinal plants species; *Rosmarinus officinalis, Zingiber officinale, Alium sativum, Alium cepa, Foeniculum vulgare, Nigella Sativa, Thymus vulgaries, Cassia Angustifolia, Mentha varidis, Syzygium aromaticum, Anethum graveolens, Eucalyptus globules, Lavandula angustifolia, Olea eurpaea* and *ziziphus spina-csisti*. The leaf (46.66%) was the most commonly used plant part followed by the fruit and clove (13.33%), root, bulb, seed and flower (6.67%). (Table 5 & Fig.1)

Table 5: Medicinal plant parts used in antifungal assessment

Plant parts used	Number of samples	Percentage (%)
Root	1	6.67%
leaf	7	46.66%
fruit	2	13.33%
bulb	1	6.67%
clove	2	13.33%
seed	1	6.67%
flower	1	6.67%
Total	15	100%

No. = Number of samples **% = Percentage of total**

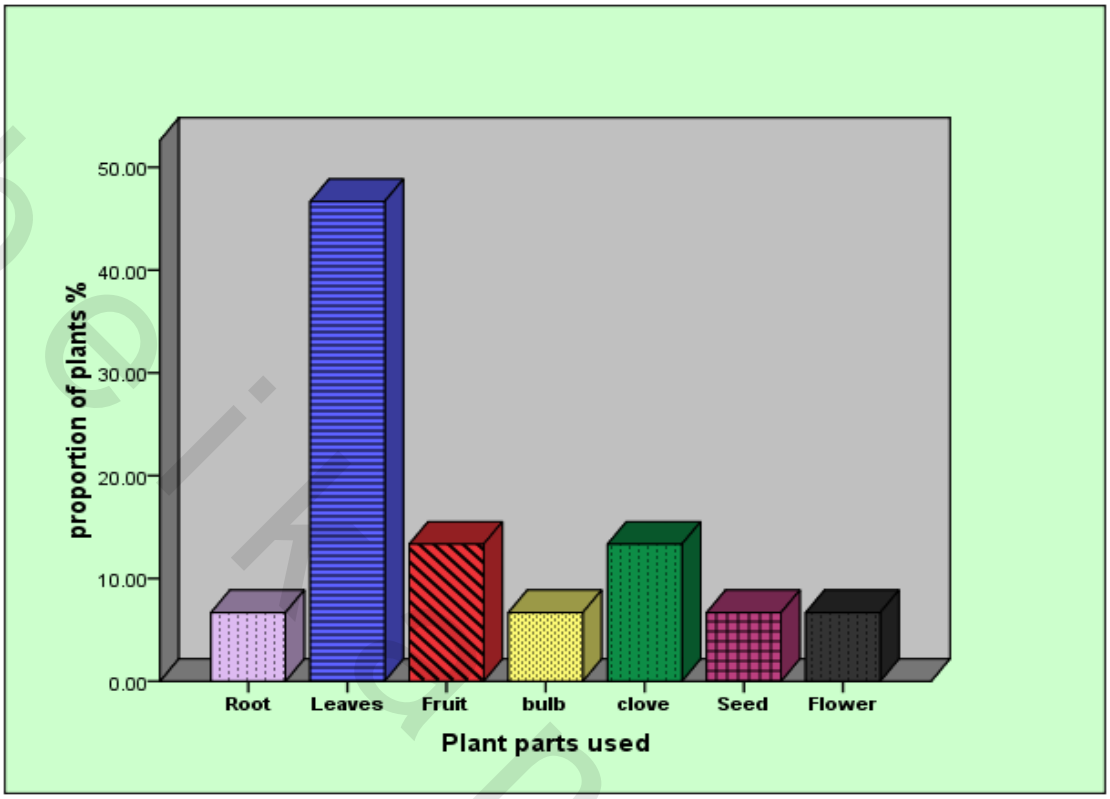


Figure 1: Diagrammatic representation of Plant parts used with antifungal activities against isolated fungi

Fourty five extracts (15 Methanolic, 15 aqueous and 15 essential oils) from fifteen selected plant species (3 different extracts from each plant material). The percentage and type of plant extracts tested are shown in (Table 6, Fig. 2)

Table 6: Different types of plant extracts used in antifungal assays

Extraction	**Number of extracts**	**Percentage (%)**
Methanolic extract Aqueous extract Essential oi	15 15 15	33.33% 33.33% 33.34%
Total extracts No.	**45**	**100%**

No. = Number of samples **% = Percentage of total**

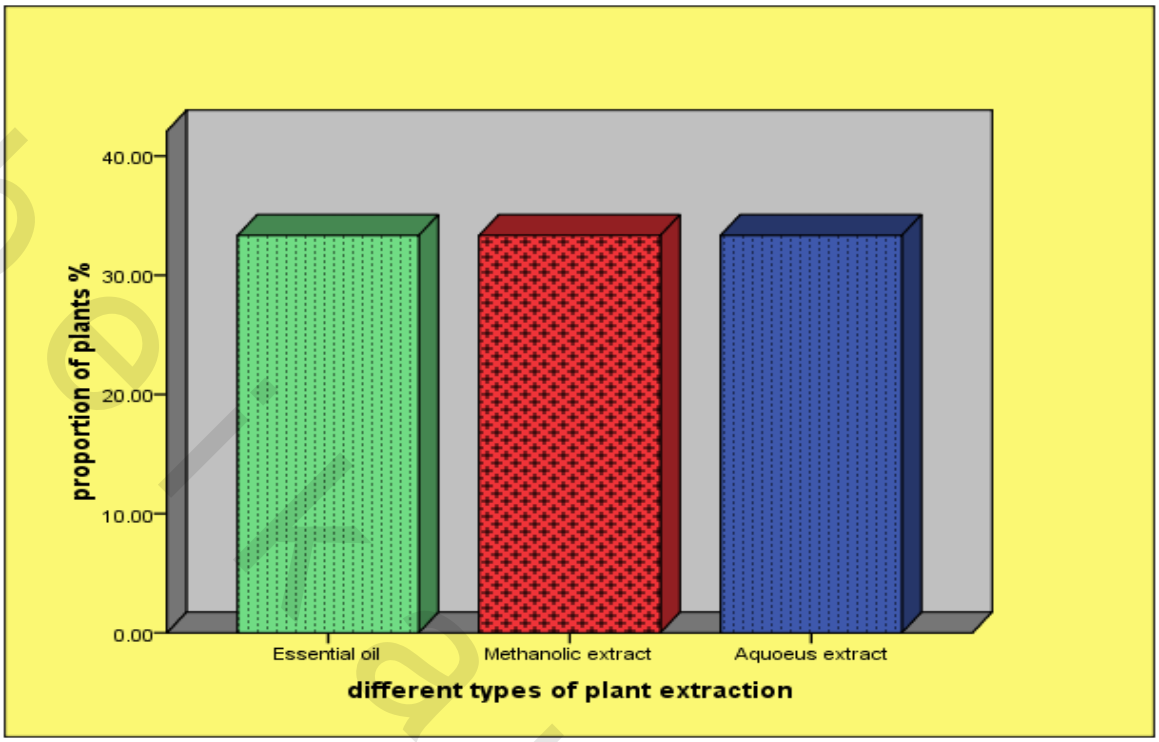


Figure 2: Diagrammatic representation of different extraction types of studied medicinal plants used in antifungal assays

VI.2. Isolation and identification of pathogens

VI.2.1. Isolation and Morphological examination

Cultivation study and morphological investigation of the different tissues of infected plant materials of economical crops (wheat, corn tomato, potato and peanut), corn silage and spoiled fruits like Banana, citrus and apples has led to isolation of nine species of soilborne and postharvest plant pathogens. Based on these features, the isolated pathogenic fungi could be classified into 3 families (Nectriaceae, Ceratobasidiaceae and Trichocomaceae and four genera (*Fusarium, Rhizoctonia, Aspergillus, and Penicillium*). The isolates are; *Fusarium oxysporum, F. solani, F. brachygibbosum, Rhizoctonia solani. Aspergillus fumigatus, A. flavus, A. oryzae, Penicillium chryogenum and p. commune*.

VI.2.2. Molecular Identification and Phylogenetic Analysis of Representative Pathogenic Fungi

In addition to the morphological characterization, molecular analysis were carried out using the ITS-rDNA sequences to confirm the identification of 9 representative fungal isolates from their hosts

VI.2.2.1. Polymerase chain reaction identification (PCR)

The amplification of ITS regions is likely to become a common approach in molecular identification strategies. In the present study Select and construction genus specific ITS-1 and ITS4 primers to identify nine fungal isolates and Amplified product with molecular size 600 bp was obtained (Fig.3).

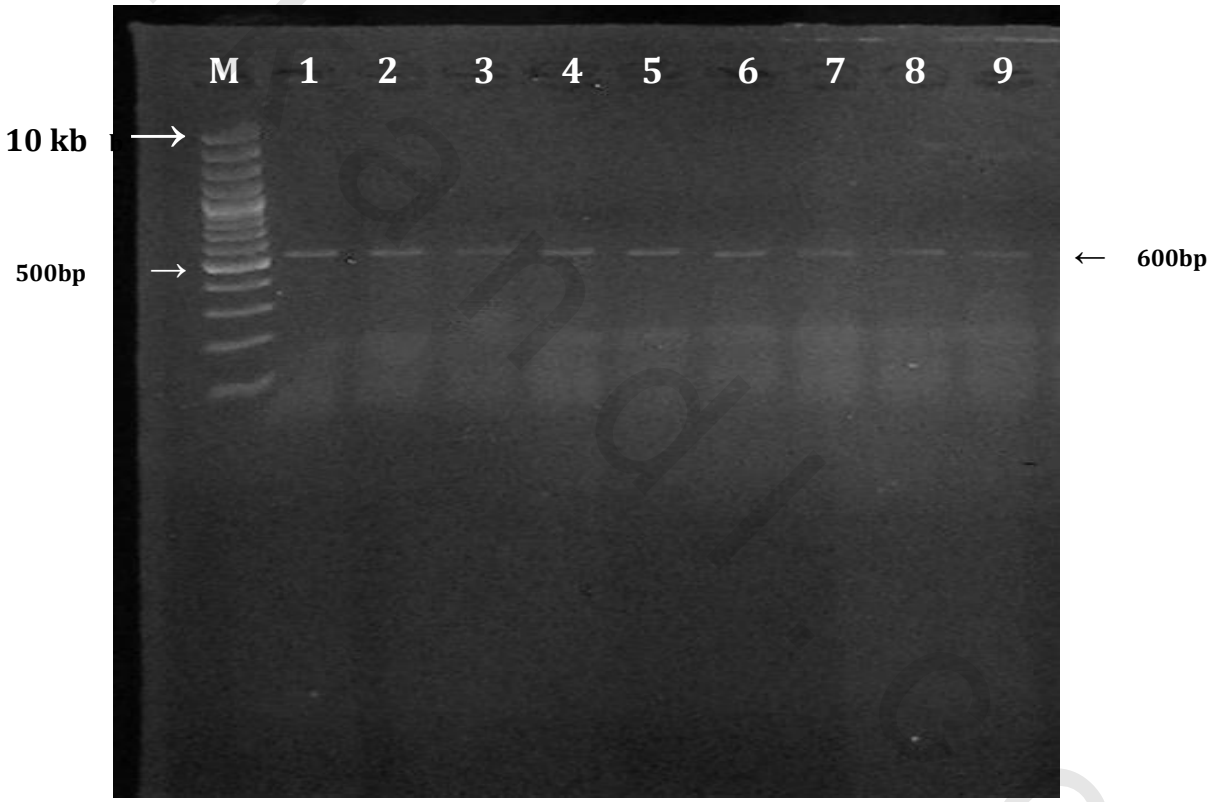


Figure 3: PCR amplification of ITS region of fungal isolates: Lane M, 10kb DNA marker & lanes 1-9 represented nine fungal species as, lane1 (Fusarium oxysporum), lane2 (*F. solani*), lane3 (*F. brachygibbosum*), lane4 (*Rhizoctonia solani*), Lane5 (*Aspergillus Fumigates*), lane6 (*A. flavus*), lane7 (*A. oryzae*), lane8 (*Penicillium chryogenum*) and lane9 (*p. commune*)

VI.2.2.2. Sequence alignment and phylogenetic analysis

Sequence analysis of ITS regions of the nuclear encoded rDNA (600 bp) of the 9 pathogenic fungi isolates were examined through BLAST and FASTA searches and through direct comparisons with potentially related taxa. The sequence analysis showed significant alignments (97–100%) in all 9 accessions for the isolated fungi and satisfactory homology with extype strains of the selected strains according to the sequences from the NCBI GenBank data base using ClustalX. Nine species were successfully identified according to the alignment analysis and accesseion numbers in Gen- Bank were listed in (Table7, 8). The phylogenetic analysis tree generated using MEGA 6.0 software (Fig. 4.1-4.4)

Table 7: Sequence and alignment of nine fungal isolates.

Isolate no.1, *Fusarium oxysporum* (KJ831189)

1 ggagtagtga cacggcggag caacccgctc ccggtaaaac gggacggccc gccagaggac
61 ccctaaactc tgtttctata tgtaacttct gagtaaaacc ataaataaat caaaactttc
121 aacaacggat ctcttggttc tggcatcgat gaagaacgca gcaaaatgcg ataagtaatg
181 tgaattgcag aattcagtga atcatcgaat ctttgaacgc acattgcgcc cgccagtatt
241 ctggcgggca tgcctgttcg agcgtcattt caaccctcaa gcacagcttg gtgttgggac
301 tcgcgttaat tcgcgttcct caaattgatt ggcggtcacg tcgagcttcc atagcgtagt
361 agtaaaaccc tcgttactgg taatcgtcgc ggccacgccg ttaaaccccc aacttctgaa
421 tgttgaccgg atcaggtagg aatacccgct gaacttaagc atatcaataa cggagga

Isolate no.2, *Fusarium solani* (KJ831188)

1 ttgcctcgcg gaacatactg ccccgtaaca cgggccgccc ccgccagagg acccccctaac
61 tctgtttcta taatgtttct tctgagtaaa caagcaaata aattaaaact ttcaacaacg
121 gatctcttgg ctctggcatc gatgaagaac gcagcgaaat gcgataagta atgtgaattg
181 cagaattcag tgaatcatcg aatctttgaa cgcacattgc gcccgccagt attctggcgg
241 gcatgcctgt tcgagcgtca ttacaaccct caggcccccg ggcctggcgt tggggatcgg
301 cggaagcccc gtgcgggcac tacgctcccc caaatacagt ggtggtcccg ccgcatcttc
361 cattgcttag gagataaaac ctcgcaact

Seq3, *Fusarium brachygibbosum* (KJ831190)

1 ggagtagtga cacggcggag caacccgctc ccggtaaaac gggacggccc gccagaggac
61 ccctaaactc tgtttctata tgtaacttct gagtaaaacc ataaataaat caaaactttc
121 aacaacggat ctcttggttc tggcatcgat gaagaacgca gcaaaatgcg ataagtaatg
181 tgaattgcag aattcagtga atcatcgaat ctttgaacgc acattgcgcc cgccagtatt
241 ctggcgggca tgcctgttcg agcgtcattt caaccctcaa gcacagcttg gtgttgggac
301 tcgcgttaat tcgcgttcct caaattgatt ggcggtcacg tcgagcttcc atagcgtagt
361 agtaaaaccc tcgttactgg taatcgtcgc ggccacgccg ttaaaccccc aacttctgaa
421 tgttgaccgg atcaggtagg aatacccgct gaacttaagc atatcaataa cggagga

Isolate no.4, *Rhizoctonia solani* (KJ831186)

Table 7: Sequence and alignment of nine fungal isolates.

1 tgtcttagtc acgttgtctc ccggggcatg gttgtgtctc tactgatgtg tgtgtcttta
61 gtcaaaatgg ttccccaatt aatggtgttt gttatcacca gcgttgcagc tgcaaggaat
121 aagttgttac cnccactttg gaagctgggg attagacagt gaactaaggc gatatcttcc
181 gctcaccatt gtagtatccg aactgaatag acctttttact ggcggcaata cccgtgtctt
241 tggggatact gatctcggcc caaacccgtc accttaggaa ggatgccctg ctattgctta
301 acccactttc tgagtaacga caataaataa taccagcc

Isolate no.5, *Aspergillus fumigatus* (KJ831194)

1 cggaggcatc agaaagagtc gcgggcccgc cgtttcgacg gccgccgggg aggccttgcg
61 cccccgggcc cgcgcccgcc gaagacccca acatgaacgc tgttctgaaa gtatgcagtc
121 tgagttgatt atcgtaatca gttaaaactt tcaacaacgg atctcttggt tccggcatcg
181 atgaagaacg cagcgaaatg cgataagtaa tgtgaattgc agaattcagt gaatcatcga
241 gtctttgaac gcacattgcg ccccctggta ttccgggggg catgcctgtc cgagcgtctt
301 gctgccctca agcacggctt gtgtgttggg ccccagcccc ctctcccggg ggacgggccg
361 aaaggaagcg gcggcaccgc gtccggtcct cagcgtatgg ggttttgtca cctgctgagg
421 cccggcccccc cgcagatacc catatttatt tttaaggtc

Isolate no.6, *Aspergillus flavus* (KJ831193)

1 gagccgtgtt tactgtacct tattgcttcg gcgggcccgc cattcatggc cgccgggggc
61 tctcagcccc gggcccgcgc ccgccggaga caccacgaac tctgtctgat ctagtgaagt
121 ctgagttgat tgtatcgcaa tcagttaaaa ctttcaacaa tggatctctt ggttccggca
181 tcgatgaaga acgcagcgaa atgcgataac tagtgtgaat tgcagaattc cgtgaatcat
241 cgagtctttg aacgcacatt gcgcccccctg gtattccggg gggcatgcct gtccgagcgt
301 cattgctgcc catcaagcac ggcttgtgtg ttgggtcgtc gtcccctctc cgggggggac
361 gggccccaaa ggcagcggcg gcaccgcgtc cgatcctcga gcgtatgggg ctttgtcacc
421 cgctctgtag gcccggccgg cgcttgccga acgcaaatca atctttttcc aggttgacct
481 cggatcaggt agggataccc gctgaactta agcatatcaa taacggagga

Isolate no.7, *Aspergillus oryzae* (KJ831192)

1 acgaacagga cggaaaatgc ccgggcccca ttcatggccg ccgggggctc tcacccctgt
61 gactttcccg ccggaacgac tctaactctg tctgatctag tgattgaatt gattgatctg
121 aatcaggtaa aacttacaca atggatctct tggatccggc atgtgtgaag aacaaatcaa
181 atgccataac tactgtgaat tgacaattcc ccgaatcatc tagtctttga atgcctgttg
241 caccccctgg tattcccggg ggcctgcctg tttgagggtg gttactgccc atcaagcacg
301 gcttgtgtgt tgggtcgtcg tcccctctcc gggggggacg ggccccaaag gcagcggcgg
361 caccgcgtcc gtcctcgagc gtatggggct ttgtcacccg ctctgagccc ggccggcgct
421 tgccgaacgc aaatcaatct ttttccaggt tgacctcgga tcagtaggga taccctgaa
481 cttaagctat c

Isolate no.8, *Penicillium chryogenum* (KJ831195)

1 cacgggtgtt tattttacct tgttgcttcg gcgggcccgc cttaactggc cgccgggggg
61 cttacgcccc cgggcccgcg cccgccgaag acaccctcga actctgtctg aagattgtag
121 tctgagtgaa aatataaatt atttaaaact ttcaacaacg gatctcttgg ttccggcatc
181 gatgaagaac gcacgaaatg cgatacgtaa tgtgaattgc aaattcagtg aatcatcgag
241 tctttgaacg cacattgcgc cccctggtat tccgggggggc atgcctgtcc aaacgtcatt
301 tctgccctca agcacggctt gtgtgttggg ccccgtcctc cgatcccggg ggacgggcaa
361 aaaggagcgg aggcccgatc cggtcctcga gtgtatggac ttcgggcaag aaagcccgcc
421 ggggacaaaa atcaaaaata ttaaggttgt cctca

Isolate no.9, *Penicillium commune* (KJ831196)

1 agagcgttta cgagtagctt cgcgggcccg ccttaactgg ccgccggggg gcttacgccc

Table 7: Sequence and alignment of nine fungal isolates.
61 ccgggcccgc gcccgccgaa gacaccctcg aactctgtct gaagattgta gtctgagtga 121 aaatataaat tatttaaaac tttcaacaac ggatctcttg gttccggcat cgatgaagaa 181 cgcagcgaaa tgcgatacgt aatgtgaatt gcaaattcag tgaatcatcg agtctttgaa 241 cgcacattgc gccccctggt attccggggg gcatgcctgt ccgagcgtca tttctgccct 301 caagcacggc ttgtgtgttg ggc

Table 8: ITS gene (600 bp) accession numbers of nine fungal isolates in the GenBank

GenBank Accession no.	Identified as	Country of origin	Identity (%)
KJ831189	*Fusarium oxysporum*	Egypt	99%
KJ831188	*Fusarium solani*	Egypt	100%
KJ831190	*Fusarium brachygibbosum*	Egypt	98%
KJ831186	*Rhizoctonia solani*	Egypt	96%
KJ831194	*Aspergillus fumigatus*	Egypt	96%
KJ831193	*Aspergillus Flavus*	Egypt	99%
KJ831192	*Aspergillus oryzae*	Egypt	95%
KJ831195	*Penicillium chryogenum*	Egypt	97%
KJ831196	*Penicillium commune*	Egypt	99%

A phylogenetic tree (Fig. 4.1) recovered three clones of Fusarium species; *Fusarium oxysporum*, *Fusarium solani* and *Fusarium brachygibbosum* and available sequences from databases. Sequences from clone types *Fusarium brachygibbosum* showed 100 similarity with ex-type *Fusarium brachygibbosum* MS-R1 and *Fusarium oxysporum* showed high similarity with *Fusarium oxysporum* Fox64

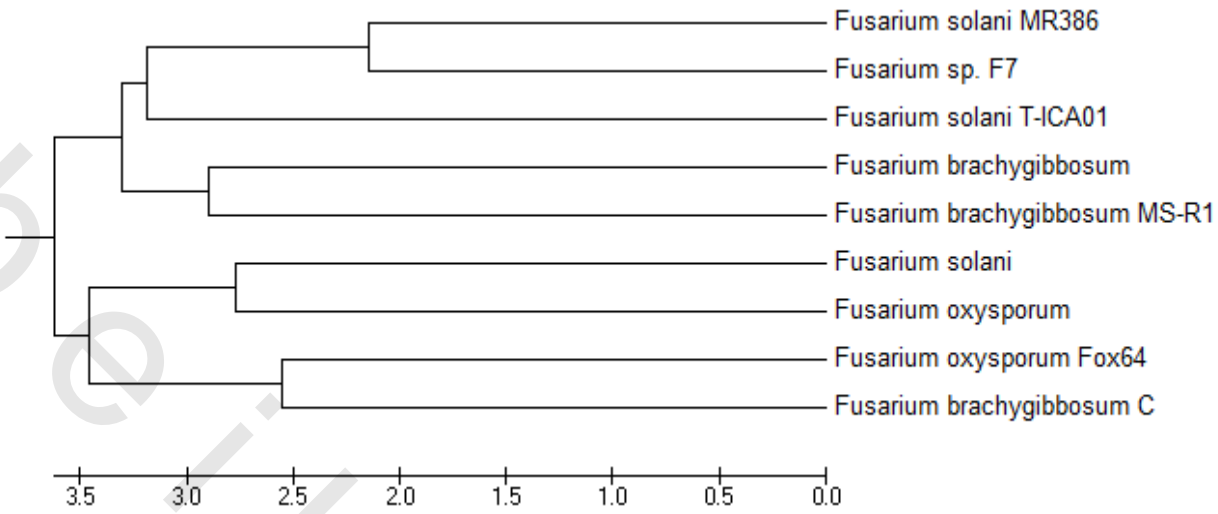


Figure 4.1: Phylogram based on the ITS- rDNA region of three Fusariums isolates and their phylogenetic analysis conducted using the UPGMA method with other ex-type isolates of F. spp. by MEGA 6.0 software

A phylogenetic tree (Fig. 4.2) recovered one clone of *Rhizoctonia solani* with two other ex-*rhizoctonia* types from databases. Sequences from clone types *Rhizoctonia solani* showed high with *Rhizoctonia solani isolate RS1*

Figure 4.2: Phylogram based on the ITS- rDNA region of one Rhizoctonia isolate and their phylogenetic analysis conducted using the UPGMA method with other ex-type isolates of *R. sola*ni by MEGA 6.0 software

A phylogenetic tree (Fig. 4.3) recovered three clones of *Aspergillus species* with six ex-type's fungal gerera from databases. Sequences from three studied clone types; *Aspergillus fumigates, flavus and oryzae* showed high similarities with the same species clones databases

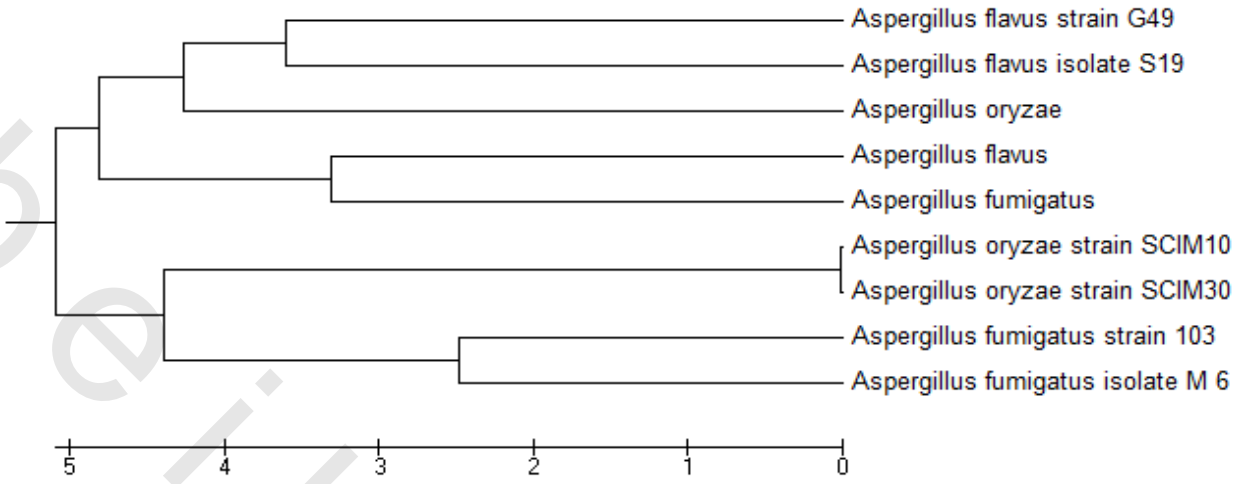


Figure 4.3: Phylogram based on the ITS- rDNA region of three *Aspergillus* isolates and their phylogenetic analysis conducted using the UPGMA method with other ex-type isolates of A. spp.by MEGA 6.0 software

A phylogenetic tree (Fig. 13.4) recovered two clones of *Penicillium species* with other five ex- type's fungal gerera from databases. Sequences from three studied clone types; *Penicillium chrysogenum and Penicillium commune* showed high similarities with the same species clones databases

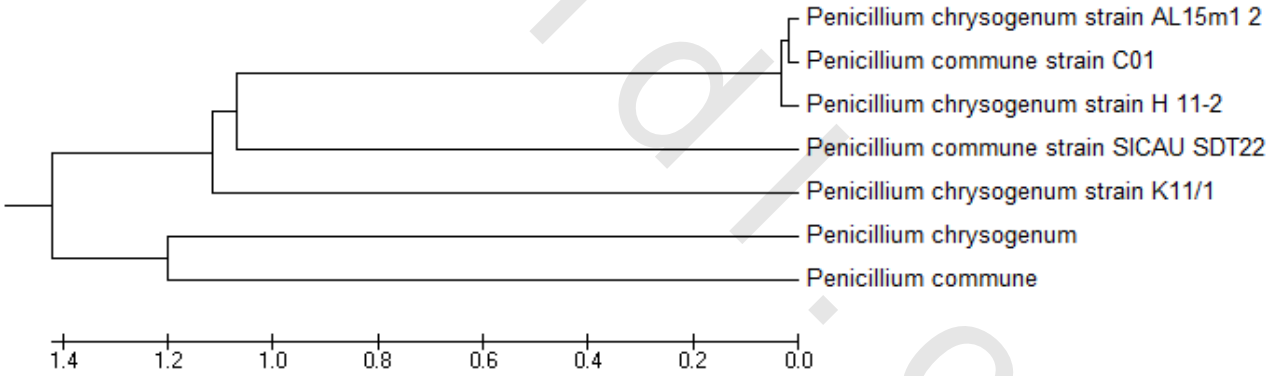


Figure 13.4: Phylogram based on the ITS- rDNA region of two penicillium isolates and their phylogenetic analysis conducted using the UPGMA method with other ex-type isolates of A. spp.by MEGA 6.0 software

VI.3. Antifungal activity of plant extracts

Any chemical with fungicidal properties could be potentially useful to inhibit fungal growth or sporulation. Some extracts derived from plants that have fungicidal properties may control plant fungal pathogens. Laboratory screening of plant extracts for their biological activity using simple procedures has given encouraging results in earlier

studies. For example, water extracts of several plants have been shown to have antifungal activity against *F. oxysporum*

VI.3.1. Antifungal activities of plant extract using antibiotic reference

The antifungal activity of different extracts derived from fifteen medicinal plants was also evaluated against nine different fungal strains, namely, *F. oxysporum, F. solani F. brachygibbosum, R. solani, A, Fumigatus, A. flavus,F.oryzae, P. chryogenum, P. commune*.

VI.3.1.1. against *Fusarium oxysporum*

The results of the effects of essential oils, aqueous and methanolic extracts of the fifteen plants at concentration 4mg/well (40µl) from the stock extracts (100 mg/ml) against the tested *Fusarium oxysporum* are presented in (Table 9, Fig. 5.1-5.3, 6)

Antifungal activity of the plant extracts against *Fusarium oxysporum*: All plant extracts exhibited highly to moderate antifungal activity against *F. oxysporum* (8±0.4 mm- 20±0.51 mm). In the present study, maximum antifungal activity was observed for many essential oils extracts against *F. oxysporum; Alium sativum* (20±0.51mm), *Anethum graveolens* (18±0.22 mm) *Syzygium aromaticum* (18±0.34mm), *Alium cepa* (17±0.65 mm), *Foeniculum vulgare* (17±0.52 mm), *Mentha varidis* (17±0.4 mm), *Eucalyptus globules* (17±0.00 mm), *ziziphus spina-csisti* (17±0.4 mm) and *Zingiber officinale* (16±0.93 mm)

Whereas, many Aqueous extracts was found to be very effective against *F. oxysporum; Nigella Sativa* (20±0.37 mm), *Mentha varidis* (19±0.44 mm), *Cassia Angustifolia* (19±0.37 mm), *Thymus vulgaries* (18±0.74 mm), *Zingiber officinale* (16±0.45 mm), *Foeniculum vulgare* (15±0.51 mm), *ziziphus spina-csisti* (14±0.43 mm) and *Olea eurpaea* (14±0.2 mm)

In addition some methanol extracts was found to be very effective against *F. oxysporum; Zingiber officinale* (18±0.71 mm), *Alium sativum* (17±0.47 mm), *Nigella Sativa* (17±0.27 mm), *Alium cepa* (16±0.27 mm), *Syzygium aromaticum* (16±0.81 mm) and 16±0.81 *(*16±0.1 mm)

Table 9: Antifungal activity of essential oils, aqueous and methanolic extracts derived from fifteen medicinal plants at 4mg/disc against *Fusarium oxysporum*

Extraction / Plant	Zone of Inhibition (mm)				
	Aq. (4mg/disc)	**Me.** (4mg/disc)	**Oil** (4mg/disc)	**Control**	**Std.** (0.1 mg/disc)
Rosmarinus officinalis	**20±0.57**	**11±0.15**	**13±0.62**	**NA**	**21±0.31**
Zingiber officinale	**16±0.45**	**18±0.71**	**16±0.93**	**NA**	**19±0.36**
Alium sativum	**13±0.37**	**17±0.47**	**20±0.51**	**NA**	**19±0.30**
Alium cepa	**10±0.4**	**16±0.27**	**17±0.65**	**NA**	**18±0.23**
Foeniculum vulgare	**15±0.51**	**15±0.31**	**17±0.52**	**NA**	**18±0.26**
Nigella Sativa	**20±0.37**	**17±0.27**	**14±0.53**	**NA**	**22±0.19**
Thymus vulgaries	**18±0.74**	**13±0.17**	**15±0.42**	**NA**	**18±0.22**
Cassia Angustifolia	**19±0.37**	**15±0.51**	**14±0.28**	**NA**	**20±0.42**
Mentha varidis	**19±0.44**	**13±0.76**	**17±0.4**	**NA**	**19±0.32**
Syzygium aromaticum	**8±0.4**	**16±0.81**	**18±0.34**	**NA**	**20±0.2**
Anethum graveolens	**9±0.17**	**16±0.1**	**18±0.22**	**NA**	**20±0.63**
Eucalyptus globules	**8±0.32**	**14±0.3**	**17±0.00**	**NA**	**20±0.43**
Lavandula angustifolia	**13±0.34**	**15±0.55**	**16±0.16**	**NA**	**21±0.24**
Olea eurpaea	**14±0.2**	**12±0.46**	**15±0.73**	**NA**	**18±0.6**
ziziphus spina-csisti	**14±0.43**	**15±0.18**	**17±0.4**	**NA**	**20±0.32**

*** Inhibition zone** measured in **(mm)**, **NA**= no inhibition zone:
*** Control**= DMSO (-ve control), **Std. =** Nystatin (positive control),
***Method of extraction**: **Me**= methanol, **Oil**= essential oil, **Aq**= Aqeous
*** ±Values** are mean= SD of three separate experiments, P<0.05 **significant**

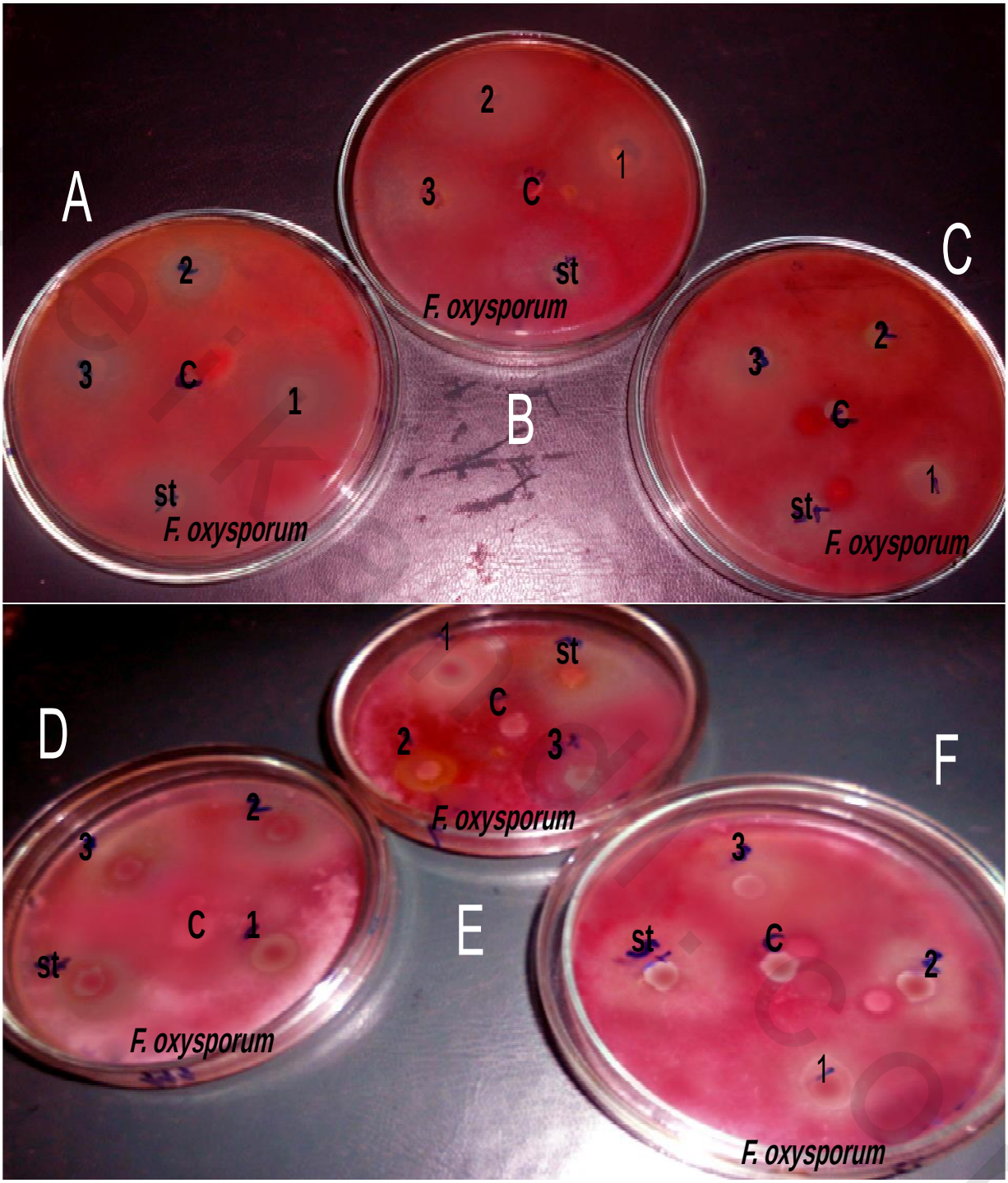


Figure 5.1: The effect of essential oils, aqueous and methanolic extracts derived from six medicinal plants; A=*Rosmarinus officinalis*, B=*Zingiber officinale*, C=*Alium sativum*, D=*Alium cepa*, E=*Foeniculum vulgare* and F=*Nigella Sativa* at 4mg/well against *Fusarium oxysporum*

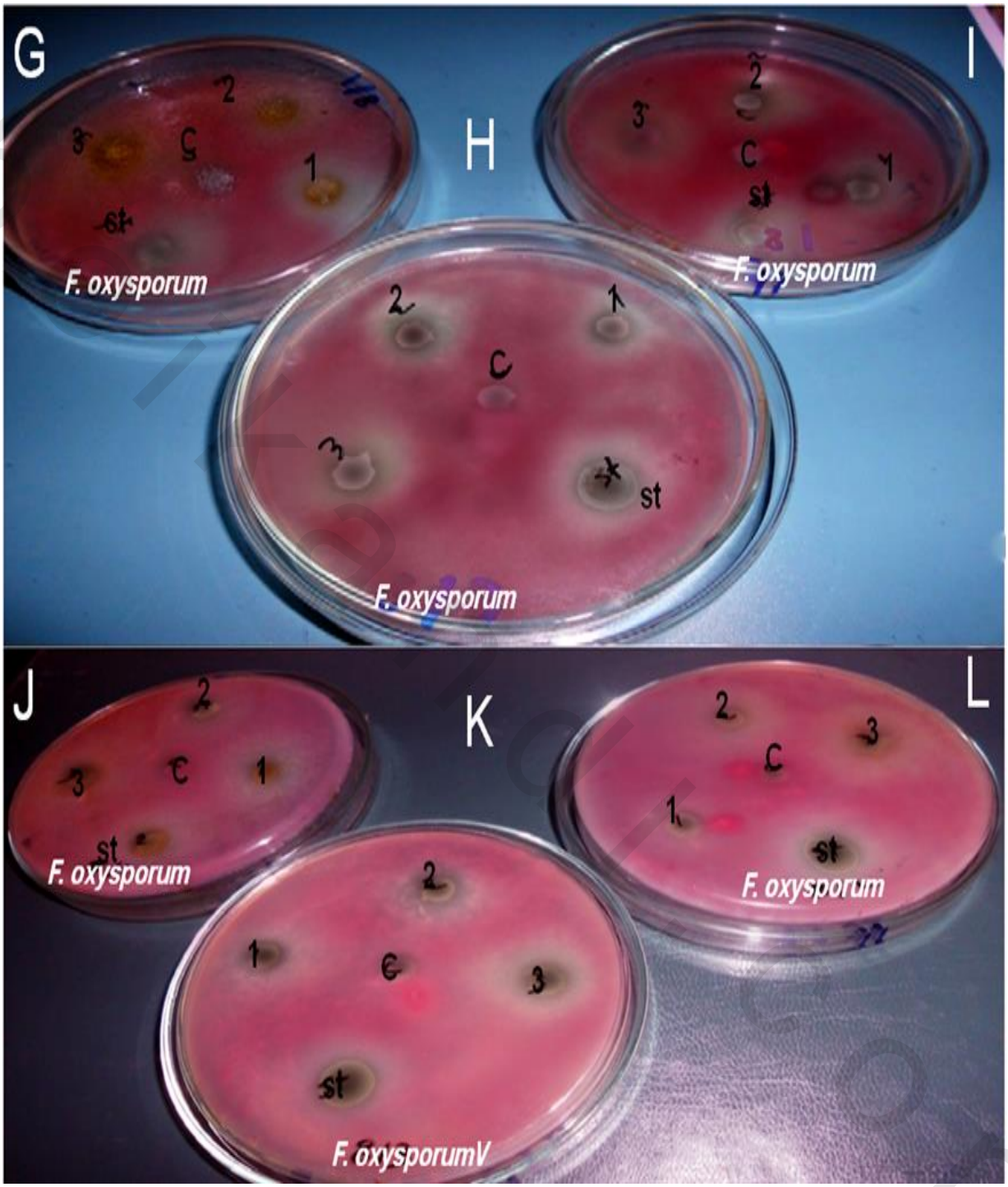


Figure 5.2: The effect of essential oils, aqueous and methanolic extracts derived from six medicinal plants; G=*Thymus vulgaries*, *H=Cassia Angustifolia*, *I=Mentha varidis*, *J=Syzygium aromaticum*, *K=Anethum graveolens* and *L=Eucalyptus globules* at 4mg/well against *Fusarium oxysporum*

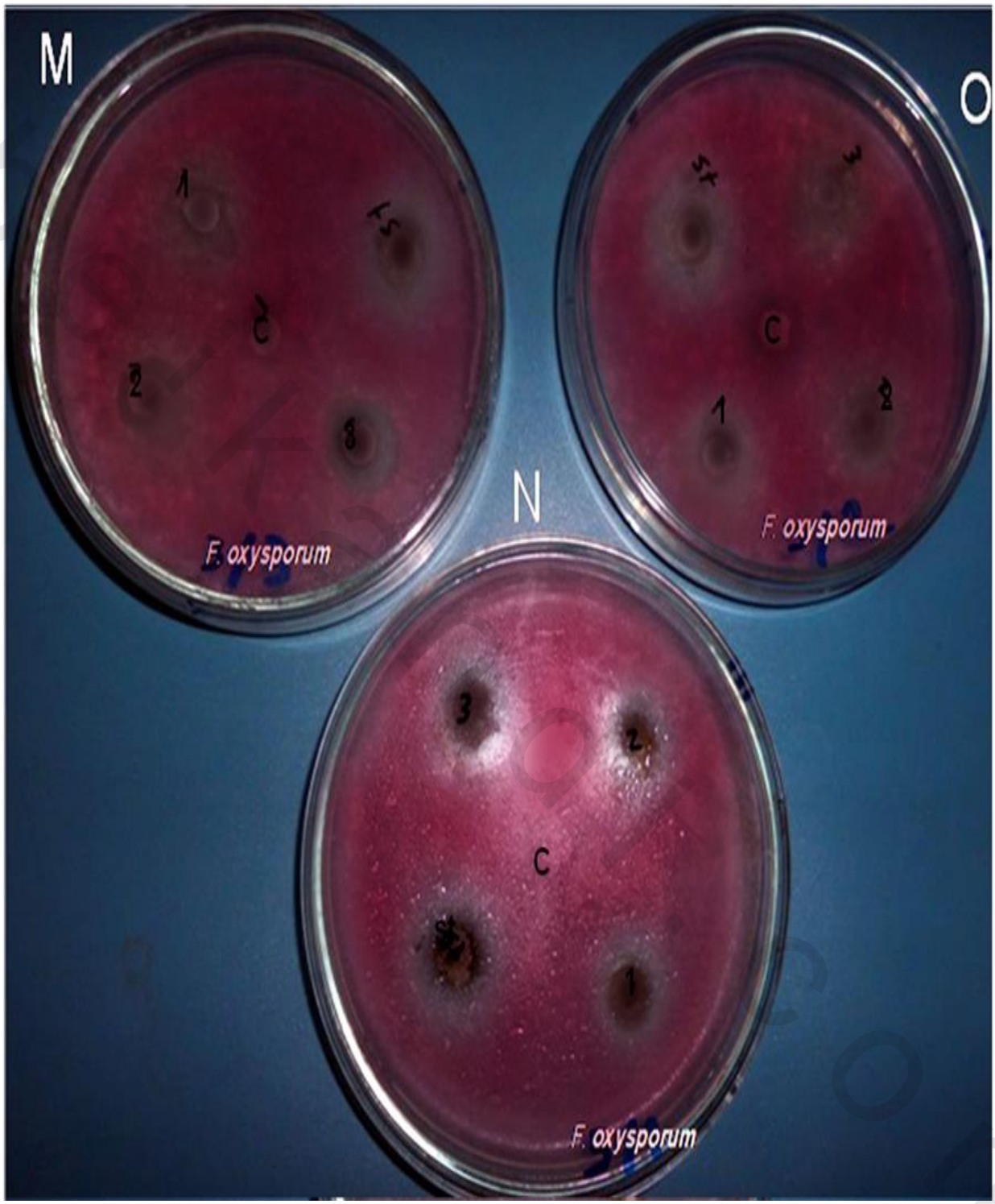


Figure 5.3: The effect of essential oils, aqueous and methanolic extracts derived from three medicinal plants; M=*Lavandula angustifolia, N=Olea eurpaea* and *O=ziziphus spina-csisti* at 4mg/well against *Fusarium oxysporum*

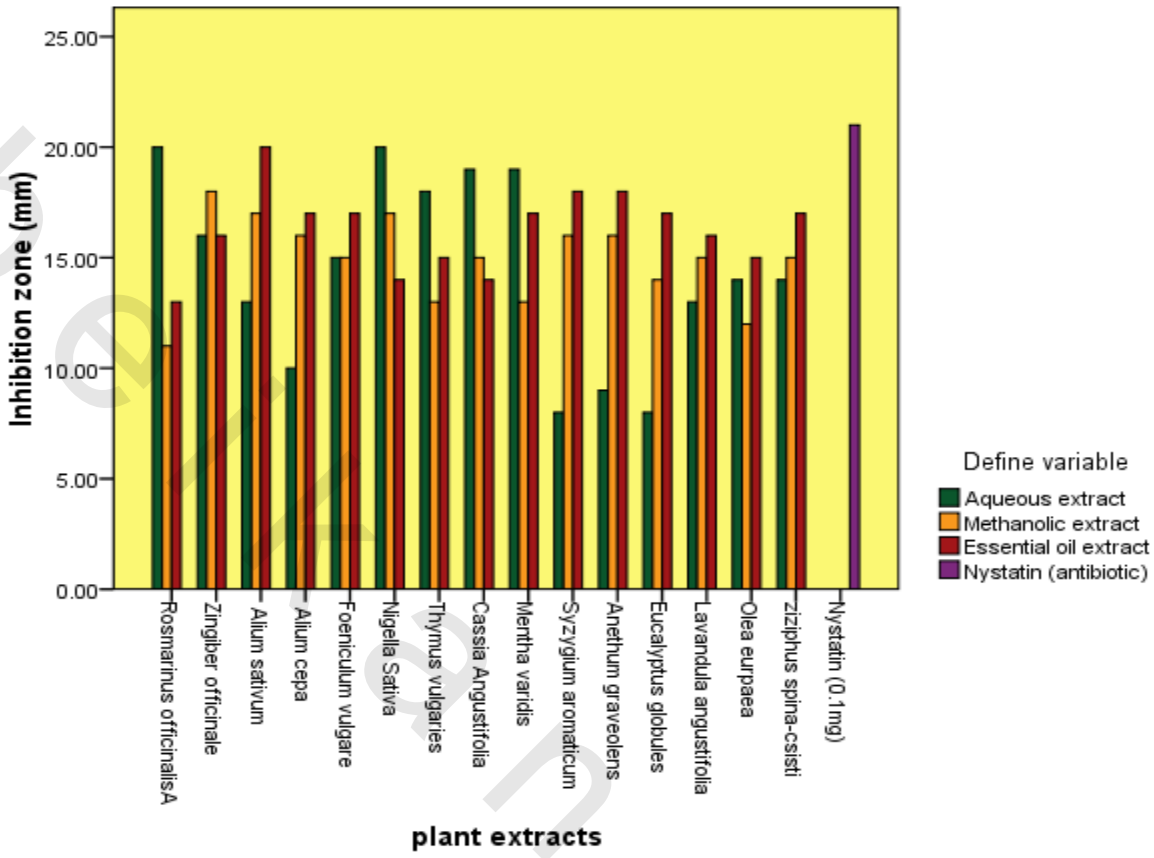


Figure 6: Diagrammatic representation of Antifungal activity of 4mg/ml concentration of aqueous, methanolic and oil extracts from 15 plant species against *Fusarium oxysporum*

VI.3.1.2. against *Fusarium solani*

The results of the effects of essential oils, aqueous and methanolic extracts of the fifteen plants at concentration 4mg/well (40µl) from the stock extracts (100 mg/ml) against the tested *Fusarium solani* are presented in (Table 10, Fig. 7.1-7.3, 8)

Antifungal activity of the plant extracts against *Fusarium solani*: All plant extracts exhibited highly to moderate antifungal activity against *F. solani* (6±0.11 mm - 19±0.06 mm). In the present study, maximum antifungal activity was observed for many essential oils extracts against *F. solani; Zingiber officinale* (19±0.06 mm), *Foeniculum vulgare* (17±0.4 mm), *Cassia Angustifolia* (17±0.61 mm), *Lavandula angustifolia* (17±0.26 mm),

Alium sativum (16±0.33 mm), *Mentha varidis* (16±0.47 mm), *Anethum graveolens* (16±0.49 mm), *Thymus vulgaries* (15±0.34 mm) and *Olea eurpaea* (15±0.18 mm)

Whereas, many methanol extracts was found to be very effective against *F. solani; Thymus vulgaries* (17±0.42 mm), *Cassia Angustifolia* (16±0.77 mm), *Foeniculum vulgare* (15±0.34 mm), *Nigella Sativa* (15±0.16 mm), *Zingiber officinale* (14±015 mm) and *Alium sativum* (14±0.15 mm)

In addition some aqueous extracts was found to be very effective against *F. solani; Mentha varidis* (16±0.16 mm), *Rosmarinus officinalis* (15±0.15 mm), *Zingiber officinale* (15±0.31 mm), *Foeniculum vulgare* (15±0.45 mm) and *Thymus vulgaries* (14±0.51 mm)

Table 10: Antifungal activity of essential oils, aqueous and methanolic extracts derived from fifteen medicinal plants at 4mg/disc against *Fusarium solani*

Extraction / Plant	Zone of Inhibition (mm)				
	Aq. (4mg/disc)	**Me.** (4mg/disc)	**Oil** (4mg/disc)	**Control**	**Std.** (0.1 mg/disc)
Rosmarinus officinalis	**15±0.15**	**13±0.4**	**16±0.30**	**NA**	**18±0.22**
Zingiber officinale	**15±0.31**	**14±015**	**19±0.06**	**NA**	**19±0.42**
Alium sativum	**NA**	**14±0.15**	**16±0.33**	**NA**	**18±0.60**
Alium cepa	**6±0.11**	**11±0.42**	**13±0.26**	**NA**	**17±0.00**
Foeniculum vulgare	**15±0.45**	**15±0.34**	**17±0.4**	**NA**	**16±0.26**
Nigella Sativa	**12±0.36**	**15±0.16**	**13±0.27**	**NA**	**17±0.30**
Thymus vulgaries	**14±0.51**	**17±0.42**	**15±0.34**	**NA**	**17±0.42**
Cassia Angustifolia	**13±0.22**	**16±0.77**	**17±0.61**	**NA**	**16±0.19**
Mentha varidis	**16±0.16**	**8±0.61**	**16±0.47**	**NA**	**17±0.47**
Syzygium aromaticum	**14±0.18**	**13±0.37**	**14±0.43**	**NA**	**17±0.55**
Anethum graveolens	**9±0.64**	**13±0.32**	**16±0.49**	**NA**	**17±0.27**
Eucalyptus globules	**8±0.44**	**9±0.15**	**13±0.5**	**NA**	**14±0.36**
Lavandula angustifolia	**14±0.42**	**12±0.6**	**17±0.26**	**NA**	**19±0.41**
Olea eurpaea	**9±0.51**	**11±0.24**	**15±0.18**	**NA**	**21±0.22**
ziziphus spina-csisti	**9±0.32**	**11±0.26**	**14±0.13**	**NA**	**18±0.25**

*** Inhibition zone** measured in **(mm)**, **NA**= no inhibition zone:
*** Control**= DMSO (-ve control), **Std. =** Nystatin (positive control),
***Method of extraction**: **Me**= methanol, **Oil**= essential oil, **Aq**= Aqeous
*** ±Values** are mean= SD of three separate experiments, P<0.05 **significant**

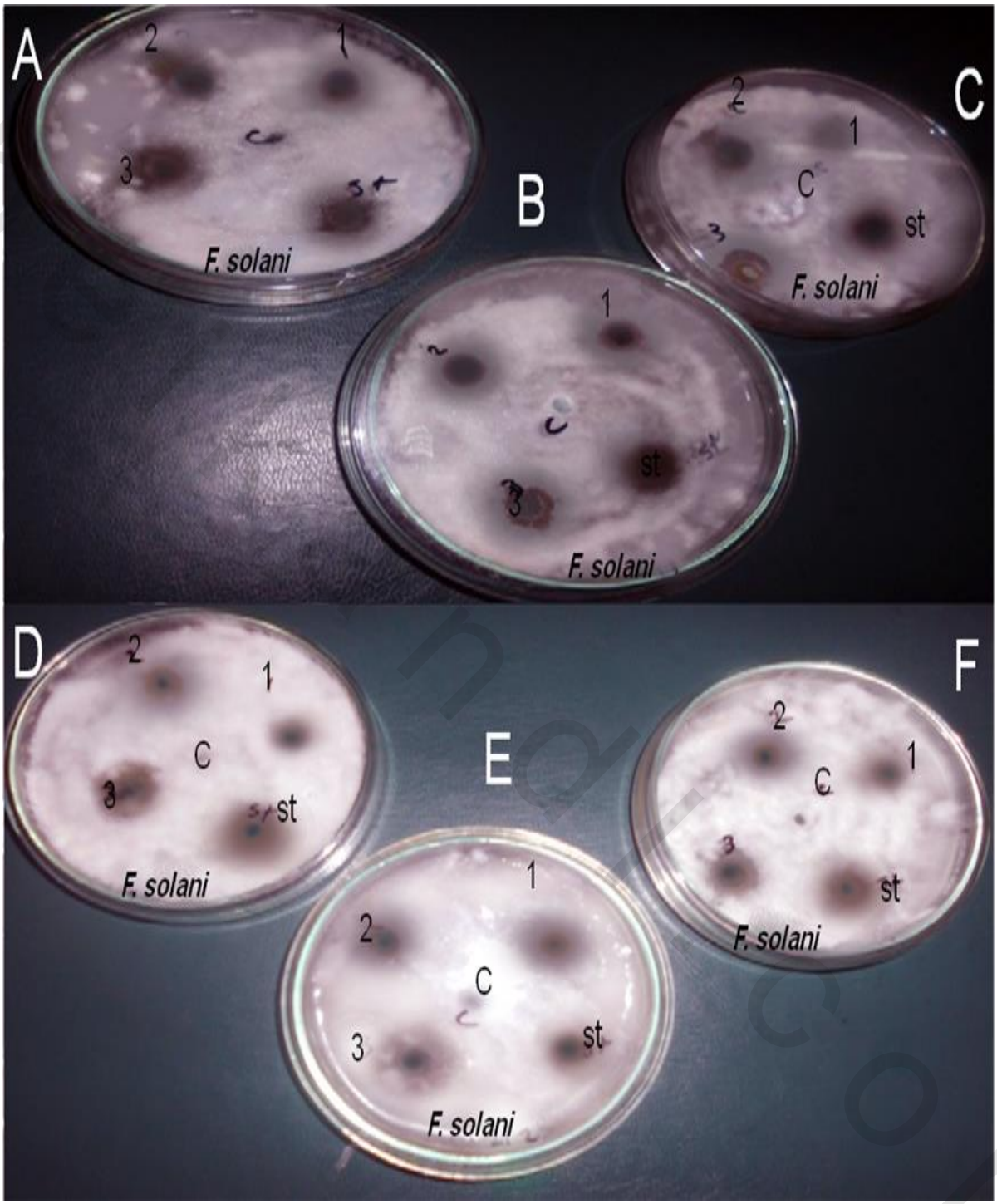


Figure 7.1: The effect of essential oils, aqueous and methanolic extracts derived from six medicinal plants; A=*Rosmarinus officinalis, B=Zingiber officinale, C=Alium sativum, D=Alium cepa, E=Foeniculum vulgare* and *F=Nigella Sativa* at 4mg/well against *Fusarium solani*

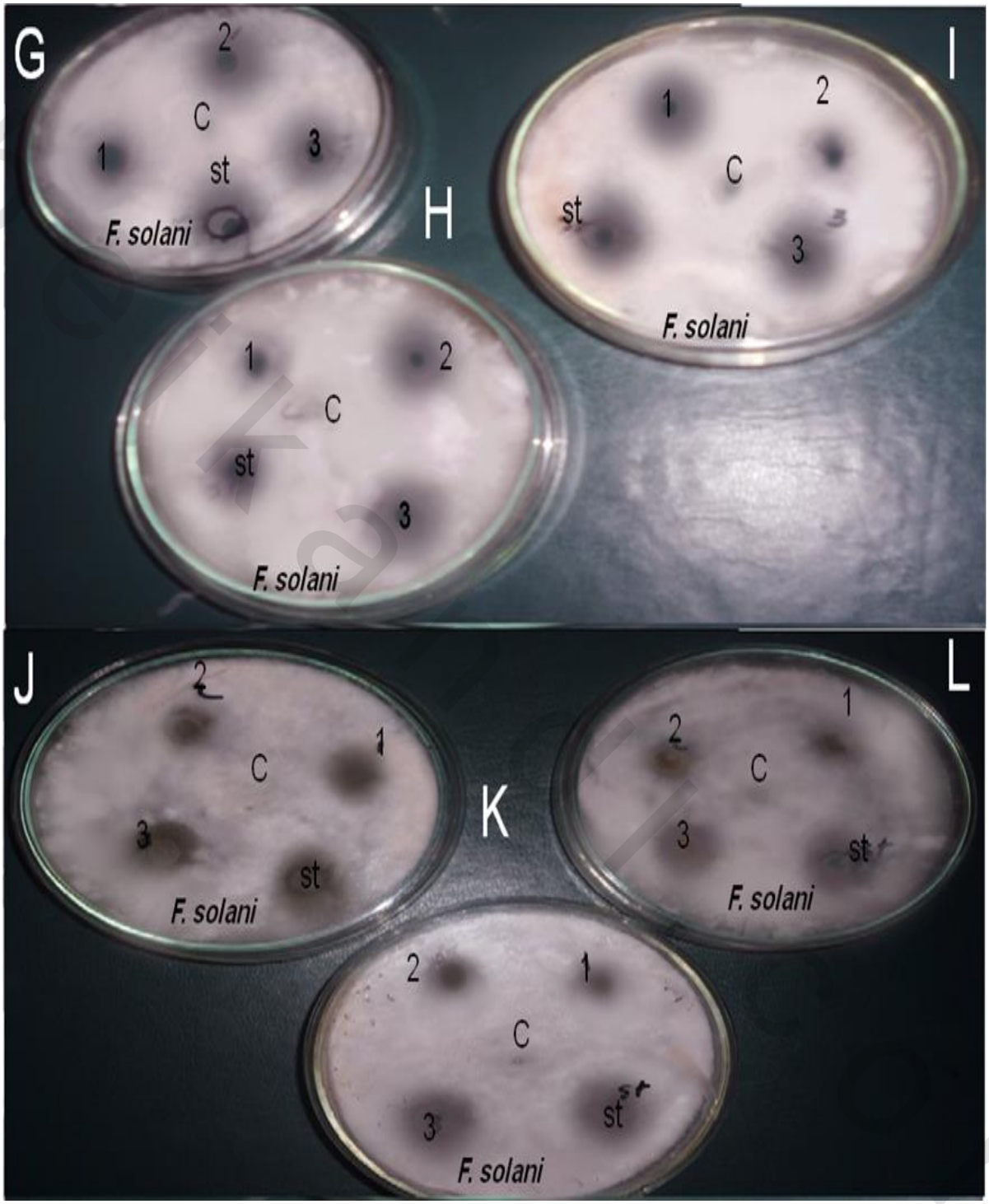


Figure 7.2: The effect of essential oils, aqueous and methanolic extracts derived from six medicinal plants; G=*Thymus vulgaries*, *H=Cassia Angustifolia*, *I=Mentha varidis*, *J=Syzygium aromaticum*, *K=Anethum graveolens* and *L=Eucalyptus globules* at 4mg/well against *Fusariu solani*

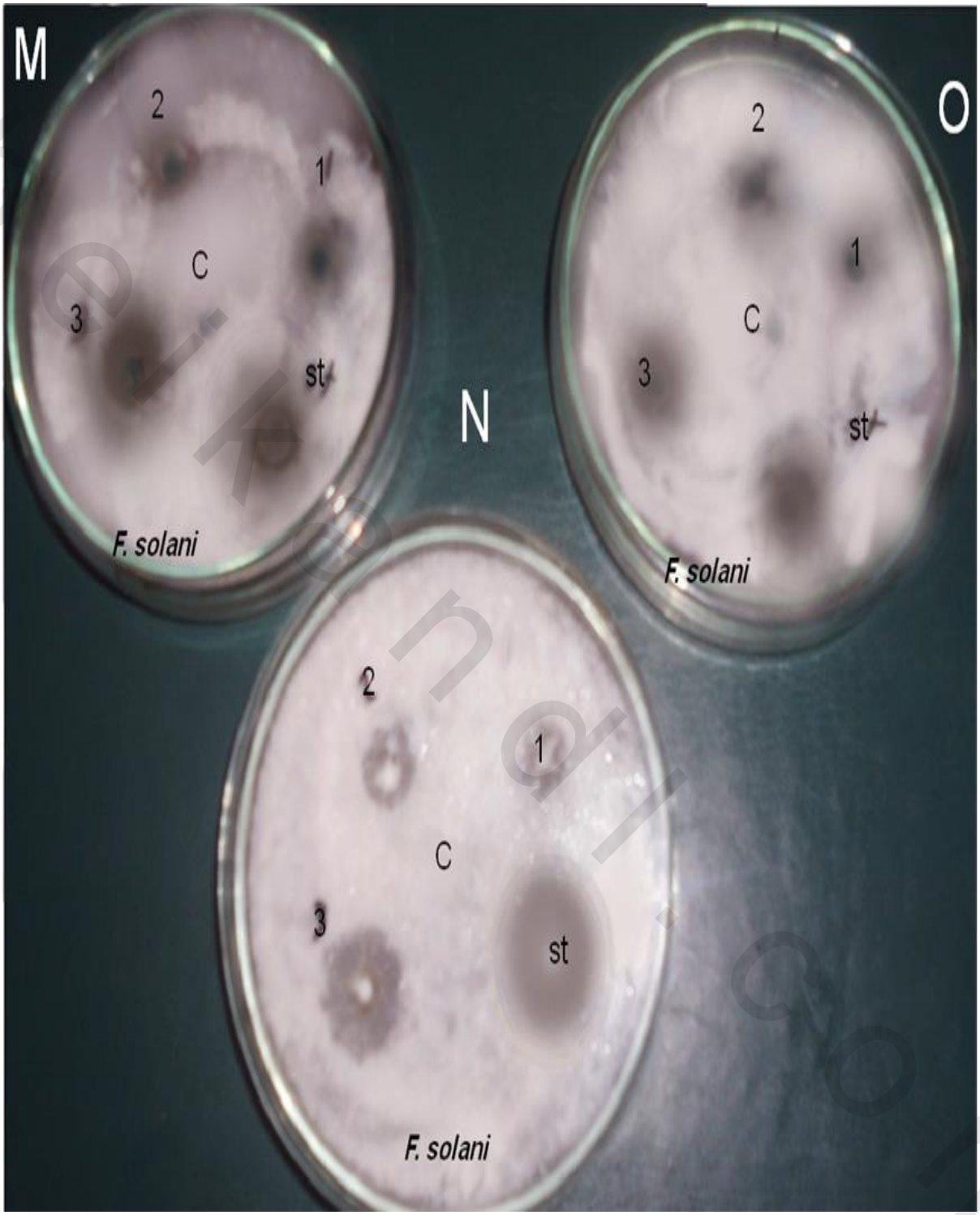


Figure 7.3: The effect of essential oils, aqueous and methanolic extracts derived from three medicinal plants; M=*Lavandula angustifolia, N=Olea eurpaea* and *O=ziziphus spina-csisti* at 4mg/well against *Fusarium solani*

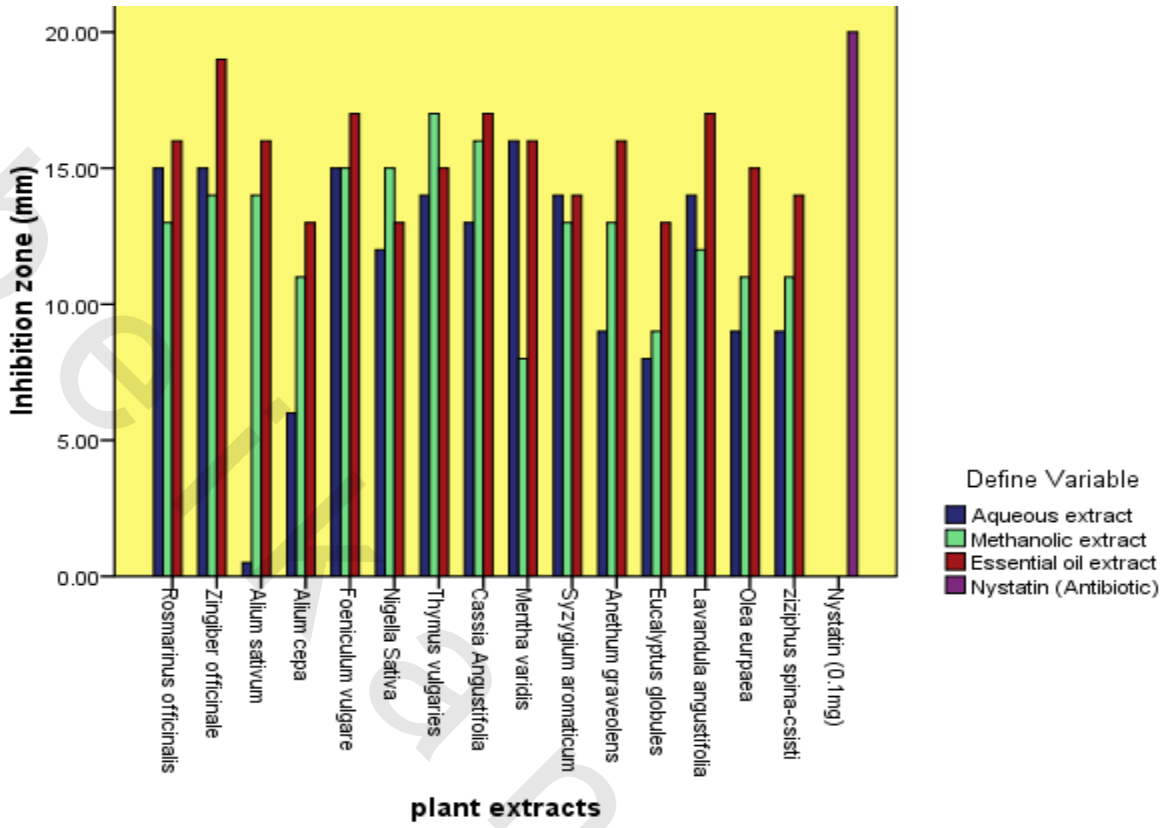


Figure 8: Diagrammatic representation of Antifungal activity of 100mg/ml concentration of aqueous, methanolic and oil extracts from 15 plant species against *Fusarium solani*

VI.3.1.3. against *Fusarium brachygibbosum*

The results of the effects of essential oils, aqueous and methanolic extracts of the fifteen plants at concentration 4mg/well (40µl) from the stock extracts (100 mg/ml) against the tested *Fusarium brachygibbosum* are presented in (Table 11, Fig. 9.1-9.3, 10)

Antifungal activity of the plant extracts against *Fusarium brachygibbosum*: All plant extracts exhibited highly to moderate antifungal activity against *F. brachygibbosum* (5±0.35 mm- 18±0.47 mm). In the present study, maximum antifungal activity was observed for many essential oils extracts against *F. brachygibbosum; Foeniculum vulgare* (18±0.47 mm), *ziziphus spina-csisti* (18±0.29 mm), *Lavandula angustifolia* (18±0.29 mm), *Rosmarinus officinalis* (17±0.25 mm), *Olea eurpaea* (16±0.63 mm),

Zingiber officinale (15±0.45 mm), *Mentha varidis* (15±0.34 mm) and *Anethum graveolens* (15±0.17 mm)

Whereas, many methanol extracts was found to be very effective against *F. brachygibbosum; Foeniculum vulgare* (16±0.52 mm), *Thymus vulgaries* (15±0.71 mm), *ziziphus spina-csisti* (15±0.70 mm), *Nigella Sativa* (15±0.36 mm) and *Syzygium aromaticum* (14±0.30 mm)

In addition aqueous extracts was found to be very effective against *F. brachygibbosum; Rosmarinus officinalis* (16±0.47 mm), *Zingiber officinale* (14±0.25 mm), *Foeniculum vulgare* (13±0.81 mm), *Mentha varidis* (13±0.36 mm), *Anethum graveolens* (13±0.21 mm) and *Cassia Angustifolia* (13±0.16 mm)

Table 11: Antifungal activity of essential oils, aqueous and methanolic extracts derived from fifteen medicinal plants at 4mg/disc against *Fusarium brachygibbosum*

Extraction / Plant	Zone of Inhibition (mm)				
	Aq. (4mg/disc)	**Me.** (4mg/disc)	**Oil** (4mg/disc)	**Control**	**Std.** (0.1 mg/disc)
Rosmarinus officinalis	**16±0.47**	**13±0.44**	**17±0.25**	**NA**	**19±0.55**
Zingiber officinale	**14±0.25**	**12±0.17**	**15±0.45**	**NA**	**15±0.47**
Alium sativum	**8±0.36**	**12±0.31**	**13±0.43**	**NA**	**15±0.19**
Alium cepa	**NA**	**14±0.25**	**13±0.65**	**NA**	**16±0.32**
Foeniculum vulgare	**13±0.81**	**16±0.52**	**18±0.47**	**NA**	**15±0.23**
Nigella Sativa	**8±0.66**	**15±0.36**	**10±0.16**	**NA**	**16±0.50**
Thymus vulgaries	**12±0.30**	**15±0.71**	**13±0.30**	**NA**	**16±0.32**
Cassia Angustifolia	**13±0.16**	**9±0.62**	**9±0.43**	**NA**	**15±0.14**
Mentha varidis	**13±0.36**	**NA**	**15±0.34**	**NA**	**17±0.23**
Syzygium aromaticum	**12±0.20**	**14±0.30**	**13±0.50**	**NA**	**16±0.53**
Anethum graveolens	**13±0.21**	**5±0.35**	**15±0.17**	**NA**	**15±0.25**
Eucalyptus globules	**9±0.56**	**9±0.31**	**13±0.35**	**NA**	**16±0.27**
Lavandula angustifolia	**11±0.42**	**13±0.17**	**18±0.21**	**NA**	**18±0.37**
Olea eurpaea	**10±0.40**	**12±0.52**	**16±0.63**	**NA**	**18±0.63**
ziziphus spina-csisti	**6±0.16**	**15±0.70**	**18±0.29**	**NA**	**19±0.21**

*** Inhibition zone** measured in **(mm)**, **NA**= no inhibition zone:
*** Control**= DMSO (-ve control), **Std. =** Nystatin (positive control),
***Method of extraction**: **Me**= methanol, **Oil**= essential oil, **Aq**= Aqeous
*** ±Values** are mean= SD of three separate experiments, P<0.05 **significant**

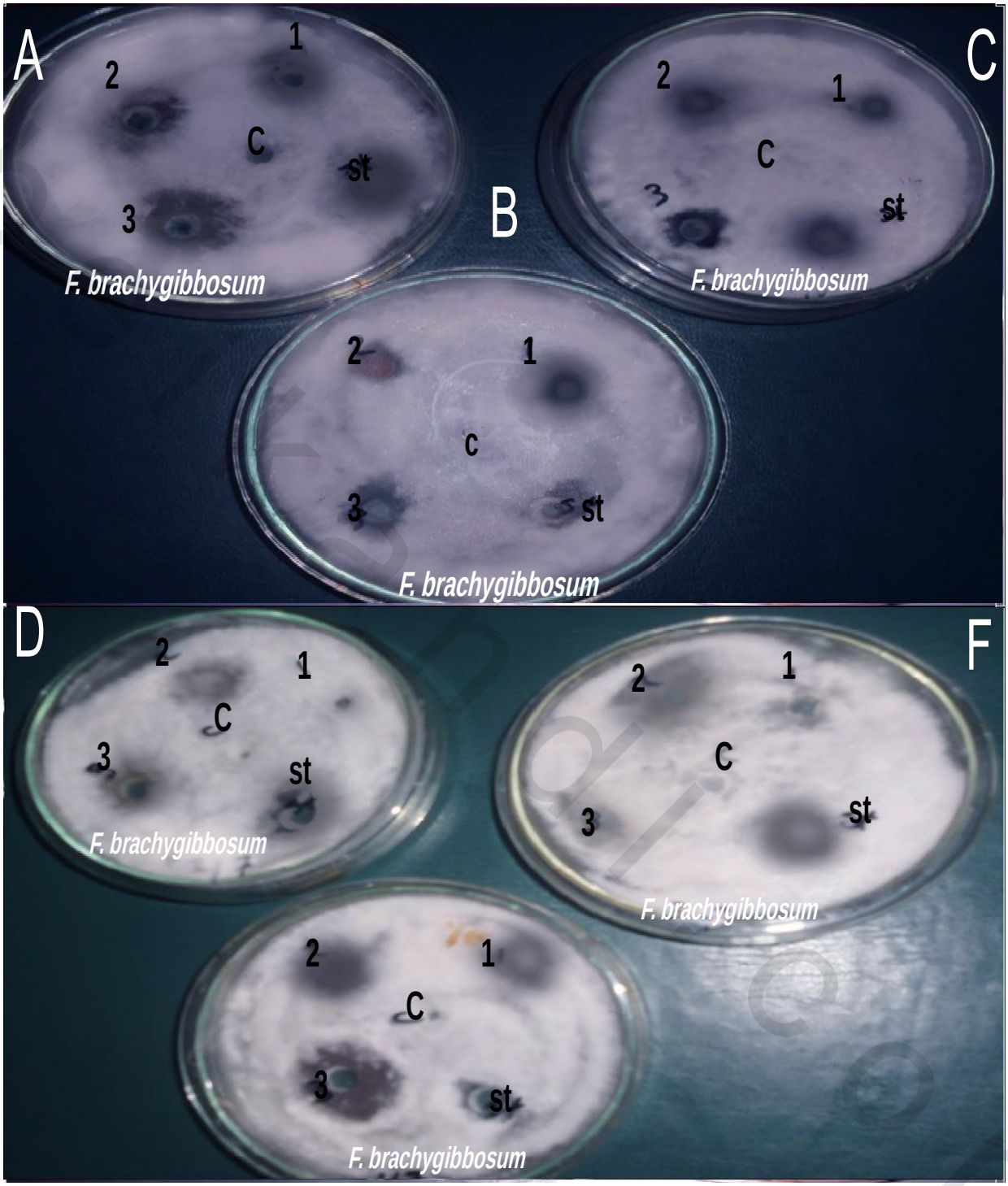


Figure 9.1: The effect of essential oils, aqueous and methanolic extracts derived from six medicinal plants; A=*Rosmarinus officinalis, B=Zingiber officinale, C=Alium sativum, D=Alium cepa, E=Foeniculum vulgare* and *F=Nigella Sativa* at 4mg/well against *Fusarium brachygibbosum*

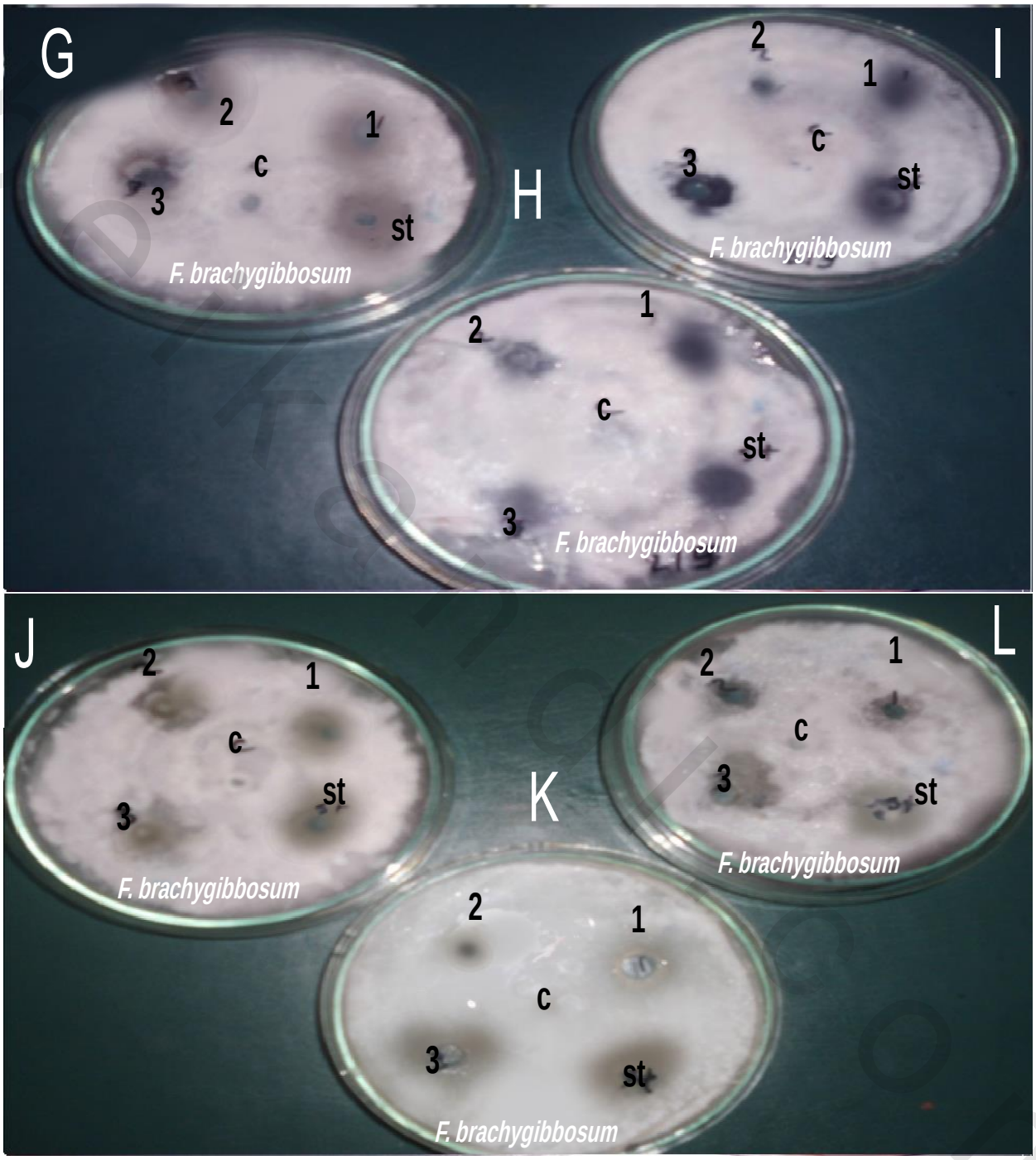


Figure 9.2: The effect of essential oils, aqueous and methanolic extracts derived from six medicinal plants; G=*Thymus vulgaries*, *H=Cassia Angustifolia*, *I=Mentha varidis*,

J=Syzygium aromaticum, K=Anethum graveolens **and** ***L=Eucalyptus globules*** **at 4mg/well against** ***Fusarium brachygibbosum***

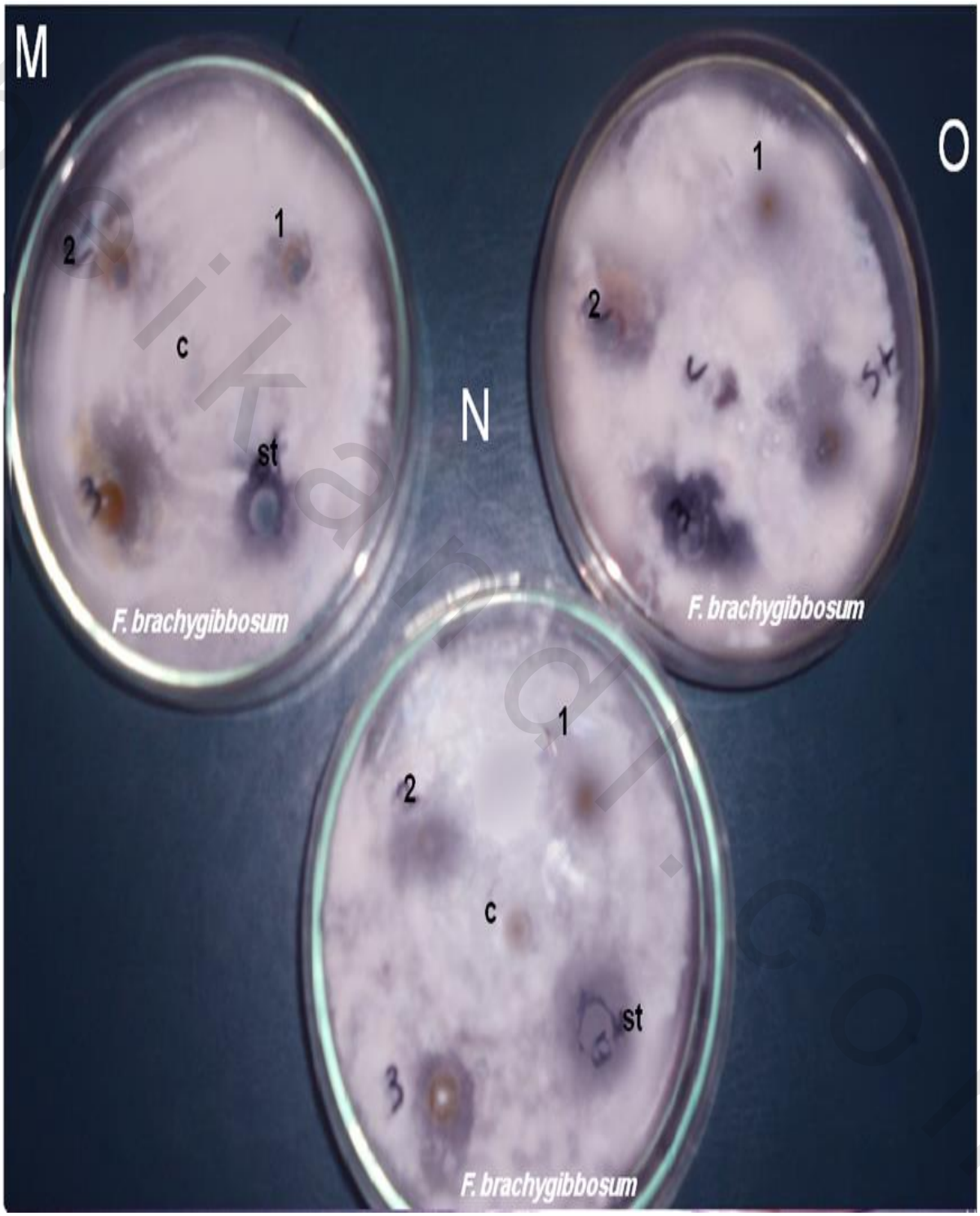


Figure 9.3: The effect of essential oils, aqueous and methanolic extracts derived from three medicinal plants; M=*Lavandula angustifolia, N=Olea eurpaea* and *O=ziziphus spina-csisti* at 4mg/well against *Fusarium brachygibbosum*

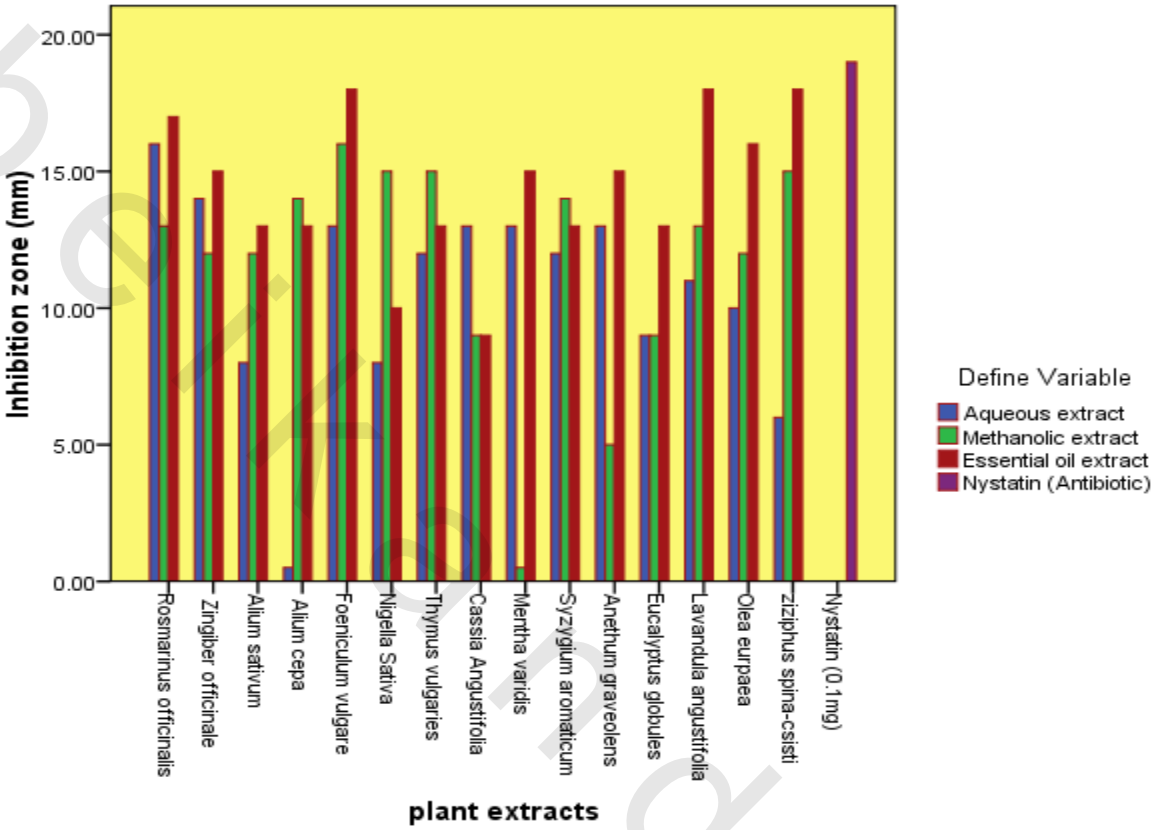


Figure 10: Diagrammatic representation of Antifungal activity of 100mg/ml concentration of aqueous, methanolic and oil extracts from 15 plant species against *Fusarium brachygibbosum*

VI.3.1.4. against *Rhizoctonia solani*

The results of the effects of essential oils, aqueous and methanolic extracts of the fifteen plants at concentration 4mg/well (40µl) from the stock extracts (100 mg/ml) against the tested *Rhizoctonia solani* are presented in (Table 12, Fig. 11.1-11.3, 12)

Antifungal activity of the plant extracts against *Rhizoctonia solani*: All plant extracts exhibited highly to moderate antifungal activity against *R. solani* (8±0.4mm-20±0.51 mm). In the present study, maximum antifungal activity was observed for many essential oils extracts against *R. solani; Alium sativum* (17±0.37 mm), *Anethum*

graveolens (16±0.42 mm), *Cassia Angustifolia* (15±0.71 mm), *Nigella Sativa* (15±0.50 mm), *Alium cepa* (15±0.16 mm) and *Syzygium aromaticum* (15±0.12 mm)

Whereas, many methanol extracts was found to be very effective against *R. solani; Cassia Angustifolia* (17±0.23 mm), *Zingiber officinale* (16±0.34 mm) *Alium sativum* (*Alium sativum mm*), *Anethum graveolens* (14±0.43 mm) and *Syzygium aromaticum* (14±0.27 mm)

In addition some aqueous extracts was found to be very effective against *R. solani; Cassia Angustifolia* (15±0.47 mm), *Zingiber officinale* (14±0.51 mm), *Rosmarinus officinalis* (14±0.20 mm) and *Alium cepa* (13±0.50 mm)

Table 12: Antifungal activity of essential oils, aqueous and methanolic extracts derived from fifteen medicinal plants at 4mg/disc against *Rhizoctonia solani*

Extraction / Plant	Zone of Inhibition (mm)				
	Aq. (4mg/disc)	**Me.** (4mg/disc)	**Oil** (4mg/disc)	**Control**	**Std.** (0.1 mg/disc)
Rosmarinus officinalis	**14±0.20**	**8±0.61**	**15±0.34**	**NA**	**19±0.32**
Zingiber officinale	**14±0.51**	**16±0.34**	**17±0.23**	**NA**	**18±0.65**
Alium sativum	**12±0.26**	**15±0.50**	**17±0.37**	**NA**	**19±0.45**
Alium cepa	**13±0.50**	**9±0.23**	**15±0.16**	**NA**	**18±0.72**
Foeniculum vulgare	**9±0.16**	**8±0.41**	**13±0.21**	**NA**	**15±0.16**
Nigella Sativa	**10±0.23**	**12±0.13**	**15±0.50**	**NA**	**17±0.30**
Thymus vulgaries	**11±0.65**	**13±0.10**	**11±0.35**	**NA**	**16±0.45**
Cassia Angustifolia	**15±0.47**	**17±0.23**	**15±0.71**	**NA**	**17±0.37**
Mentha varidis	**9±0.23**	**NA**	**11±0.54**	**NA**	**11±0.27**
Syzygium aromaticum	**12±0.40**	**14±0.27**	**15±0.12**	**NA**	**16±0.60**
Anethum graveolens	**12±0.23**	**14±0.43**	**16±0.42**	**NA**	**16±0.34**
Eucalyptus globules	**NA**	**8±0.12**	**11±0.20**	**NA**	**12±0.61**
Lavandula angustifolia	**NA**	**12±0.24**	**14±0.24**	**NA**	**16±0.25**
Olea eurpaea	**11±0.18**	**9±0.46**	**13±0.27**	**NA**	**13±0.21**
ziziphus spina-csisti	**NA**	**8±0.30**	**14±0.14**	**NA**	**15±0.34**

*** Inhibition zone** measured in **(mm)**, **NA**= no inhibition zone:
*** Control**= DMSO (-ve control), **Std. =** Nystatin (positive control),
***Method of extraction**: **Me**= methanol, **Oil**= essential oil, **Aq**= Aqeous
*** ±Values** are mean= SD of three separate experiments, P<0.05 **significant**

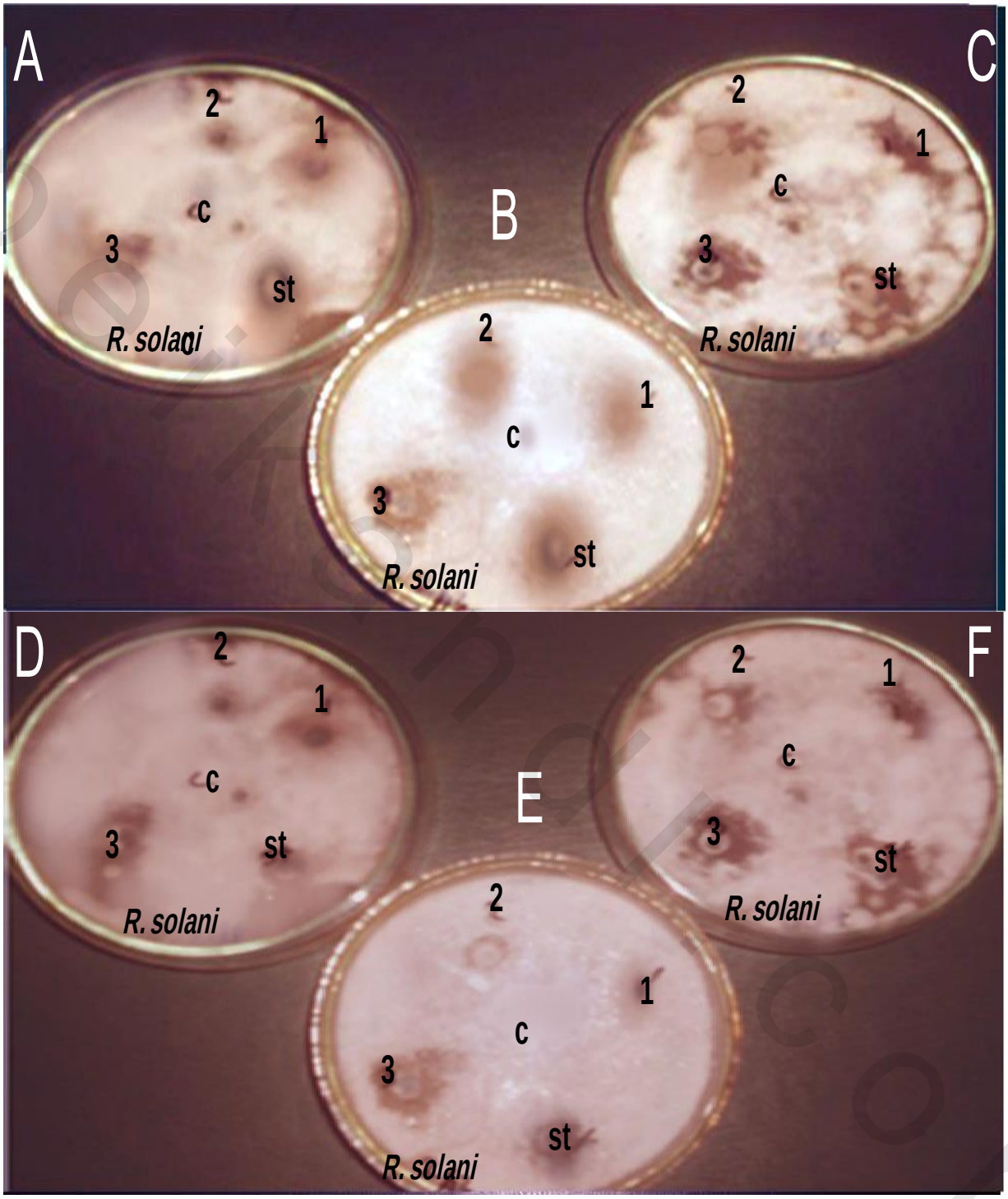


Figure 11.1: The effect of essential oils, aqueous and methanolic extracts derived from six medicinal plants; A=*Rosmarinus officinalis, B=Zingiber officinale, C=Alium sativum, D=Alium cepa, E=Foeniculum vulgare* and *F=Nigella Sativa* at 4mg/well against *Rhizoctonia solani*

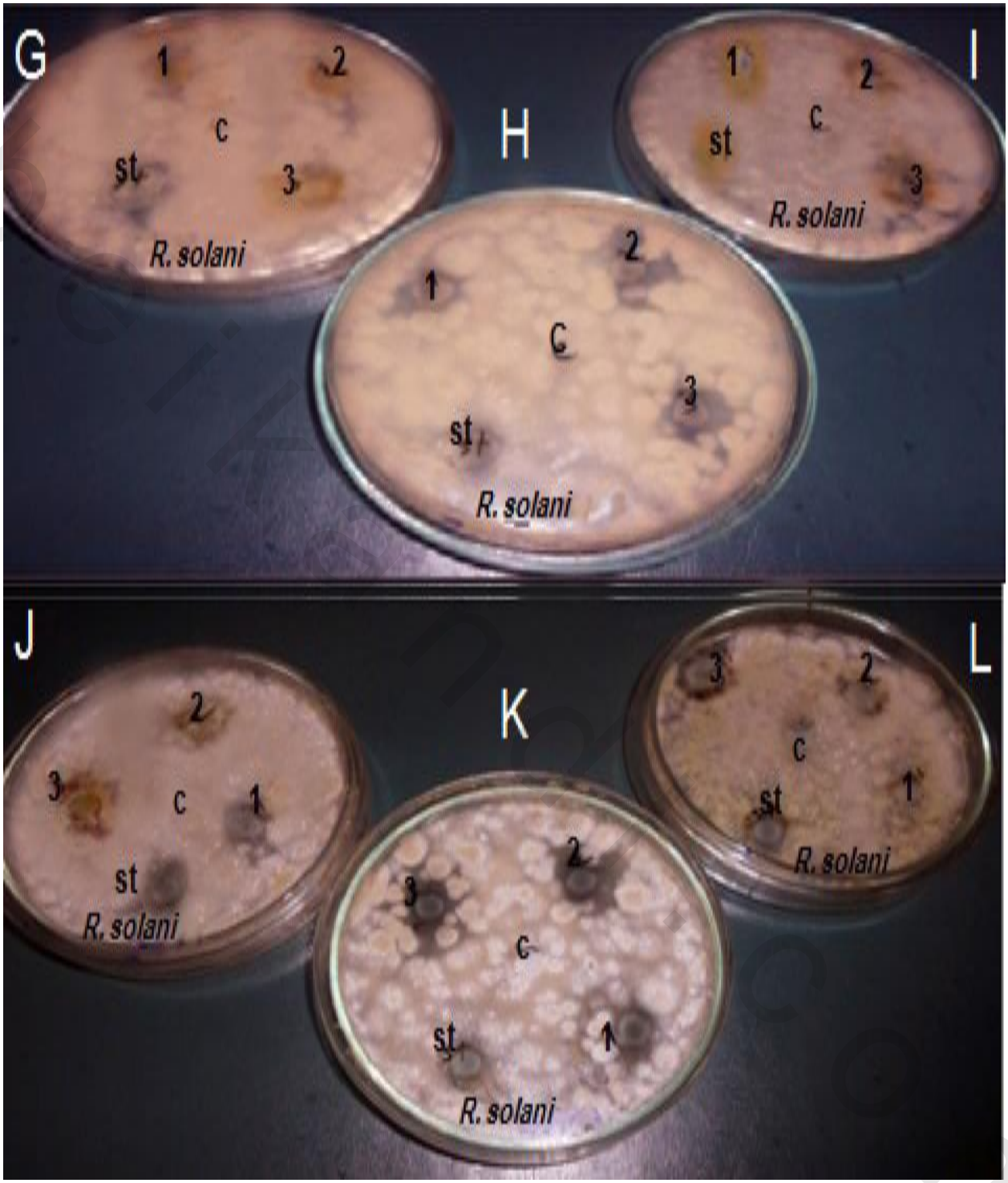


Figure 11.2: The effect of essential oils, aqueous and methanolic extracts derived from six medicinal plants; G=*Thymus vulgaries*, *H=Cassia Angustifolia*, *I=Mentha varidis*, *J=Syzygium aromaticum*, *K=Anethum graveolens* and *L=Eucalyptus globules* at 4mg/well against *Rhizoctonia solani*

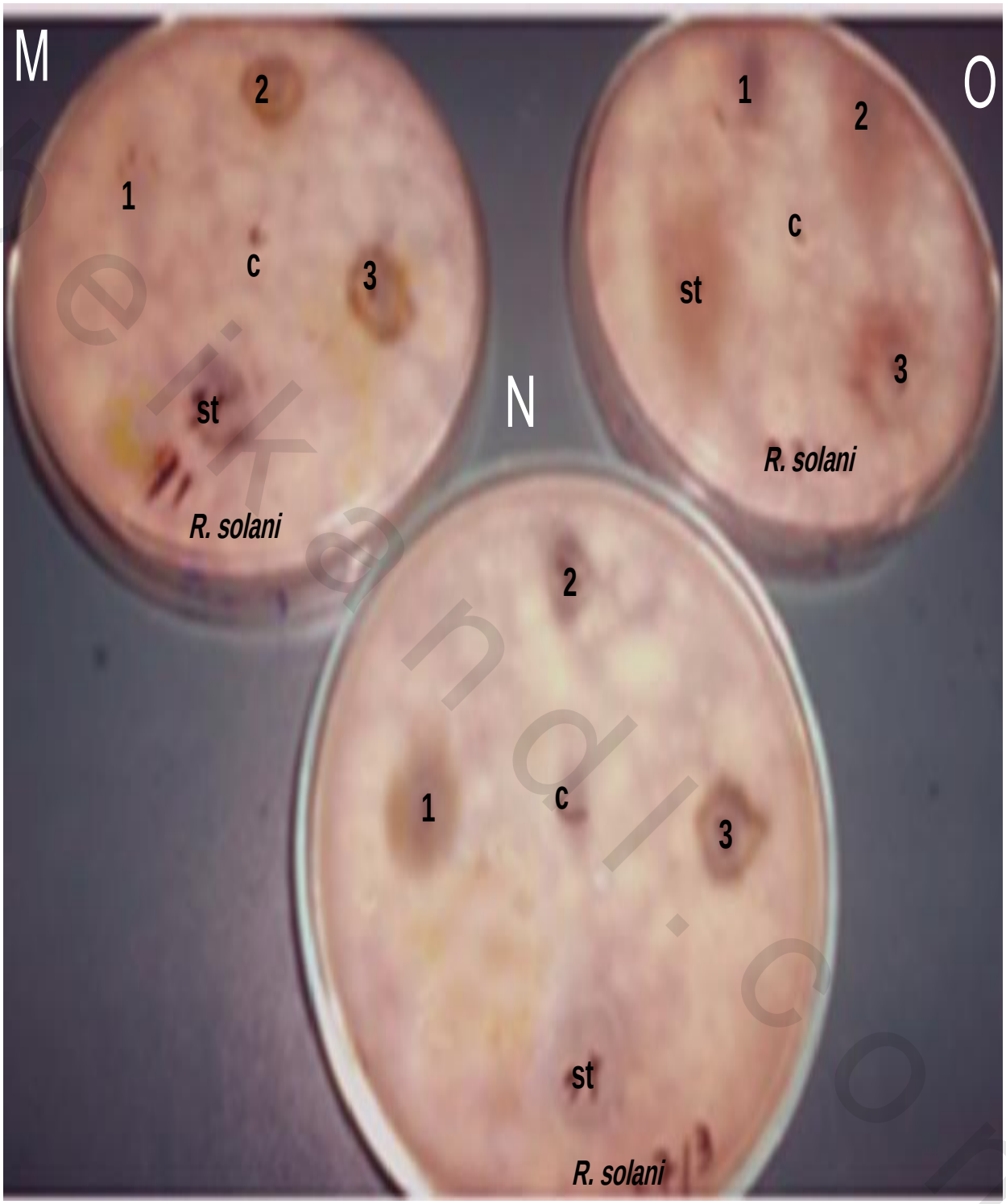


Figure 11.3: The effect of essential oils, aqueous and methanolic extracts derived from three medicinal plants; M=*Lavandula angustifolia, N=Olea eurpaea* and *O=ziziphus spina-csisti* at 4mg/well against *Rhizoctonia solani*

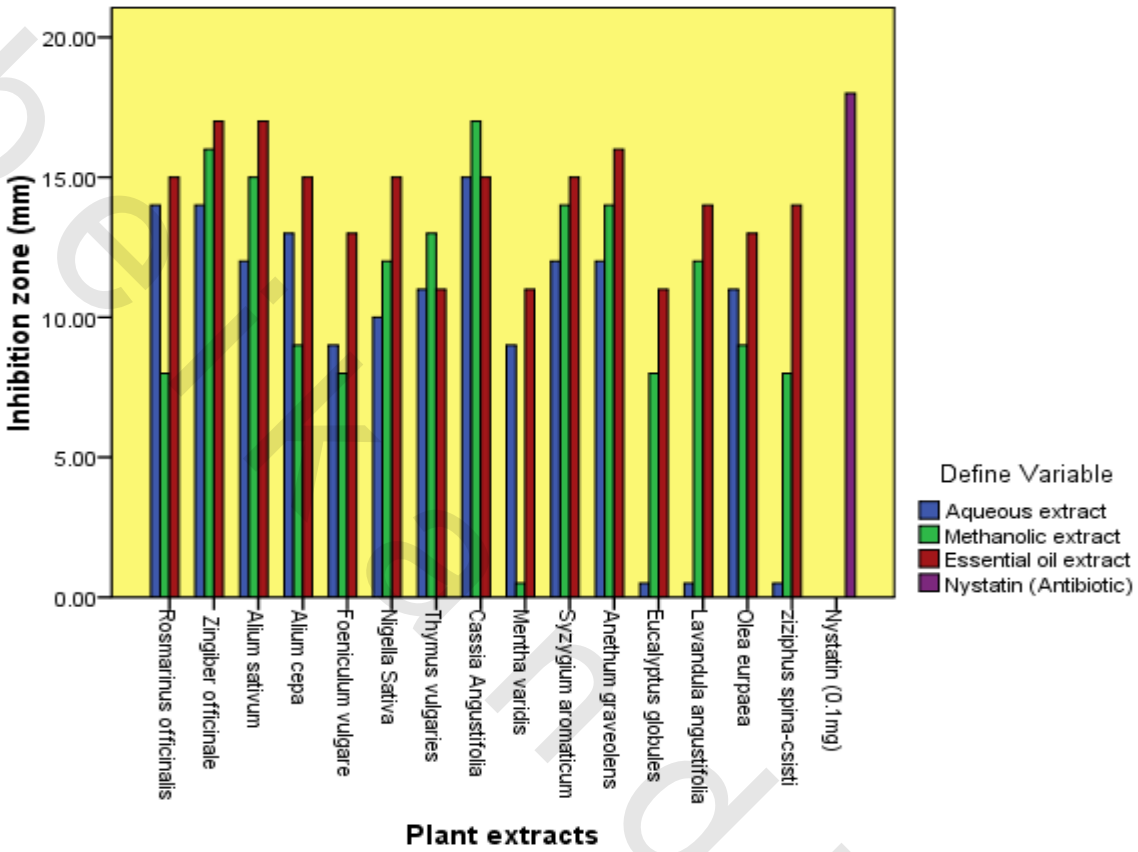


Figure 12: Diagrammatic representation of Antifungal activity of 100mg/ml concentration of aqueous, methanolic and oil extracts from 15 plant species against *Rhizoctonia solani*

VI.3.1.5. against *Aspergillus fumigatus*

The results of the effects of essential oils, aqueous and methanolic extracts of the fifteen plants at concentration 4mg/well (40µl) from the stock extracts (100 mg/ml) against the tested *Aspergillus fumigatus* are presented in (Table 13, Fig. 13.1-13.3, 14)

Antifungal activity of the plant extracts against *Aspergillus fumigatus*: All plant extracts exhibited highly to moderate antifungal activity against *A. fumigatus* (5±0.20 mm- 17±0.73 mm). In the present study, maximum antifungal activity was observed for many essential oils extracts against *A. fumigatus; Thymus vulgaries* (17±0.73 mm),

Olea eurpaea (17±0.66 mm), *Foeniculum vulgare* (17±0.56 mm), *Lavandula angustifolia* (17±0.43 mm), *Mentha varidis* (17±0.32 mm) and *Alium cepa* (17±0.23 mm)

Whereas, many aqueous extracts was found to be very effective against *A. fumigatus; Cassia Angustifolia* (17±0.52 mm), *Lavandula angustifolia* (17±0.41 mm), *Thymus vulgaries* (15±0.37 mm), *Foeniculum vulgare* (14±0.70 mm), *Mentha varidis* (14±0.35 mm) and *Rosmarinus officinalis* (14±0.25 mm)

In addition some methanol extracts was found to be very effective against *A. fumigatus; Zingiber officinale* (16±0.65 mm), *Foeniculum vulgare* (15±0.86 mm), *Lavandula angustifolia* (15±0.54 mm) and *Alium sativum* (14±0.41 mm)

Table 13: Antifungal activity of essential oils, aqueous and methanolic extracts derived from fifteen medicinal plants at 4mg/disc against *Aspergillus fumigatus*

Extraction / Plant	Zone of Inhibition (mm)				
	Aq. (4mg/disc)	**Me.** (4mg/disc)	**Oil** (4mg/disc)	**Control**	**Std.** (0.1 mg/disc)
Rosmarinus officinalis	**14±0.25**	**10±0.34**	**14±0.34**	**NA**	**21±0.40**
Zingiber officinale	**10±0.54**	**16±0.65**	**NA±0.21**	**NA**	**16±0.61**
Alium sativum	**NA±0.34**	**14±0.41**	**12±0.54**	**NA**	**15±0.32**
Alium cepa	**NA±0.17**	**14±0.23**	**17±0.23**	**NA**	**17±0.16**
Foeniculum vulgare	**14±0.70**	**15±0.86**	**17±0.56**	**NA**	**16±0.21**
Nigella Sativa	**12±0.22**	**12±0.45**	**16±0.43**	**NA**	**15±0.34**
Thymus vulgaries	**15±0.37**	**11±0.37**	**17±0.73**	**NA**	**17±0.52**
Cassia Angustifolia	**17±0.52**	**NA**	**14±0.51**	**NA**	**18±0.32**
Mentha varidis	**14±0.35**	**NA**	**17±0.32**	**NA**	**18±0.47**
Syzygium aromaticum	**6±0.55**	**12±0.43**	**15±0.17**	**NA**	**15±0.54**
Anethum graveolens	**11±0.32**	**6±0.41**	**13±0.32**	**NA**	**14±0.35**
Eucalyptus globules	**NA**	**NA**	**13±0.45**	**NA**	**15±0.67**
Lavandula angustifolia	**17±0.41**	**15±0.54**	**17±0.43**	**NA**	**17±0.34**
Olea eurpaea	**9±0.56**	**7±0.62**	**17±0.66**	**NA**	**17±0.52**
ziziphus spina-csisti	**NA**	**5±0.20**	**14±0.41**	**NA**	**15±0.27**

*** Inhibition zone** measured in **(mm)**, **NA**= no inhibition zone:
*** Control**= DMSO (-ve control), **Std. =** Nystatin (positive control),
***Method of extraction**: **Me**= methanol, **Oil**= essential oil, **Aq**= Aqeous
*** ±Values** are mean= SD of three separate experiments, P<0.05 **significant**

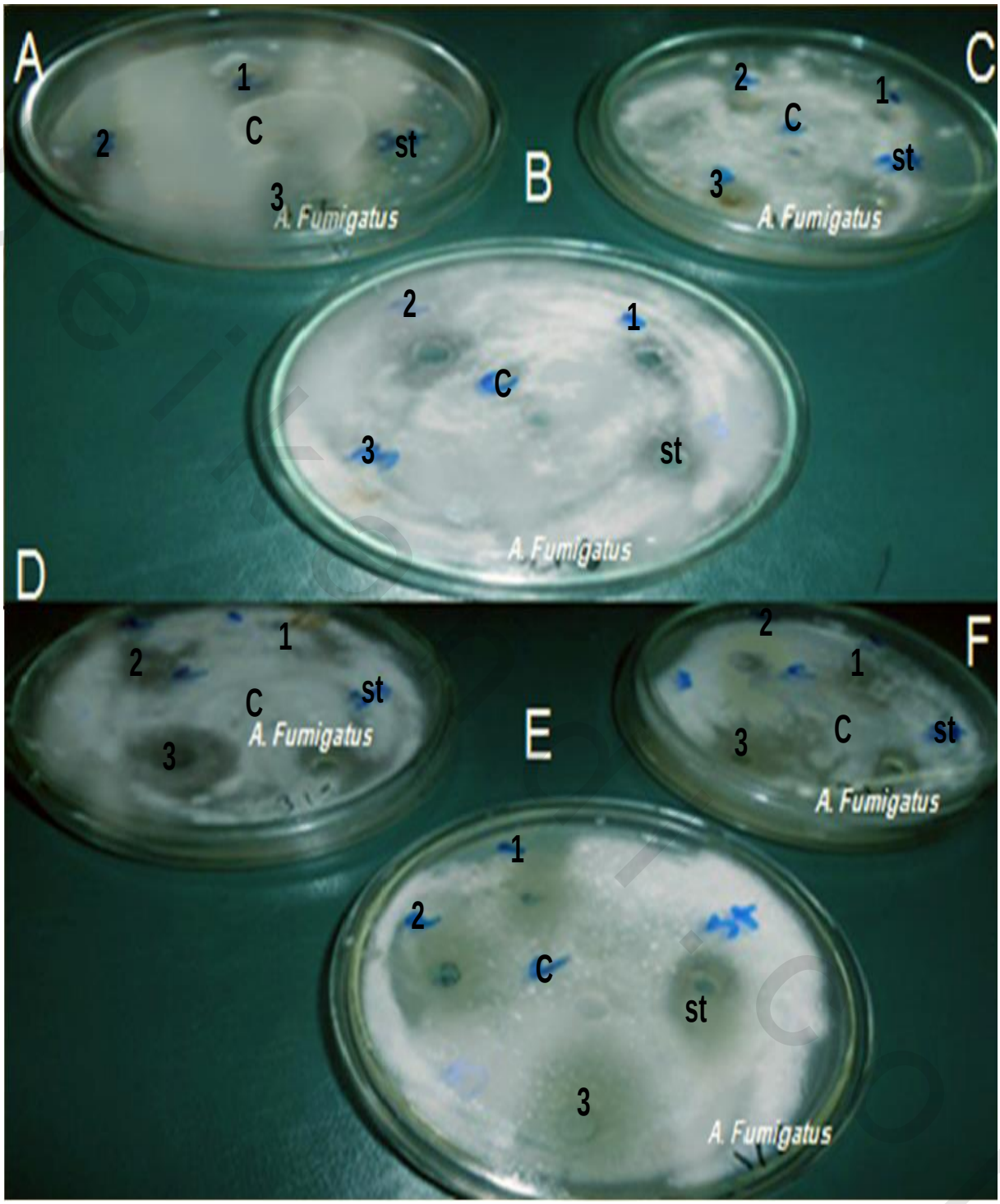


Figure 13.1: The effect of essential oils, aqueous and methanolic extracts derived from six medicinal plants; A=*Rosmarinus officinalis, B=Zingiber officinale, C=Alium sativum,*

D=Alium cepa, E=Foeniculum vulgare **and** ***F=Nigella Sativa*** **at 4mg/well against** ***Aspergillus fumigatus***

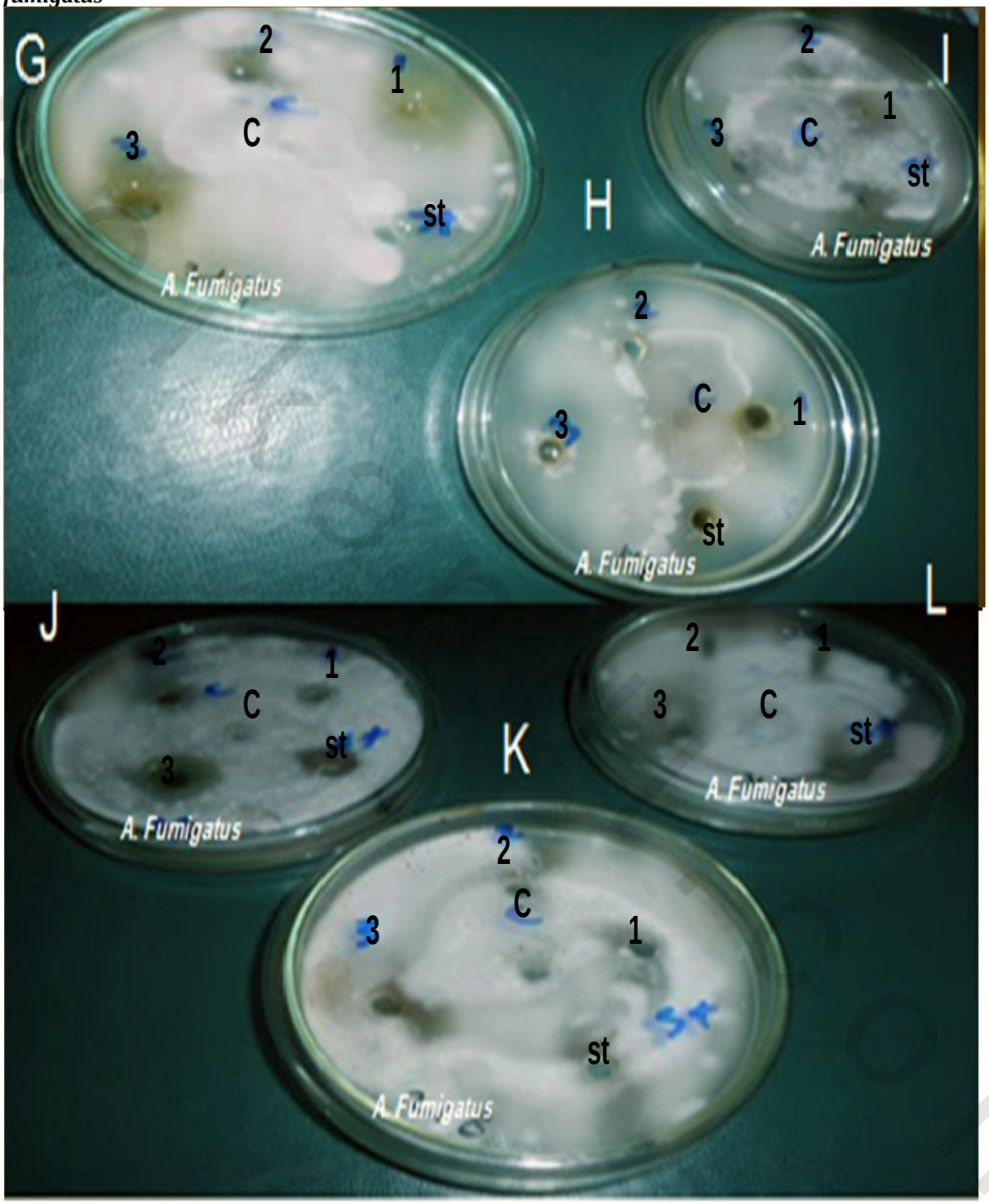
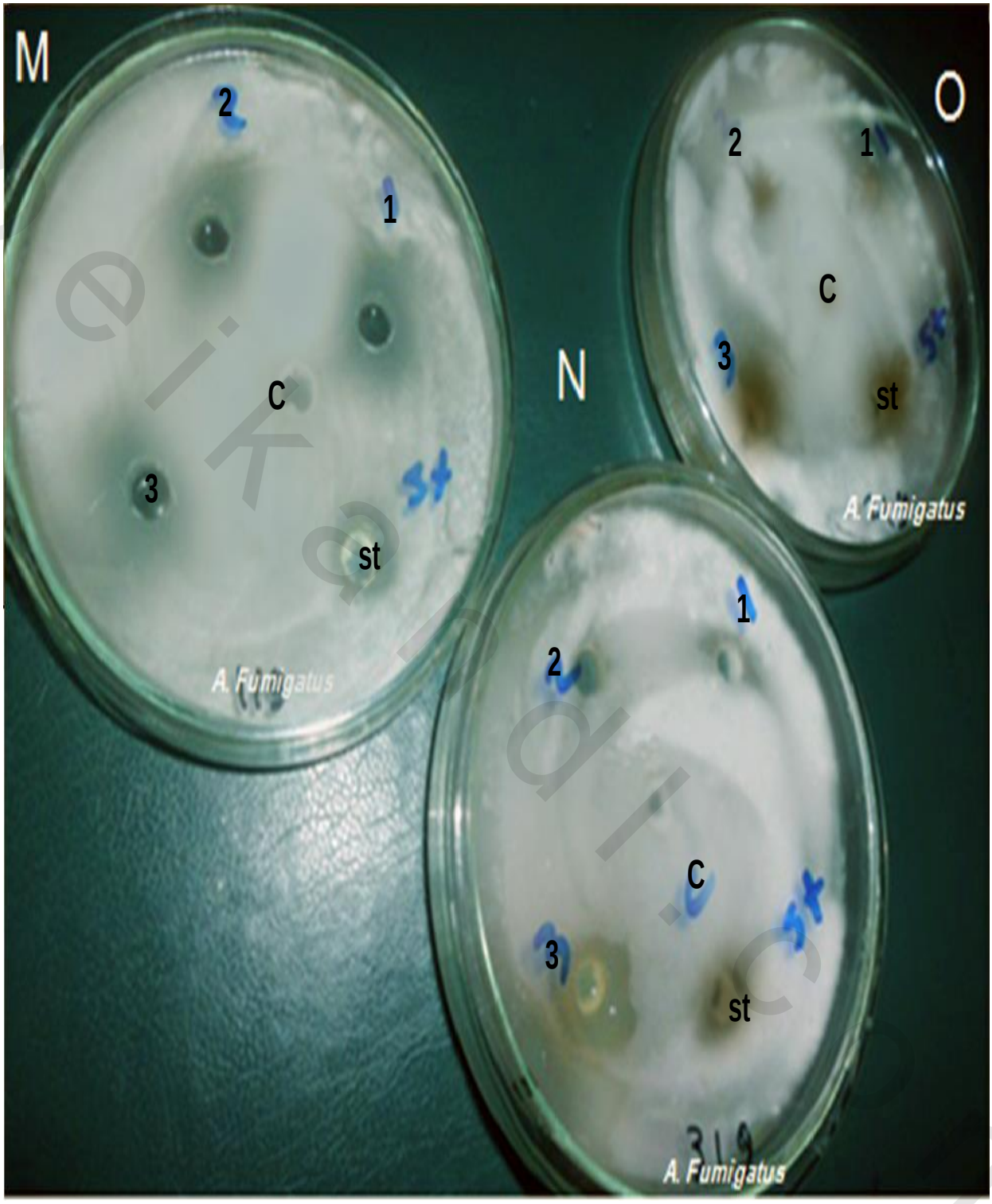


Figure 13.2: The effect of essential oils, aqueous and methanolic extracts derived from six medicinal plants; G=*Thymus vulgaries, H=Cassia Angustifolia, I=Mentha varidis, J=Syzygium aromaticum, K=Anethum graveolens* and *L=Eucalyptus globules* at 4mg/well against *Aspergillus fumigatus*


M
2
1
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A. Fumigatus
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A. Fumigatus
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A. Fumigatus

Figure 13.3: The effect of essential oils, aqueous and methanolic extracts derived from three medicinal plants; M=*Lavandula angustifolia, N=Olea eurpaea* and *O=ziziphus spina-csisti* at 4mg/well against *Aspergillus fumigates*

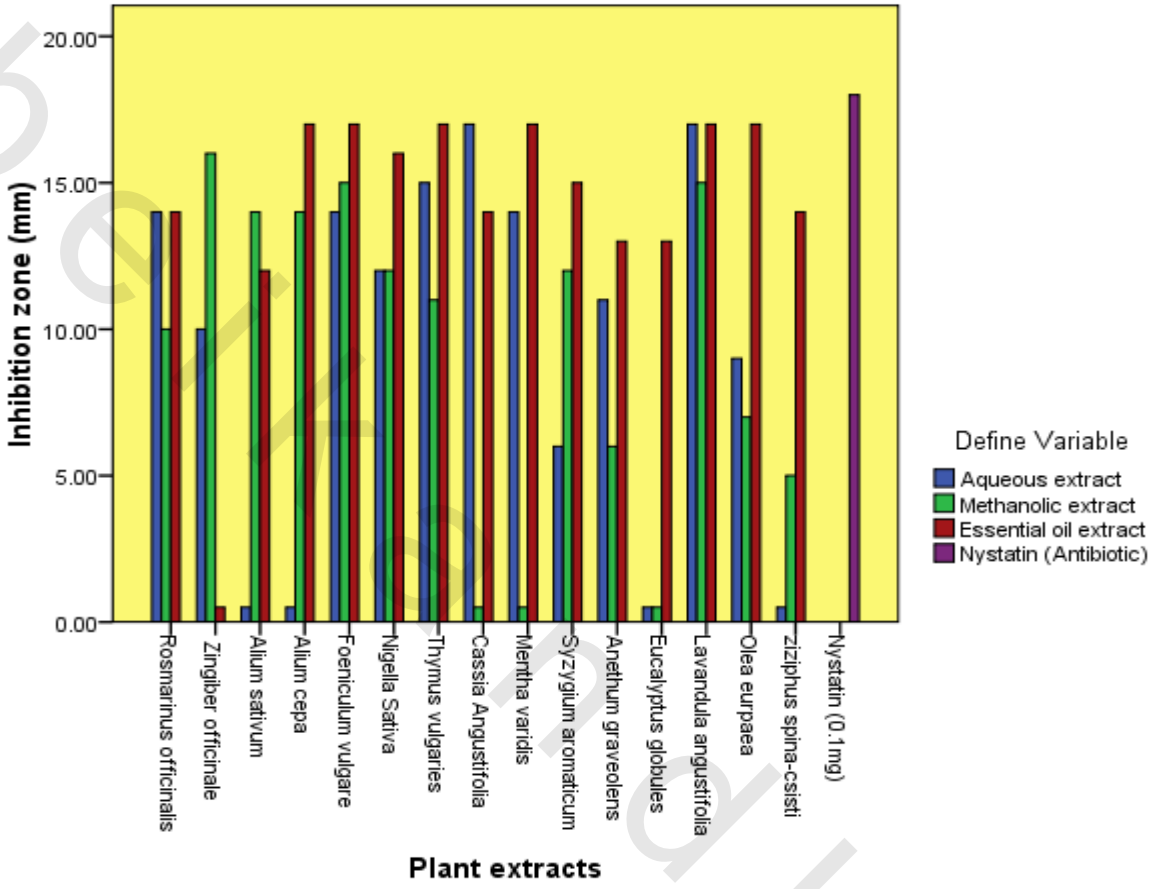


Figure 14: Diagrammatic representation of Antifungal activity of 100mg/ml concentration of aqueous, methanolic and oil extracts from 15 plant species against *Aspergillus fumigates*

VI.3.1.6. against *Aspergillus flavus*

The results of the effects of essential oils, aqueous and methanolic extracts of the fifteen plants at concentration 4mg/well (40µl) from the stock extracts (100 mg/ml) against the tested *Aspergillus flavus* are presented in (Table 14, Fig. 15.1154.3, 16)

Antifungal activity of the plant extracts against *Aspergillus flavus*: All plant extracts exhibited highly to moderate antifungal activity against *A. flavus* (6±0.23 mm- 19±0.47 mm). In the present study, maximum antifungal activity was observed for many essential

oils extracts against *A. flavus; Alium sativum (*19±0.47mm), *Lavandula angustifolia* (16±0.27 mm), *Anethum graveolens* (15±0.35 mm) and *Rosmarinus officinalis* (13±0.52 mm)

Whereas, many Aqueous extracts was found to be very effective against *A. flavus; Lavandula angustifolia* (16±0.21 mm), *Zingiber officinale* (14±0.62 mm) and *Cassia Angustifolia* (13±0.36 mm)

In addition some methanol extracts was found to be very effective against *A. flavus; Anethum graveolens* (14±0.35 mm), *Syzygium aromaticum* (13±0.40 mm), *Lavandula angustifolia* (13±0.35 mm) and *Zingiber officinale* (13±0.24 mm)

Table 15: Antifungal activity of essential oils, aqueous and methanolic extracts derived from fifteen medicinal plants at 4mg/disc against *Aspergillus flavus*

Extraction / Plant	Zone of Inhibition (mm)				
	Aq. (4mg/disc)	**Me.** (4mg/disc)	**Oil** (4mg/disc)	**Control**	**Std.** (0.1 mg/disc)
Rosmarinus officinalis	**NA**	**7±0.83**	**13±0.52**	**NA**	**16±0.62**
Zingiber officinale	**14±0.62**	**13±0.24**	**8±0.35**	**NA**	**15±0.35**
Alium sativum	**10±0.33**	**NA**	**19±0.47**	**NA**	**19±0.16**
Alium cepa	**8±0.53**	**10±0.53**	**11±0.42**	**NA**	**13±0.50**
Foeniculum vulgare	**NA**	**7±0.26**	**11±0.54**	**NA**	**12±0.42**
Nigella Sativa	**6±0.54**	**8±0.36**	**9±0.57**	**NA**	**12±0.62**
Thymus vulgaries	**8±0.45**	**11±0.44**	**12±0.82**	**NA**	**13±0.43**
Cassia Angustifolia	**13±0.36**	**8±0.38**	**10±0.16**	**NA**	**13±0.44**
Mentha varidis	**6±0.23**	**NA**	**NA**	**NA**	**11±0.27**
Syzygium aromaticum	**12±0.73**	**13±0.40**	**14±0.21**	**NA**	**15±0.32**
Anethum graveolens	**12±0.19**	**14±0.35**	**15±0.35**	**NA**	**15±0.53**
Eucalyptus globules	**NA**	**NA**	**9±0.22**	**NA**	**10±0.63**
Lavandula angustifolia	**16±0.21**	**13±0.35**	**16±0.27**	**NA**	**16±0.73**
Olea eurpaea	**8±0.67**	**NA**	**10±0.45**	**NA**	**12±0.46**
ziziphus spina-csisti	**NA**	**7±0.32**	**12±0.62**	**NA**	**13±0.54**

*** Inhibition zone** measured in **(mm)**, **NA**= no inhibition zone:
*** Control**= DMSO (-ve control), **Std. =** Nystatin (positive control),
***Method of extraction**: **Me**= methanol, **Oil**= essential oil, **Aq**= Aqeous
*** ±Values** are mean= SD of three separate experiments, P<0.05 **significant**

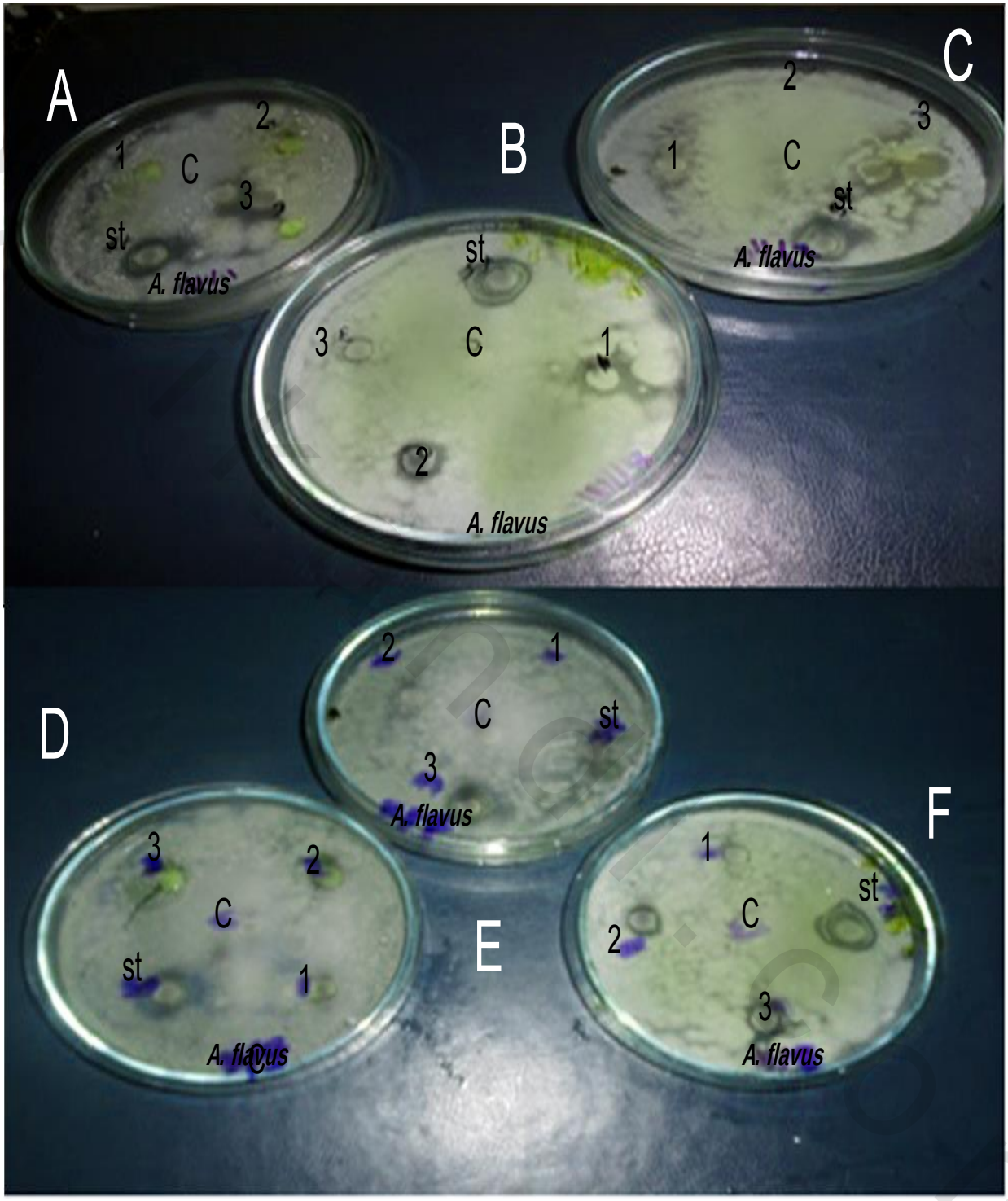


Figure 15.1: The effect of essential oils, aqueous and methanolic extracts derived from six medicinal plants; A=*Rosmarinus officinalis*, *B=Zingiber officinale*, *C=Alium sativum*,

D=Alium cepa, E=Foeniculum vulgare **and** ***F=Nigella Sativa*** **at 4mg/well against** ***Aspergillus flavus***

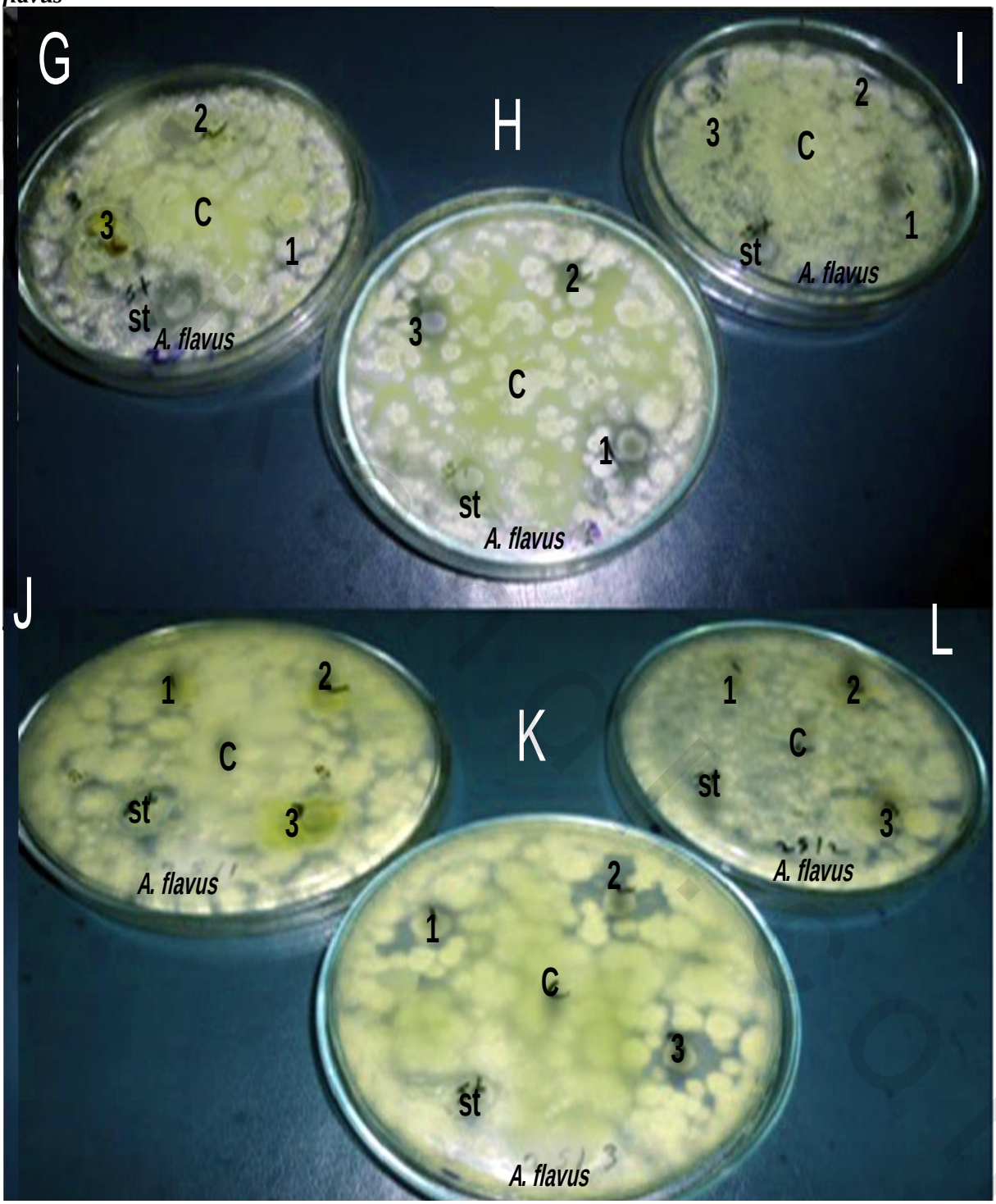


Figure 15.2: The effect of essential oils, aqueous and methanolic extracts derived from six medicinal plants; G=*Thymus vulgaries, H=Cassia Angustifolia, I=Mentha varidis, J=Syzygium aromaticum, K=Anethum graveolens* and *L=Eucalyptus globules* at 4mg/well against *Aspergillus flavus*

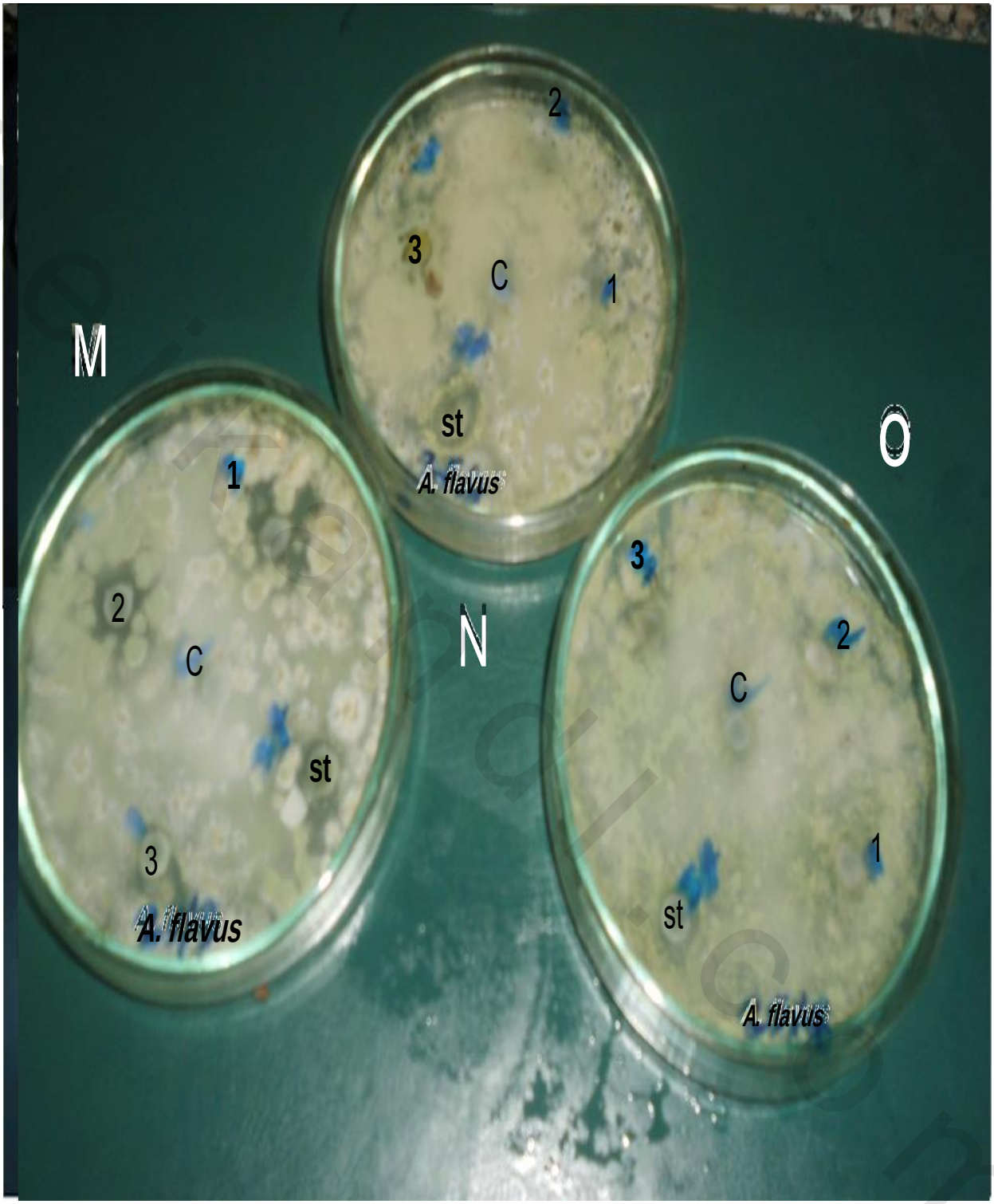


Figure 15.3: The effect of essential oils, aqueous and methanolic extracts derived from three medicinal plants; M=*Lavandula angustifolia, N=Olea eurpaea* and *O=ziziphus spina-csisti* at 4mg/well against *Aspergillus flavus*

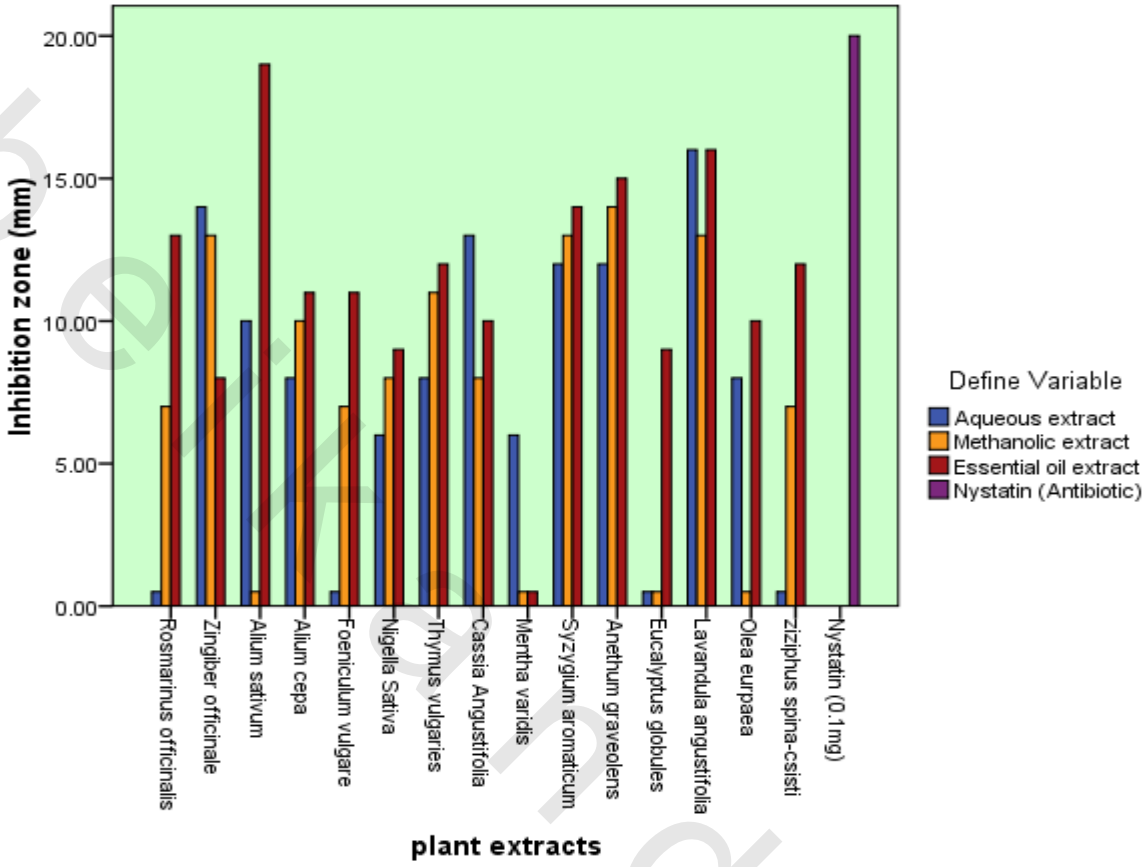


Figure 16: Diagrammatic representation of Antifungal activity of 100mg/ml concentration of aqueous, methanolic and oil extracts from 15 plant species against *Aspergillus flavus*

VI.3.1.7. against *Aspergillus oryzae*

The results of the effects of essential oils, aqueous and methanolic extracts of the fifteen plants at concentration 4mg/well (40µl)12 from the stock extracts (100 mg/ml) against the tested *Aspergillus oryzae* are presented in (Table15, Fig. 17.1-17.3, 18)

Antifungal activity of the plant extracts against *Aspergillus oryzae*: All plant extracts exhibited highly to moderate antifungal activity against *A. oryzae* (6±0.32 mm-17±0.35 mm). In the present study, maximum antifungal activity was observed for many essential oils extracts against *A. oryzae; Zingiber officinale (*17±0.43 mm), *Lavandula*

angustifolia (17±0.35 mm), *Olea eurpaea* (15±0.56 mm), *Syzygium aromaticum* (15±0.33 mm) and *Thymus vulgaries* (15±0.16 mm)

Whereas, many methanol extracts was found to be very effective against *A. oryzae; Zingiber officinale* (16±0.43 mm), *Nigella Sativa* (15±0.63 mm), *Thymus vulgaries* (15±0.42 mm) and *Alium sativum* (13±0.43 mm)

In addition some aqueous extracts was found to be very effective against *A. oryzae; Thymus vulgaries* (16±0.25 mm), *Zingiber officinale* (15±0.41 mm), *Olea eurpaea* (14±0.37 mm) and *Syzygium aromaticum* (14±0.14 mm)

Table 15: Antifungal activity of essential oils, aqueous and methanolic extracts derived from fifteen medicinal plants at 4mg/disc against *Aspergillus oryzae*

Extraction / Plant	Zone of Inhibition (mm)				
	Aq. (4mg/disc)	**Me.** (4mg/disc)	**Oil** (4mg/disc)	**Control**	**Std.** (0.1 mg/disc)
Rosmarinus officinalis	**10±0.32**	**6±0.32**	**13±0.61**	**NA**	**15±0.51**
Zingiber officinale	**15±0.41**	**16±0.43**	**17±0.43**	**NA**	**18±0.23**
Alium sativum	**9±0.45**	**13±0.43**	**10±0.25**	**NA**	**13±0.52**
Alium cepa	**13±0.24**	**10±0.27**	**13±0.36**	**NA**	**13±0.18**
Foeniculum vulgare	**NA**	**7±0.14**	**9±0.46**	**NA**	**13±0.33**
Nigella Sativa	**NA**	**15±0.63**	**10±0.32**	**NA**	**15±0.25**
Thymus vulgaries	**16±0.25**	**15±0.42**	**15±0.16**	**NA**	**18±0.61**
Cassia Angustifolia	**10±0.53**	**6±0.72**	**8±0.63**	**NA**	**13±0.72**
Mentha varidis	**NA**	**6±0.34**	**9±0.42**	**NA**	**10±0.36**
Syzygium aromaticum	**14±0.14**	**12±0.21**	**15±0.33**	**NA**	**15±0.27**
Anethum graveolens	**NA**	**8±0.34**	**13±0.27**	**NA**	**14±0.32**
Eucalyptus globules	**NA**	**10±0.51**	**13±0.64**	**NA**	**14±0.15**
Lavandula angustifolia	**8±0.42**	**13±0.34**	**17±0.35**	**NA**	**14±0.36**
Olea eurpaea	**14±0.37**	**7±0.35**	**15±0.56**	**NA**	**15±0.62**
ziziphus spina-csisti	**8±0.35**	**7±0.31**	**13±0.44**	**NA**	**13±0.55**

*** Inhibition zone** measured in **(mm)**, **NA**= no inhibition zone:
*** Control**= DMSO (-ve control), **Std. =** Nystatin (positive control),
***Method of extraction**: **Me**= methanol, **Oil**= essential oil, **Aq**= Aqeous
*** ±Values** are mean= SD of three separate experiments, P<0.05 **significant**

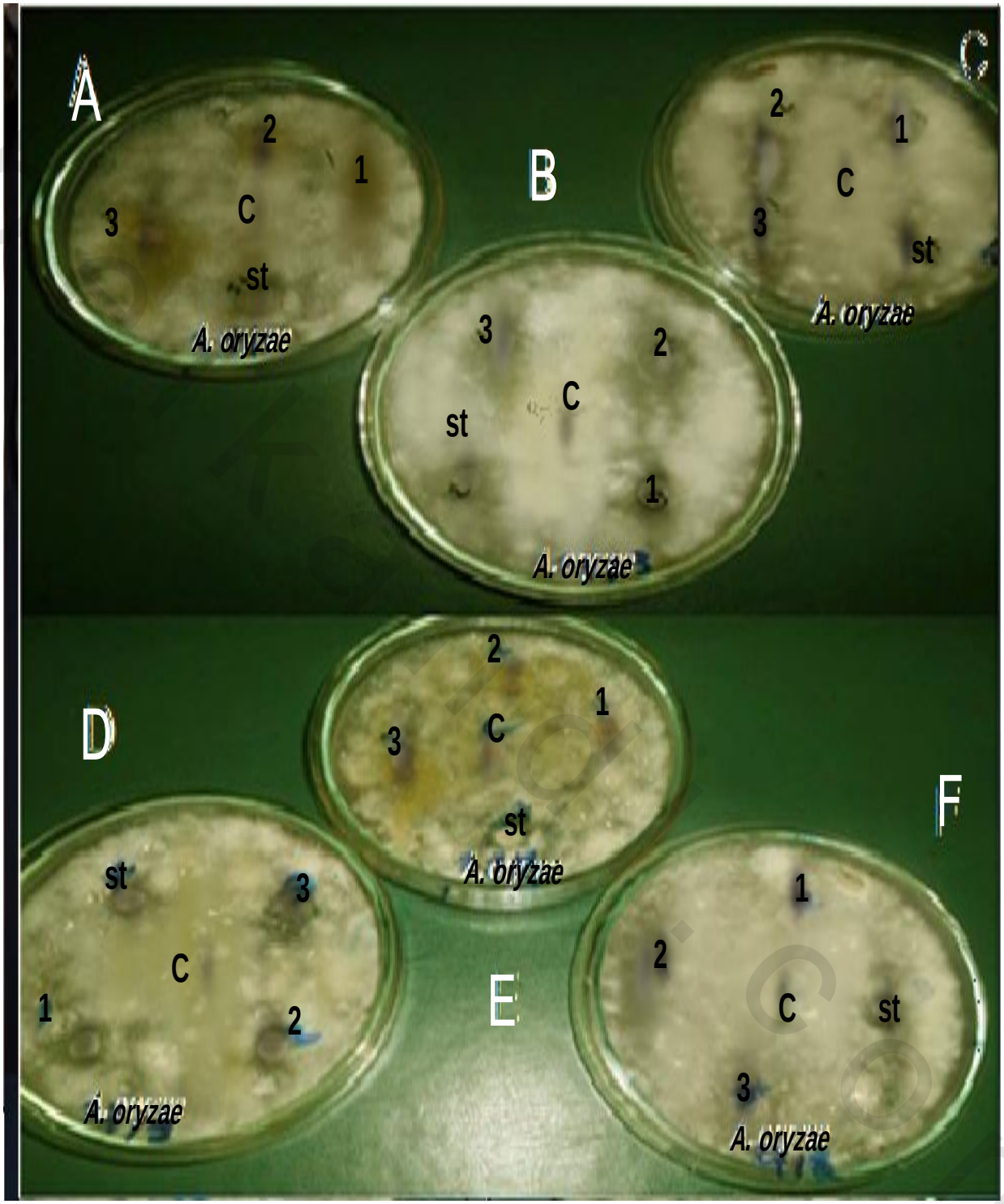


Figure 17.1: The effect of essential oils, aqueous and methanolic extracts derived from six medicinal plants; A=*Rosmarinus officinalis, B=Zingiber officinale, C=Alium sativum,*

D=Alium cepa, E=Foeniculum vulgare **and** ***F=Nigella Sativa*** **at 4mg/well against** ***Aspergillus oryzae***

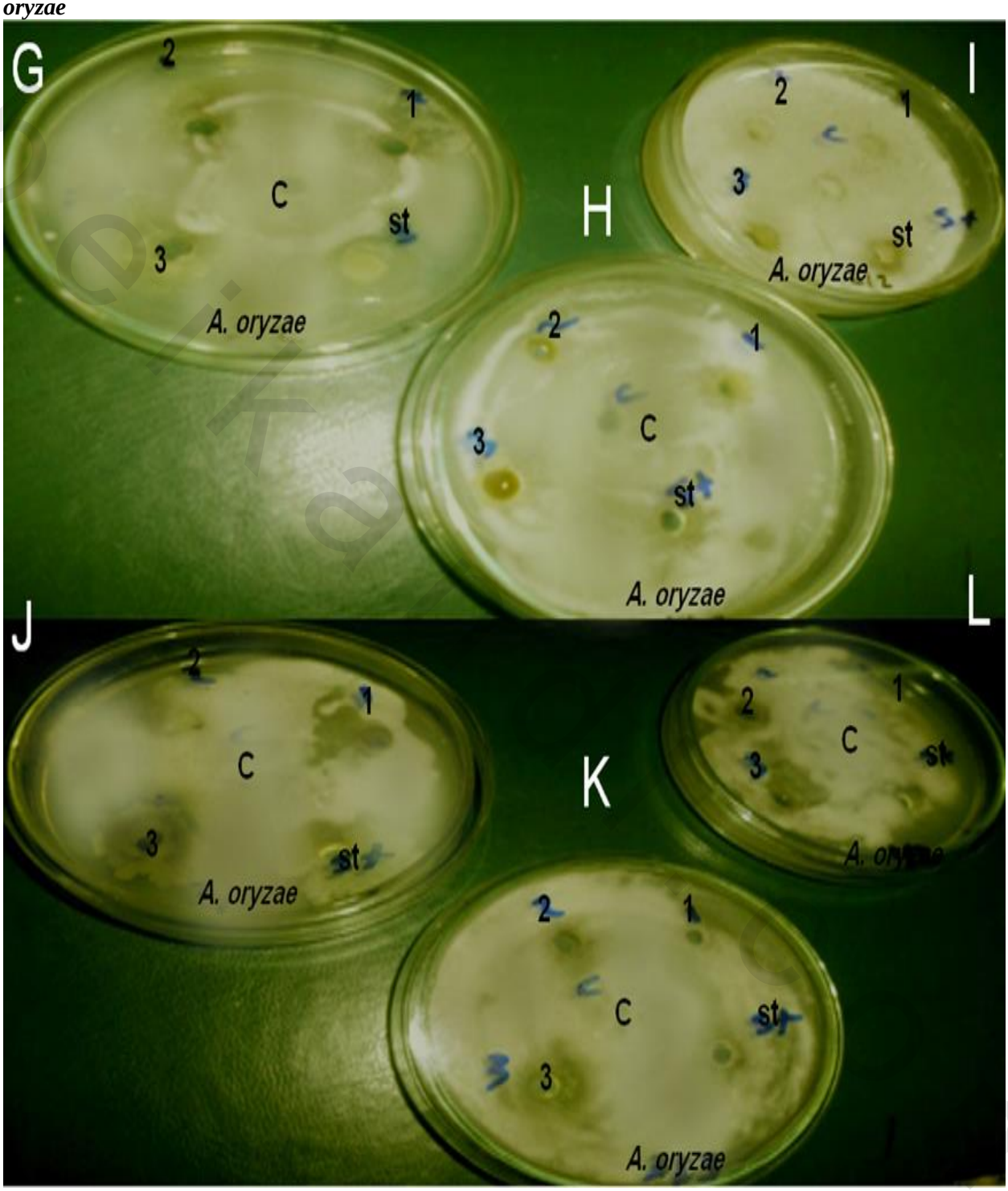


Figure 17.2: The effect of essential oils, aqueous and methanolic extracts derived from six medicinal plants; G=*Thymus vulgaries*, *H=Cassia Angustifolia*, *I=Mentha varidis*,

J=Syzygium aromaticum, K=Anethum graveolens **and** ***L=Eucalyptus globules*** **at 4mg/well against** ***Aspergillus oryzae***

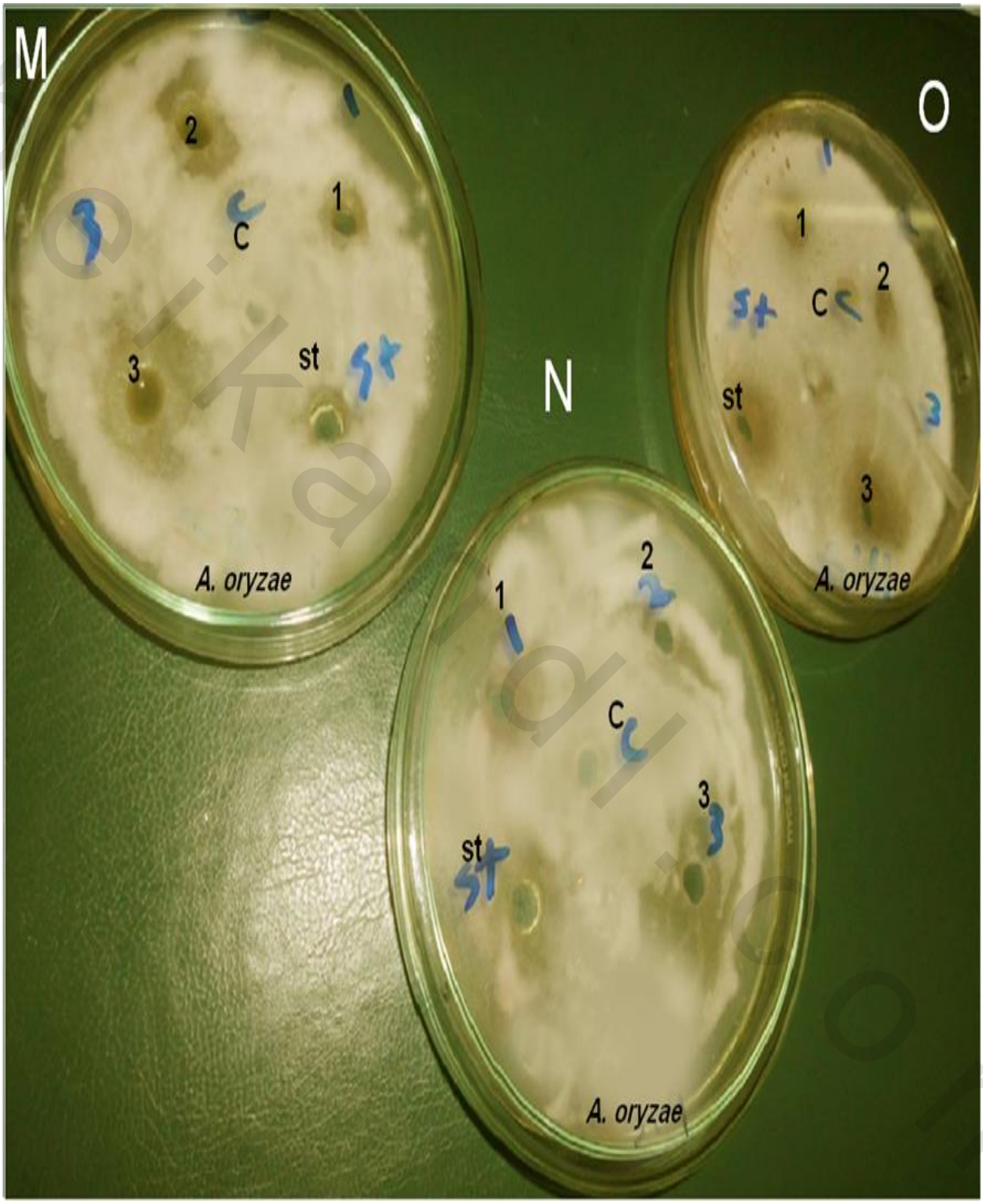


Figure 17.3: The effect of essential oils, aqueous and methanolic extracts derived from three medicinal plants; M=*Lavandula angustifolia, N=Olea eurpaea* and *O=ziziphus spina-csisti* at 4mg/well against *Aspergillus oryzae*

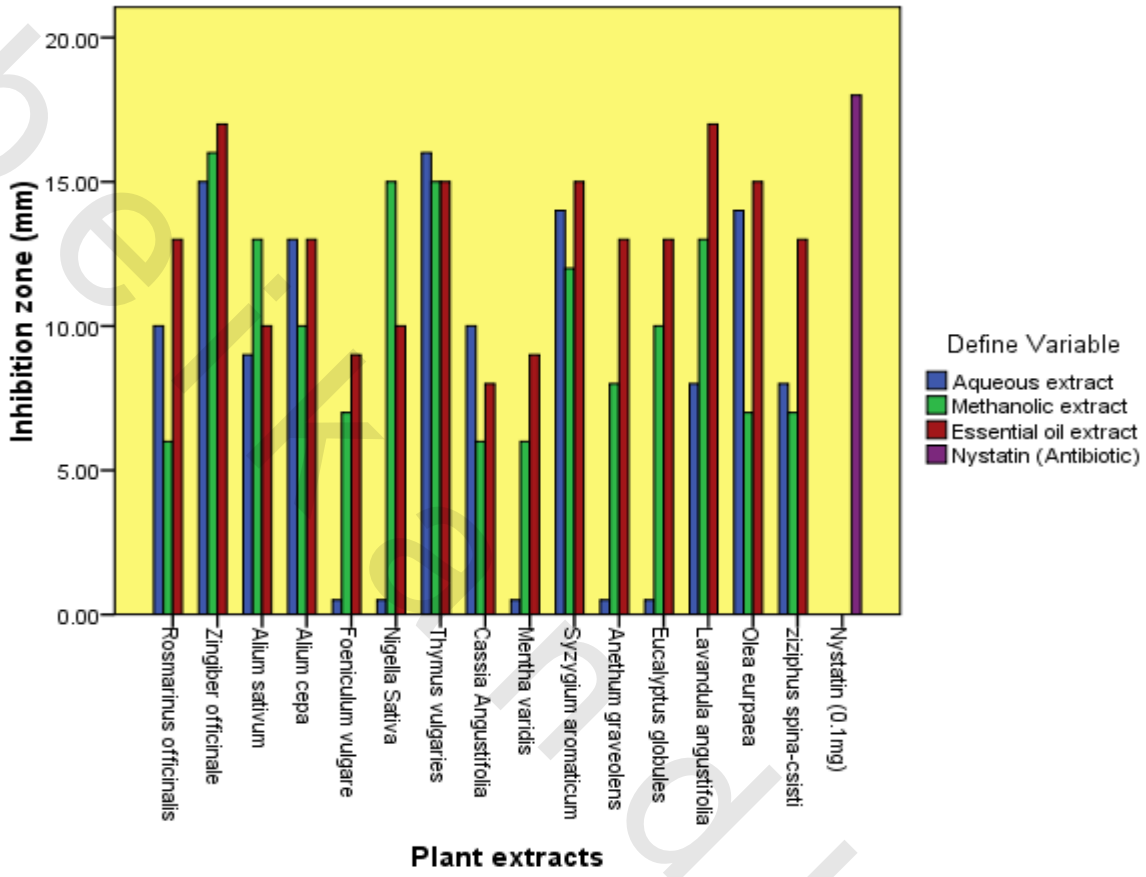


Figure 18: Diagrammatic representation of Antifungal activity of 100mg/ml concentration of aqueous, methanolic and oil extracts from 15 plant species against *Aspergillus oryzae*

VI.3.1.8. against *Penicillium chryogenum*

The results of the effects of essential oils, aqueous and methanolic extracts of the fifteen plants at concentration 4mg/well (40µl) from the stock extracts (100 mg/ml) against the tested *Penicillium chryogenum* are presented in (Table 16, Fig. 19.1-19.3, 20)

Antifungal activity of the plant extracts against *Penicillium chryogenum*: All plant extracts exhibited highly to moderate antifungal activity against *P. chryogenum* (7±0.35 mm- 20±0.35 mm). In the present study, maximum antifungal activity was observed for many essential oils extracts against *F. oxys P. chryogenum porum; Lavandula*

angustifolia (20±0.35mm), *Alium cepa*(18±0.90 mm), *Olea eurpaea* (18±0.34 mm), *Eucalyptus globules* (18±0.15 mm), *Anethum graveolens* (17±0.22 mm) and *Foeniculum vulgare* (16±0.45 mm) and *ziziphus spina-csisti* (15±0.52 mm)

Whereas, many methanol extracts was found to be very effective against *P. chryogenum; Lavandula angustifolia* (20±0.15 mm), *Foeniculum vulgar* (15±0.62 mm), *Olea eurpaea* (15±0.56 mm), *Nigella Sativa* (15±0.45 mm) and *Alium cepa* (15±0.32 mm)

In addition some aqueous extracts was found to be very effective against *P. chryogenum; Lavandula angustifolia* (19±0.33 mm), *Eucalyptus globules* (16±0.43 mm), *Foeniculum vulgare* (15±0.40 mm), *Zingiber officinale* (14±0.51 mm) and *ziziphus spina-csisti* (14±0.48 mm)

Table 16: Antifungal activity of essential oils, aqueous and methanolic extracts derived from fifteen medicinal plants at 4mg/disc against *Penicillium chryogenum*

Extraction / Plant	Zone of Inhibition (mm)				
	Aq. (4mg/disc)	**Me.** (4mg/disc)	**Oil** (4mg/disc)	**Control**	**Std.** (0.1 mg/disc)
Rosmarinus officinalis	**10±0.23**	**11±0.64**	**14±0.64**	**NA**	**21±0.32**
Zingiber officinale	**14±0.51**	**12±0.17**	**13±0.37**	**NA**	**17±0.45**
Alium sativum	**12±0.35**	**12±0.23**	**11±0.21**	**NA**	**15±0.34**
Alium cepa	**10±0.27**	**15±0.32**	**18±0.90**	**NA**	**18±0.53**
Foeniculum vulgare	**15±0.40**	**15±0.62**	**16±0.45**	**NA**	**16±0.56**
Nigella Sativa	**9±0.63**	**15±0.45**	**11±0.72**	**NA**	**16±0.36**
Thymus vulgaries	**8±0.25**	**9±0.42**	**12±0.16**	**NA**	**14±0.52**
Cassia Angustifolia	**13±0.17**	**7±0.37**	**NA**	**NA**	**14±0.34**
Mentha varidis	**13±0.53**	**7±0.35**	**15±0.43**	**NA**	**17±0.75**
Syzygium aromaticum	**10±0.34**	**11±0.25**	**12±0.65**	**NA**	**13±0.27**
Anethum graveolens	**NA**	**11±0.42**	**17±0.22**	**NA**	**17±0.63**
Eucalyptus globules	**16±0.43**	**13±0.24**	**18±0.15**	**NA**	**18±0.23**
Lavandula angustifolia	**19±0.33**	**20±0.15**	**20±0.35**	**NA**	**21±0.54**
Olea eurpaea	**12±0.52**	**15±0.56**	**18±0.34**	**NA**	**18±0.16**
ziziphus spina-csisti	**14±0.48**	**13±0.36**	**15±0.52**	**NA**	**15±0.23**

*** Inhibition zone** measured in **(mm)**, **NA**= no inhibition zone:
*** Control**= DMSO (-ve control), **Std. =** Nystatin (positive control),
***Method of extraction**: **Me**= methanol, **Oil**= essential oil, **Aq**= Aqeous
*** ±Values** are mean= SD of three separate experiments, P<0.05 **significant**

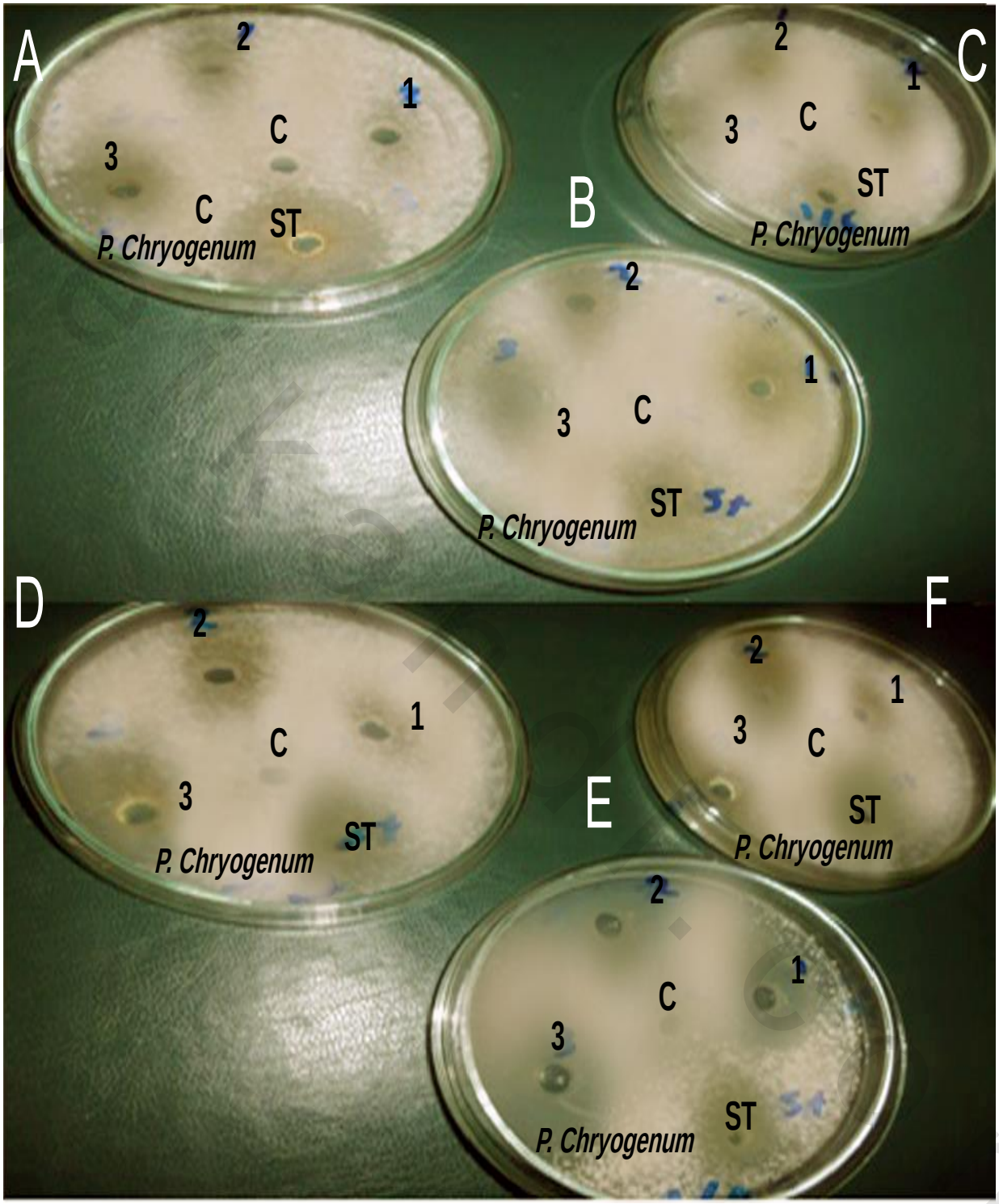


Figure 19.1: The effect of essential oils, aqueous and methanolic extracts derived from six medicinal plants; A=*Rosmarinus officinalis, B=Zingiber officinale, C=Alium sativum,*

D=Alium cepa, E=Foeniculum vulgare **and** ***F=Nigella Sativa*** **at 4mg/well against** ***Penicillium chryogenum***

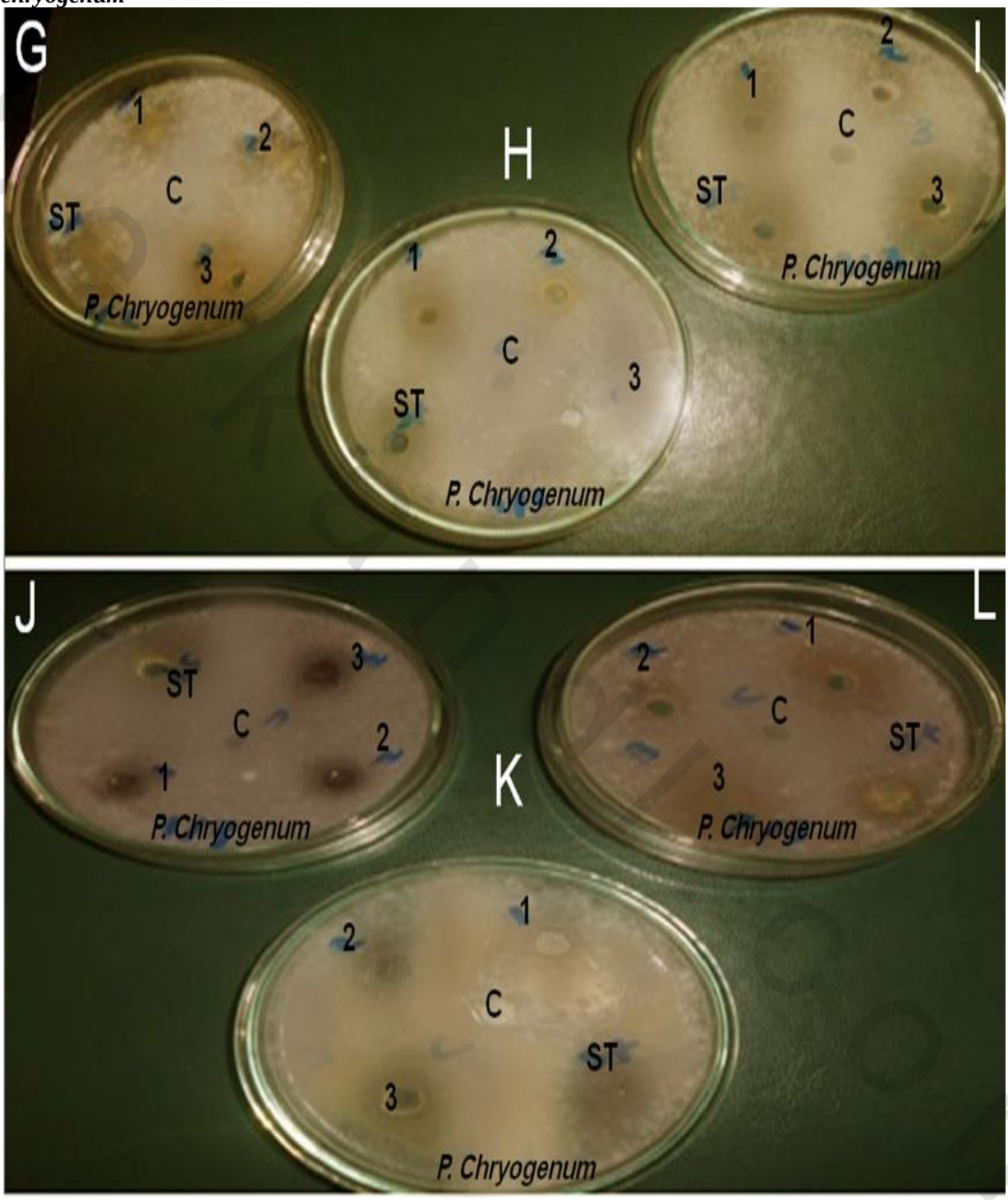


Figure 19.2: The effect of essential oils, aqueous and methanolic extracts derived from six medicinal plants; G=*Thymus vulgaries, H=Cassia Angustifolia, I=Mentha varidis,*

J=Syzygium aromaticum, K=Anethum graveolens* and *L=Eucalyptus globules* at 4mg/well against *Penicillium chryogenum

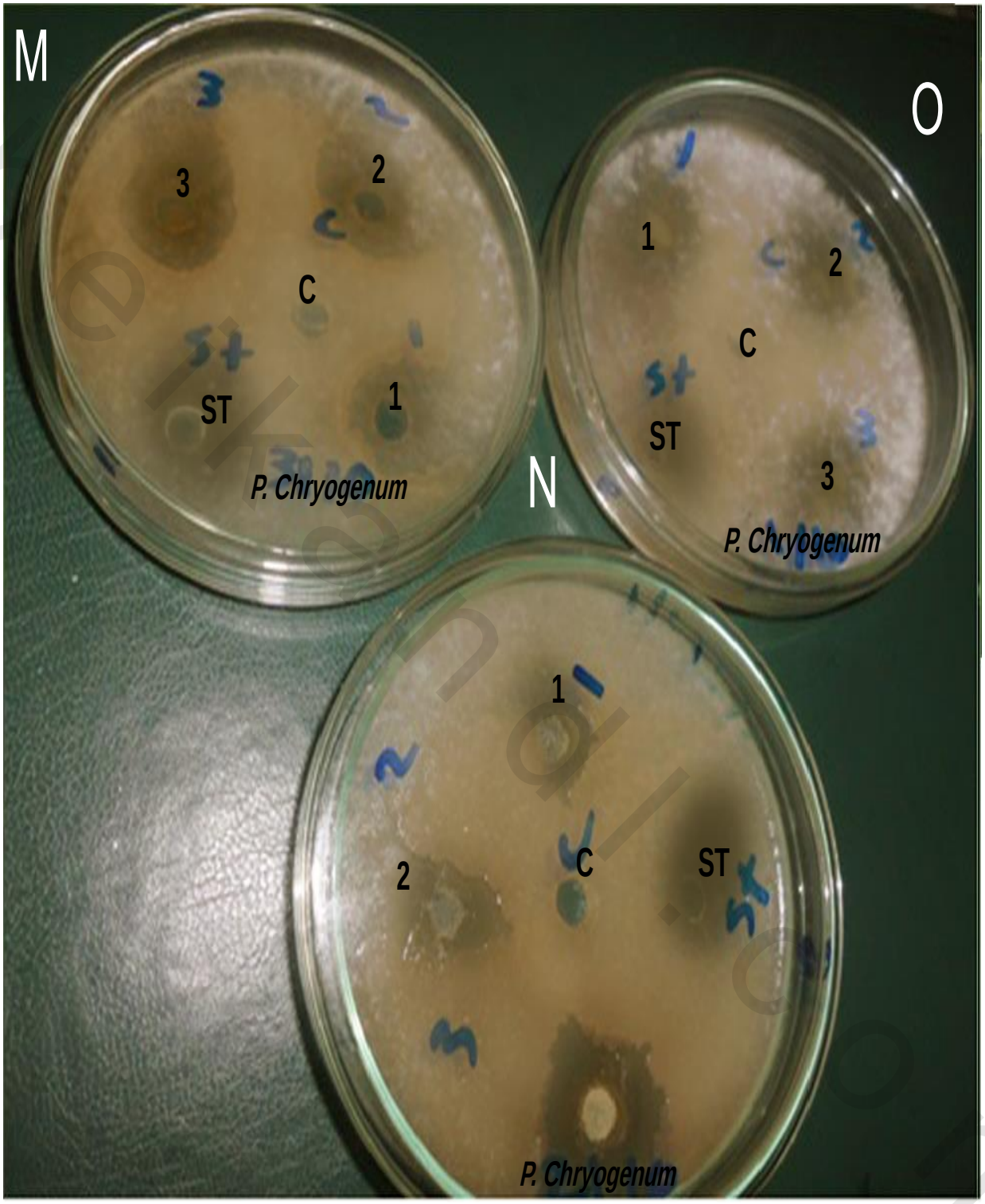

M
3
2
C
ST
1
P. Chryogenum
N
O
1
2
C
ST
3
P. Chryogenum
1
2
C
ST
P. Chryogenum

Figure 19.3: The effect of essential oils, aqueous and methanolic extracts derived from three medicinal plants; M=*Lavandula angustifolia, N=Olea eurpaea* and *O=ziziphus spina-csisti* at 4mg/well against *Penicillium chryogenum*

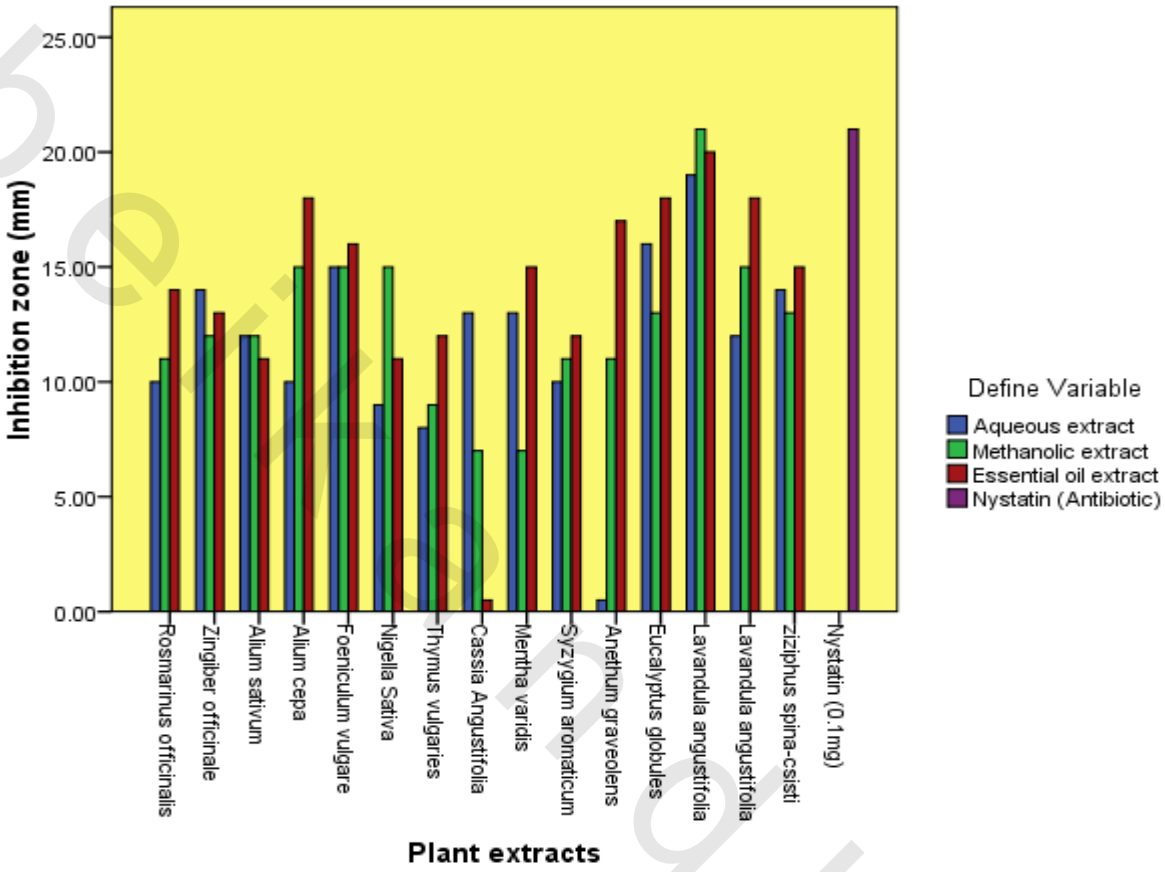


Figure 20: Diagrammatic representation of Antifungal activity of 100mg/ml concentration of aqueous, methanolic and oil extracts from 15 plant species against *Penicillium chryogenum*

VI.3.1.9. against *Penicillium commune*

The results of the effects of essential oils, aqueous and methanolic extracts of the fifteen plants at concentration 4mg/well (40µl) from the stock extracts (100 mg/ml) against the tested *Penicillium commune* are presented in (Table 17, Fig. 21.1-21.3, 22)

Antifungal activity of the plant extracts against *Penicillium commune*: All plant extracts exhibited highly to moderate antifungal activity against *P. commune* (6±0.72 mm- 18±0.33 mm). In the present study, maximum antifungal activity was observed for many essential oils extracts against *P. commune; Lavandula angustifolia* (18±0.33mm),

*Alium cepa (*15±0.57 mm), *Anethum graveolens* (15±0.43 mm), *Cassia Angustifolia (*15±0.32 mm), *Foeniculum vulgare* (15±0.23 mm) and *Mentha varidis* (15±0.15 mm)

Whereas, many methanol extracts was found to be very effective against *P. commune; Nigella Sativa* (16±0.41 mm), *Lavandula angustifolia* (16±0.36 mm), *Alium sativum* (15±0.60 mm), *Mentha varidis* (13±0.64 mm) and *Alium cepa* (13±0.26 mm)

In addition some aqueous extracts was found to be very effective against *P. commune; Mentha varidis* (16±0.34 mm), *Lavandula angustifolia* (14±0.54 mm), *Foeniculum vulgare* (14±0.35 mm) and *Alium sativum* (12±0.65 mm)

Table 17: Antifungal activity of essential oils, aqueous and methanolic extracts derived from fifteen medicinal plants at 4mg/disc against *Penicillium commune*

Extraction / Plant	Zone of Inhibition (mm)				
	Aq. (4mg/disc)	**Me.** (4mg/disc)	**Oil** (4mg/disc)	**Control**	**Std.** (0.1 mg/disc)
Rosmarinus officinalis	**10±0.56**	**7±0.43**	**13±0.25**	**NA**	**15±0.37**
Zingiber officinale	**11±0.34**	**8±0.45**	**13±0.63**	**NA**	**17±0.42**
Alium sativum	**12±0.65**	**15±0.60**	**8±0.45**	**NA**	**15±0.14**
Alium cepa	**8±0.63**	**13±0.26**	**15±0.57**	**NA**	**16±0.64**
Foeniculum vulgare	**14±0.35**	**11±0.21**	**15±0.23**	**NA**	**17±0.34**
Nigella Sativa	**12±0.16**	**16±0.41**	**13±0.40**	**NA**	**17±0.55**
Thymus vulgaries	**NA**	**NA**	**14±0.15**	**NA**	**14±0.30**
Cassia Angustifolia	**12±0.24**	**13±0.25**	**15±0.32**	**NA**	**15±0.27**
Mentha varidis	**16±0.34**	**13±0.64**	**15±0.15**	**NA**	**16±0.31**
Syzygium aromaticum	**10±0.75**	**8±0.36**	**12±0.27**	**NA**	**14±0.37**
Anethum graveolens	**NA**	**6±0.72**	**15±0.43**	**NA**	**15±0.32**
Eucalyptus globules	**7±0.34**	**8±0.46**	**12±0.25**	**NA**	**14±0.47**
Lavandula angustifolia	**14±0.54**	**16±0.36**	**18±0.33**	**NA**	**18±0.45**
Olea eurpaea	**8±0.46**	**10±0.41**	**14±0.54**	**NA**	**16±0.34**
ziziphus spina-csisti	**NA**	**7±0.46**	**14±0.33**	**NA**	**18±0.61**

*** Inhibition zone** measured in **(mm)**, **NA**= no inhibition zone:
*** Control**= DMSO (-ve control), **Std. =** Nystatin (positive control),
***Method of extraction**: **Me**= methanol, **Oil**= essential oil, **Aq**= Aqeous
*** ±Values** are mean= SD of three separate experiments, P<0.05 **significant**

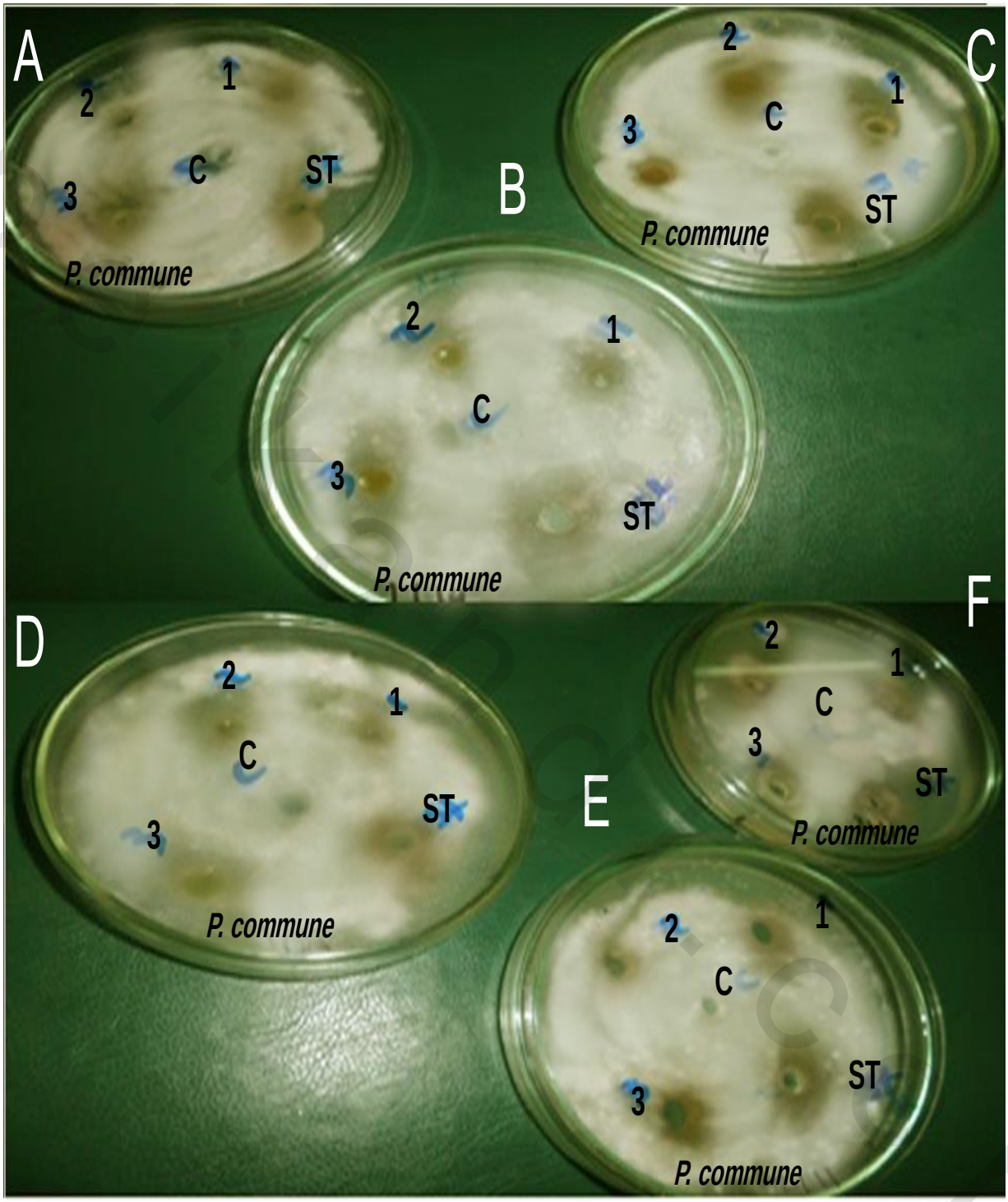


Figure 21.1: The effect of essential oils, aqueous and methanolic extracts derived from six medicinal plants; A=*Rosmarinus officinalis, B=Zingiber officinale, C=Alium sativum, D=Alium cepa, E=Foeniculum vulgare* and *F=Nigella Sativa* at 4mg/well against *Penicillium commune*

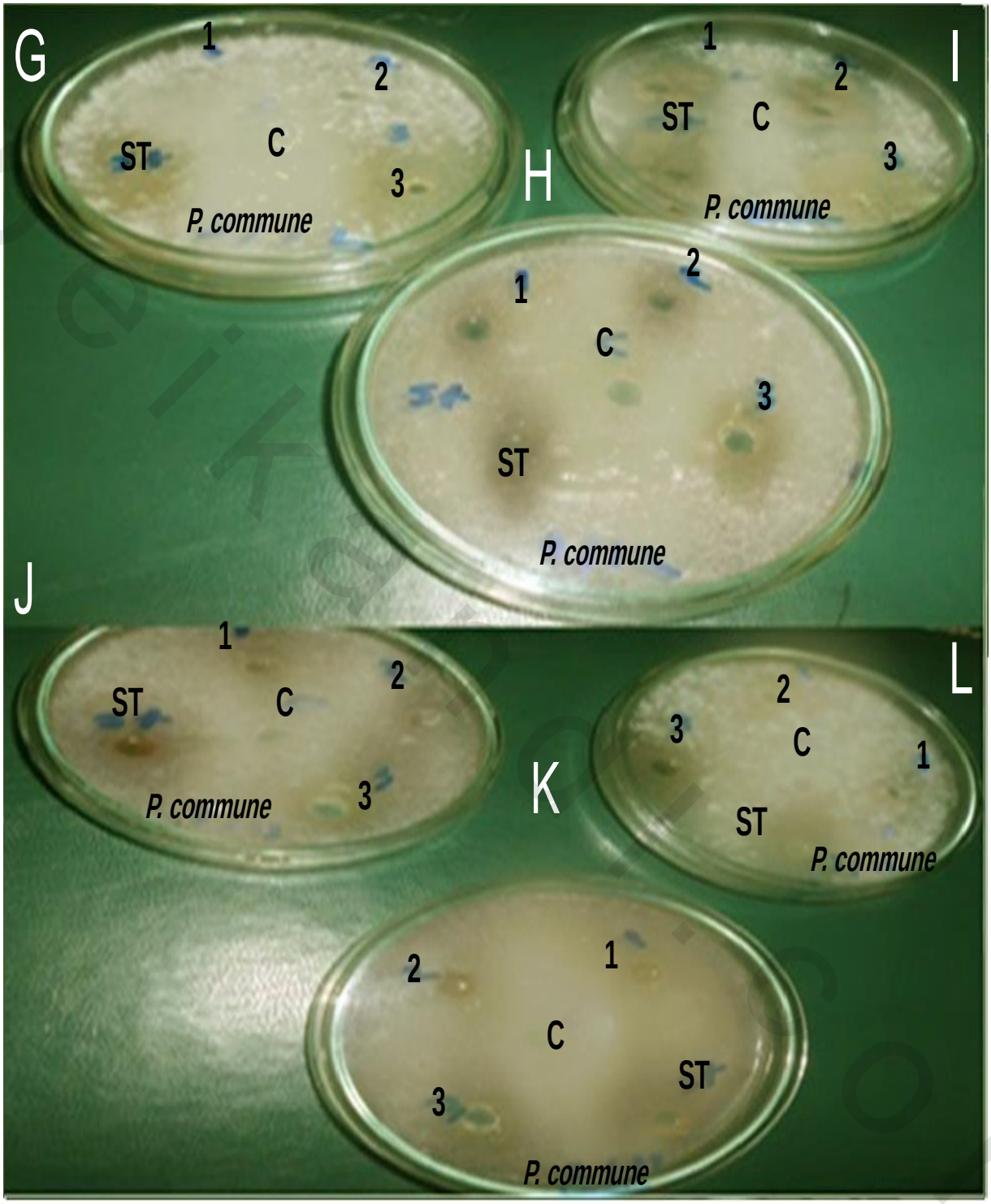


Figure 21.2: The effect of essential oils, aqueous and methanolic extracts derived from six medicinal plants; G=*Thymus vulgaries*, H=*Cassia Angustifolia*, I=*Mentha varidis*,

J=Syzygium aromaticum, K=Anethum graveolens **and** ***L=Eucalyptus globules*** **at 4mg/well against** ***Penicillium commune***

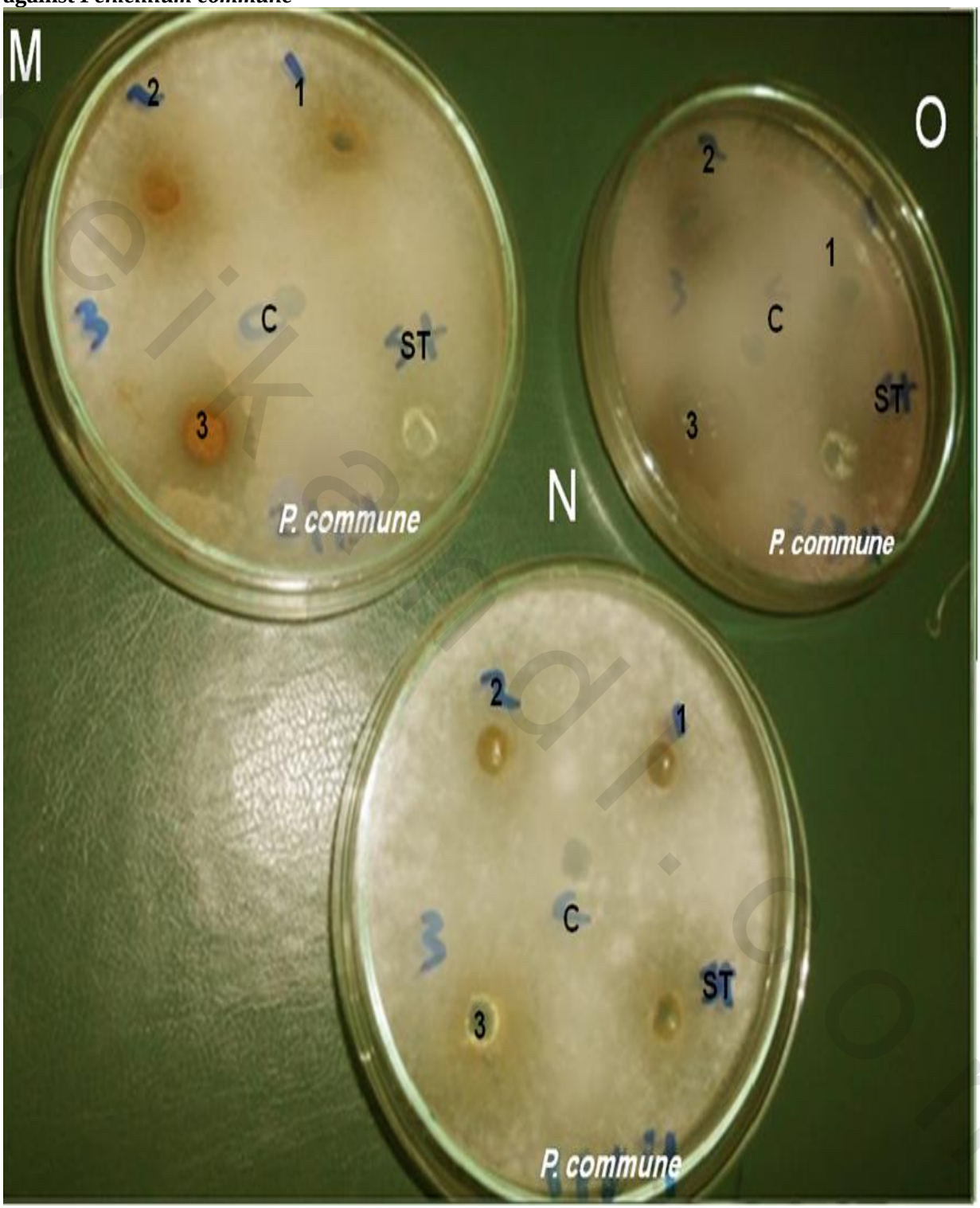


Figure 21.3: The effect of essential oils, aqueous and methanolic extracts derived from three medicinal plants; M=*Lavandula angustifolia, N=Olea eurpaea* and *O=ziziphus spina-csisti* at 4mg/well against *Penicillium commun*

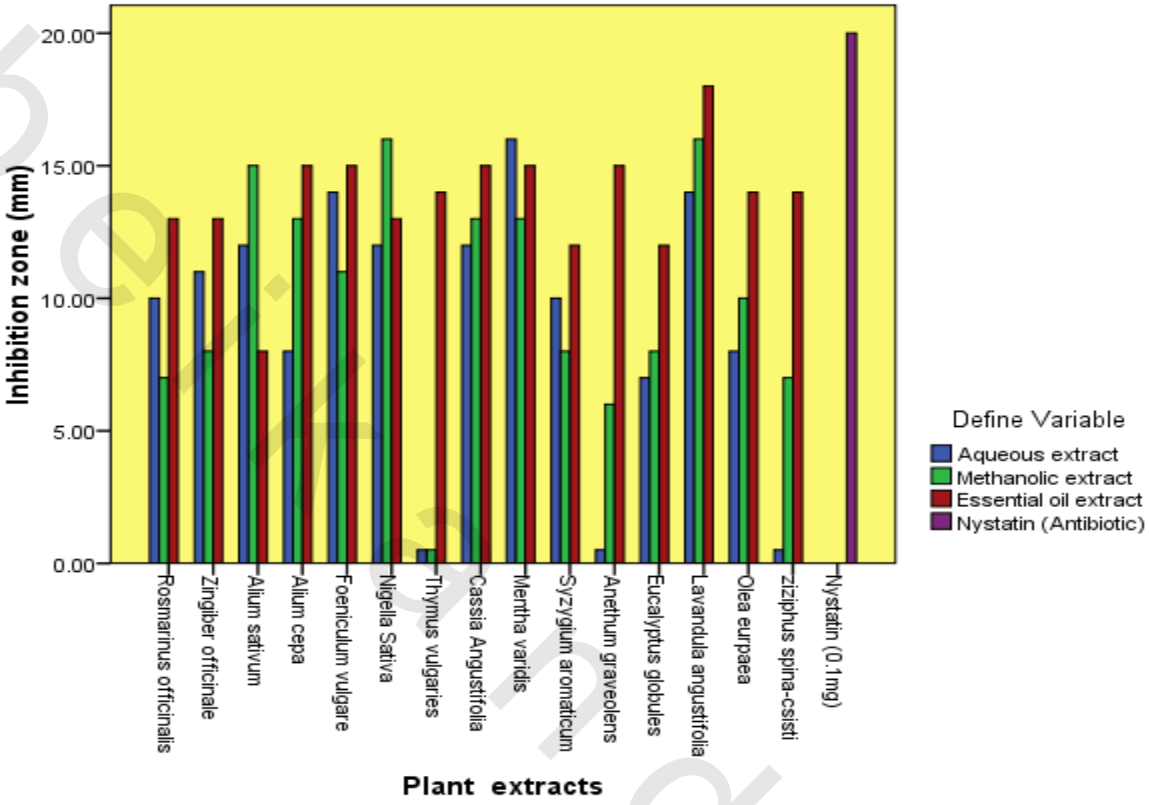


Figure 22: Diagrammatic representation of Antifungal activity of 100mg/ml concentration of aqueous, methanolic and oil extracts from 15 plant species against *Penicillium commune*

VI.3.2. Determination of Minimum Inhibitory concentration using Broth dilution Method

The minimum inhibitory concentration values of the crude extracts of fifteen plants by broth dilution technique and The MIC results showed that all tested plant extracts were showed antifungal activity against nine plant pathogenic fungi with MIC values ranging from 0.25 to 4mg/ml. The tested extracts showed different levels of antifungal activity depending on tested species as shown in (Table 18). *Rosmarinus* officinalis extracts showed considerable inhibitory activity against *F. oxysporum, F. solani, A.fumigates, A. oryzae and P. chryogenum* (1mg/ml), *F. brachygibbosum, R. solani and P. commune* (2 mg/ml) and *A. flavus* (0.5mg/ml). *Zingiber officinale* extracts showed considerable inhibitory activity against *F. oxysporum, F. solani, P. chryogenum*

and *p. commune* (2 mg/ml) and *brachygibbosum, R. solani. , A. fumigatus, A. flavus and A. oryzae* (1 mg/ml). *Alium sativum* extracts showed considerable inhibitory activity against *F. oxysporum, F. solani, R. solani. , A. fumigates* and *A. oryzae* (1 mg/ml), *P. chryogenum* and *p. commune* (2 mg/ml) and *F. brachygibbosumand A. flavus* (0.5mg/ml). *Alium cepa* extracts showed considerable inhibitory activity against *F. oxysporum, R. solani, A. fumigates* and *P. chryogenum* (1 mg/ml) and *F. solani, F. brachygibbosum, A. flavus* and *A. oryzae* (2 mg/ml). *Foeniculum vulgare* extracts showed considerable inhibitory activity against *F. oxysporum, F. brachygibbosum, A. fumigates* and *A. oryzae* (0.5mg/ml) and *F. solani, R. solani , A. flavus, A. oryzae* and *p. commune* (1 mg/ml), *Nigella Sativa* extracts showed considerable inhibitory activity against *F. solani, R. solani , A. flavus, P. chryogenum*(0.5mg/ml) and *F. oxysporum, F. brachygibbosum, A. fumigatus, A. oryzae* and *p. commune* (1 mg/ml). *Thymus vulgaries* extracts showed considerable inhibitory activity against *F. oxysporum, F. solani, R. solani, A. fumigates* and *A. oryzae* (1 mg/ml), *F. brachygibbosum, A. flavus* and *p. commune* (2 mg/ml) and *P. chryogenum* (0.5mg/ml). *Cassia Angustifolia* extracts showed considerable inhibitory activity against *F. solani, F. brachygibbosum, A. flavus, A. oryzae* and *p. commune* (1 mg/ml), *F. oxysporum, R. solani* and *A. fumigates* (2 mg/ml) and *P. chryogenum* (0.5mg/ml). *Mentha varidis* extracts showed considerable inhibitory activity against *F. oxysporum, F. brachygibbosum, A. flavus* and *A. oryzae* (1 mg/ml), *F. solani, A. fumigates* and *p. commune* (0.5mg/ml) and *R. solani* and *P. chryogenum* (2 mg/ml). *Syzygium aromaticum* extracts showed considerable inhibitory activity against *F. oxysporum, R. solani, A. fumigates P. chryogenum* and *p. commune* (2 mg/ml), *F. solani, F. brachygibbosum* and *A. flavus* (1 mg/ml) and *A. oryzae* (0.5mg/ml). *Anethum graveolens* extracts showed considerable inhibitory activity against *F. oxysporum, F. brachygibbosum, A. flavus, A. oryzae, P. chryogenum* (0.5mg/ml) and *F. solani, R. solani, A. fumigates* and *p. commune* (1 mg/ml). *Eucalyptus globules* extracts showed considerable inhibitory activity against *F. oxysporum, F. solani, F. brachygibbosum, R. solani, A. fumigatus, A. oryzae, P. chryogenum* and *p. commune* (1 mg/ml) and *A. flavus* (2 mg/ml). *Lavandula angustifolia* extracts showed considerable inhibitory activity against

F. oxysporum, R. solani, A. fumigatus, A. flavus, A. oryzae and *P. chryogenum* (1 mg/ml), *F. solani* and *F. brachygibbosum* (2 mg/ml) and *p. commune* (0.5mg/ml). *Olea eurpaea* extracts showed considerable inhibitory activity against *F. brachygibbosum, R. solani, A. fumigatus, A. flavus* and *P. chryogenum* (1 mg/ml), *F. oxysporum, A. oryzae* and *p. commune* (2mg/ml) and *F. solani* (0.5mg/ml). *Ziziphus spina-csisti* extracts showed considerable inhibitory activity against *F. oxysporum, F. brachygibbosum, A. fumigates* and *P. chryogenum* (1 mg/ml), *F. solani, A. oryzae* and *p. commune* (2 mg/ml) and *R. solani* and *A. flavus* (0.5mg/ml)

Table 18: Minimal inhibitory concentrations (MIC) of the crude extracts of fifteen medicinal plants on nine fungal species

Plant	Concentration of extracts (mg/ml)	Minimum inhibitory concentration (mg/ml)								
		F.o	F.s	F.b	R.s	A.fu	Afl	A.o	P.ch	P.co
Rosmarinus officinalis	o.25	-	-	-	-	-	-	-	-	-
	0.5	-	-	-	-	-	-	-	-	-
	1	-	-	-	-	-	+	-	-	-
	2	+	+	-	-	+	+	+	+	-
	4	+	+	+	+	+	+	+	+	+
Zingiber officinale	o.25	-	-	-	-	-	-	-	-	-
	0.5	-	-	-	-	-	-	-	-	-
	1	-	-	-	-	-	-	-	-	-
	2	-	+	-	+	+	+	+	-	-
	4	+	+	+	+	+	+	+	+	+
Alium sativum	o.25	-	-	-	-	-	-	-	-	-
	0.5	-	-	-	-	-	-	-	-	-
	1	-	-	+	-	-	+	-	-	-
	2	+	+	+	+	+	+	+	-	-
	4	+	+	+	+	+	+	+	+	+
Alium cepa	o.25	-	-	-	-	-	-	-	-	-
	0.5	-	-	-	-	-	-	-	-	-
	1	-	-	-	-	-	-	-	-	-
	2	+	-	-	+	+	-	-	+	-
	4	+	+	+	+	+	+	+	+	+
Foeniculum vulgare	o.25	-	-	-	-	-	-	-	-	-
	0.5	-	-	-	-	-	-	-	-	-
	1	+	-	+	-	+	-	-	+	-
	2	+	+	+	+	+	+	+	+	+
	4	+	+	+	+	+	+	+	+	+
Nigella Sativa	o.25	-	-	-	-	-	-	-	-	-
	0.5	-	-	-	-	-	-	-	-	-
	1	-	+	-	+	-	+	-	+	-
	2	+	+	+	+	+	+	+	+	+

Plant	Concentration of extracts (mg/ml)	Minimum inhibitory concentration (mg/ml)								
		F.o	F.s	F.b	R.s	A.fu	Afl	A.o	P.ch	P.co
	4	+	+	+	+	+	+	+	+	+
Thymus vulgaries	o.25	-	-	-	-	-	-	-	-	-
	0.5	-	-	-	-	-	-	-	-	-
	1	-	-	-	-	-	-	-	+	-
	2	+	+	-	+	+	-	+	+	-
	4	+	+	+	+	+	+	+	+	+
Cassia Angustifolia	o.25	-	-	-	-	-	-	-	-	-
	0.5	-	-	-	-	-	-	-	-	-
	1	-	-	-	-	-	-	-	+	-
	2	-	+	+	-	-	+	+	+	+
	4	+	+	+	+	+	+	+	+	+
Mentha varidis	o.25	-	-	-	-	-	-	-	-	-
	0.5	-	-	-	-	-	-	-	-	-
	1	-	+	-	-	+	-	-	-	+
	2	+	+	+	-	+	+	+	-	+
	4	+	+	+	+	+	+	+	+	+
Syzygium aromaticum	o.25	-	-	-	-	-	-	-	-	-
	0.5	-	-	-	-	-	-	-	-	-
	1	-	-	-	-	-	-	+	-	-
	2	-	+	+	-	-	+	+	-	-
	4	+	+	+	+	+	+	+	+	+
Anethum graveolens	o.25	-	-	-	-	-	-	-	-	-
	0.5	-	-	-	-	-	-	-	-	-
	1	+	-	+	-	-	+	+	+	-
	2	+	+	+	+	+	+	+	+	+
	4	+	+	+	+	+	+	+	+	+
Eucalyptus globules	o.25	-	-	-	-	-	-	-	-	-
	0.5	-	-	-	-	-	-	-	-	-
	1	-	-	-	-	-	-	-	-	-
	2	+	+	+	+	+	-	+	+	+
	4	+	+	+	+	+	+	+	+	+
Lavandula angustifolia	o.25	-	-	-	-	-	-	-	-	-
	0.5	-	-	-	-	-	-	-	-	-
	1	-	-	-	-	-	-	-	-	+
	2	+	-	-	+	+	+	+	+	+
	4	+	+	+	+	+	+	+	+	+
	o.25	-	-	-	-	-	-	-	-	-
Olea eurpaea	o.25	-	-	-	-	-	-	-	-	-
	0.5	-	-	-	-	-	-	-	-	-
	1	-	+	-	-	-	-	-	-	-
	2	-	+	+	+	+	+	-	+	-
	4	+	+	+	+	+	+	+	+	+
ziziphus spina-csisti	o.25	-	-	-	-	-	-	-	-	-
	0.5	-	-	-	-	-	-	-	-	-

Plant	Concentration of extracts (mg/ml)	Minimum inhibitory concentration (mg/ml)								
		F.o	F.s	F.b	R.s	A.fu	Afl	A.o	P.ch	P.co
	1	-	-	-	+	-	+	-	-	-
	2	+	-	+	+	+	+	-	+	-
	4	+	+	+	+	+	+	+	+	+
positive control	test organism	+	+	+	+	+	+	+	+	+
Control	broth only	-	-	-	-	-	-	-	-	-

Fungal species:** *Fusarium oxysporum* **(F.o)**; *Fusarium solani (F. s**); Fusarium *brachygibbosum* (**F. b**); *Rhizoctonia solani* (**R.s**); *Aspergillus fumigates (***A.fu**)*; Aspergillus flavus (***A.fl**)*; Aspergillus oryzae (***A.o**)*; Penicillium chryogenum* (**P.ch**); *Penicillium commune (***P.co**)

***Negative controls**; Potato Dextrose broth only; Potato: **positive control**; Potato Dextrose broth, and a test organism

*** (-)** = No inhibition at the concentrations used.

VI.4. phytochemical analysis of medicinal plant extracts

VI.4.1. Preliminary phytochemical screening

Thre preliminary phytochemical investigation carried out on crude extracts of the 15 medicinal plants showed the presence of different medically active compounds like carbohydrate, protein, phenols, saponins, tannins, terpenoids and flavanoids. Other phyto constituents like alkaloids and fixed oil. The phytochemical characteristics of selected medicinal plants tested were summarized in the (Table19)

qualitative phytochemical screening results revealed that, proteins, carbohydrates, were present in all the selected fifteen plant extracts except *mentha varidis* and *Anethum graveolens* , alkaloids were absent in five extracts; Nigella *Sativa, Thymus vulgaries, Lavandula angustifolia, Olea eurpaea* and *ziziphus spina-csisti.*

Also phytochemical analysis showed that, the presence of Flavonoids in all samples except *Lavandula angustifolia*. And the Tannins present in all study samples except *Olea eurpaea.* And the Terpenoids were fiund with different levels in all the selected extracts excpt in *Eucalyptus globules*

Finally, saponins compounds were found in all the study samples except *Lavandula angustifolia* and *Olea eurpaea.* The phenolics compounds present in most of samples except only 6 extracts; *Alium sativum, Alium cepa, Foeniculum vulgare, Anethum graveolens, Lavandula angustifolia* and *ziziphus spina-csisti.*

Table 19: phytochemical analysis of 15 herbal & medicinal plants essential oils

Plant	Sources	Alkaloids	Flaonoids	Phenols	Tannins	Saponins	Terpenoids	Protein	carbohydrate
Rosmarinus officinalis (rosemary)	leaves	_	++	+++	+++	+	_	+	+
Zingiber officinale (zinger)	Rhizome	+++	++	++	++	+++	+	+	+
Alium sativum (garlic)	clove	+++	++	_	++	+	+++	+	+
Alium cepa (onion)	onion bulb	+++	+	_	++	++	+++	+	+
Foeniculum vulgare (Fennel)	fruits	++	+	_	+++	++	++	+	+
Nigella *Sativa* (Black seed)	seeds	_	++	+	+++	++	+	+	+
Thymus vulgaries (Common, Thyme)	leaves	_	++	+	+++	++	++	+	+
Cassia Senna (*senna*)	leaves	+++	++	+++	+++	+++	+++	+	+
mentha varidis (Spearmint)	leaves	++	+++	+	+++	+	+++	_	_
Syzygium aromaticum (Syzygium)	clove	++	++	++++	++++	+++	+++	+	+
Anethum graveolens (Dill)	Leaves & Stems	+++	+++	-	++	++	++	_	_
Eucalyptus globules (Eucalyptus)	leaves	++	++	+++	+++	+	_	+	+
Lavandula angustifolia (lavandar)	flowers	_	_	_	+++	_	+++	+	+
Olea eurpaea (olive)	fruits	_	+++	+++	_	_	+	+	+
ziziphus spina-csisti (Christ,s Thorn)	leaves	_	+	_	++	+++	+	+	+

*** (+)** indicate **presence**: +++; strongly positive, ++; moderately positive, +; weakly positive

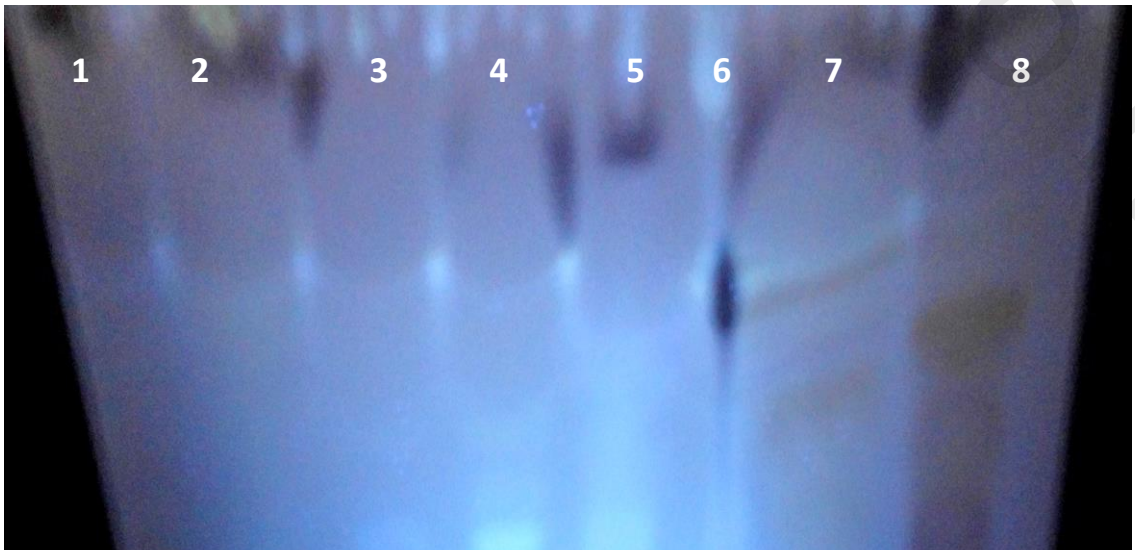
*****- negative (**absence**)

VI.4.2. Thin layer chromatography

TLC separation of methanolic extracts of the selected 15 plants using the solvent system, CHCl3: MeOH in a 4:1 ratio visualized under UV 254 nm. This qualitative techniques will give an idea of the presence or absence of bioactive compounds which shown as coloured spots in the different separated extracts of 15 plants in addition to different classes of compounds of intermediate polarity.

The result of TLC chromatographic separation fifteen herbal and medicinal plants methanolic extraction using TLC plates and shown to fluorescene light under the UV at 254 – 365nm wavelength (Fig. 23.1, 23.2) revealed that find reddish brown active spots in TLC with different Rf values in three samples (*Cassia Senna*, *mentha varidis* and *Anethum graveolens)*; these are indicated that the three samples rich in alkaloids compoumnds. And presence of spots ranged from dark to light yellow with different Rf values in five samples (*Thymus vulgaries, Cassia Senna, mentha varidis, Anethum graveolens* and *Olea eurpaea)*; these are indicated that the five samples rich in flavonoids & flavones compoumnds.

In addition tne TLC result of methaolic extracts the presence of pink to purple spots with different Rf values in five samples (*Thymus vulgaries, Cassia Senna, Syzygium aromaticum, Anethum graveolens* and *Eucalyptus globules*); these are indicated that the five samples rich in terpenoids compoumnds. And presence of blue green spots with different Rf values in eight samples (*Foeniculum vulgare, Nigella Sativa, Thymus vulgaries, Cassia Senna, Syzygium aromaticum, Anethum graveolens, Eucalyptus globules* and *Lavandula angustifolia*); these are indicated that the eight samples rich in tannins compoumnds.



Figgure 23.1: Thin layer chromatogram under UV254 of eight plant methanolic extracts in order 1= *Rosmarinus officinalis*; 2= *Zingiber officinale*; 3= *Alium sativum*; 4= *Alium cepa*; 5= *Foeniculum vulgare*; 6= *Nigella Sativa*; 7= *Thymus vulgaries* and 8= *Cassia Senn*a. Solvent system: CHCl3: MeOH (4:1)

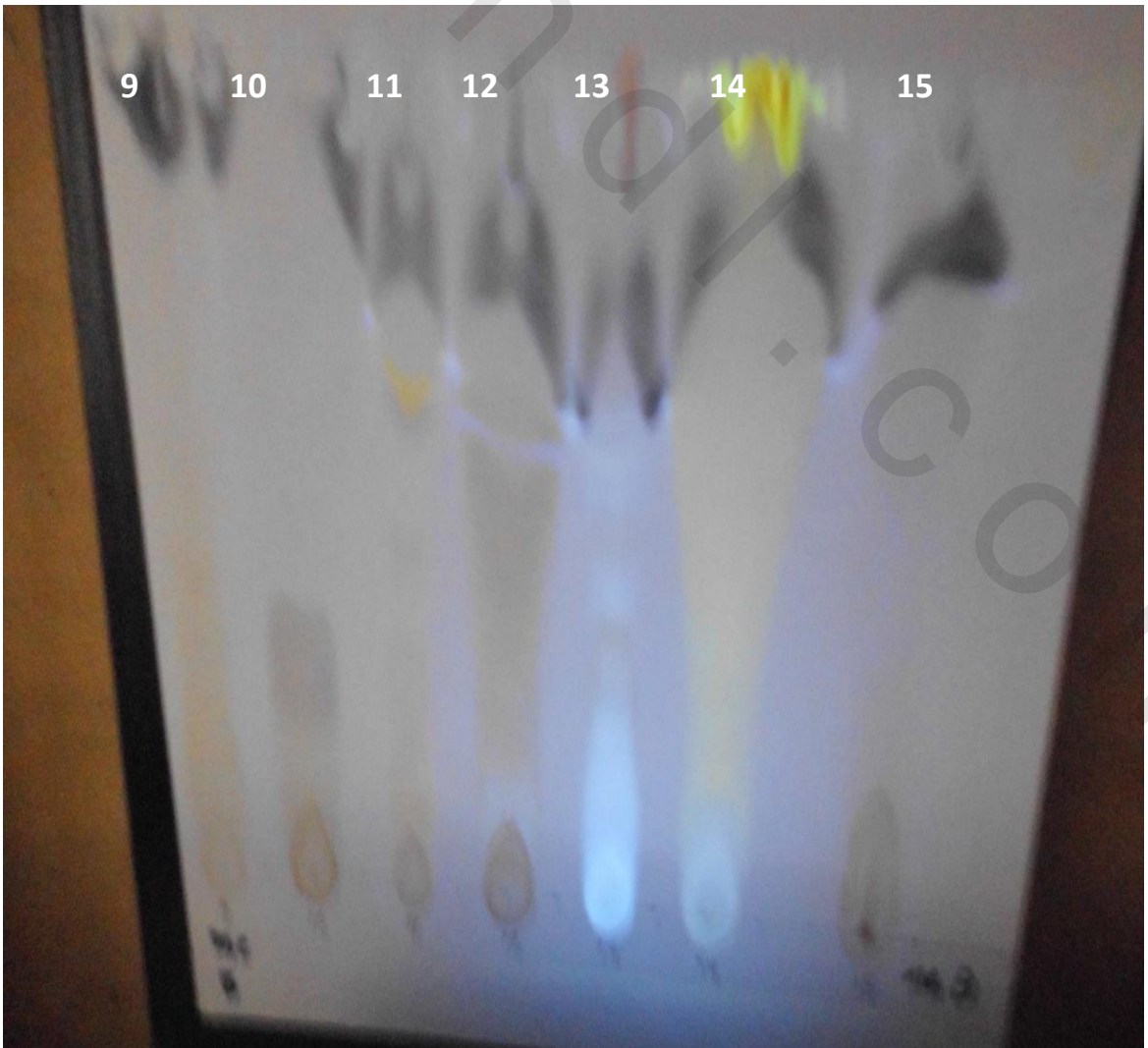


Figure 23.2: Thin layer chromatogram under UV254 of seven plant methanolic extracts in order 9= *mentha varidis*; 10= *Syzygium aromaticum*; 11= *Anethum graveolens*; 12= *Eucalyptus globules*; 13= *Lavandula angustifolia*; 14= *Olea eurpaea* and 15= *ziziphus spina-csisti*. Solvent system: CHCl3: MeOH (4:1).

VI.4.3. Gas Chromatographic-Mass Spectroscopic (GC-MS) analysis for plant essential oils

The compounds identified by GC-MS analysis from essential oils samples extracted from fifteen herbal and medicinal plants were enumerated with retention time (The interval between the instant of injection and the detection of the*)* and peak area (%). The result of GC-MS analysis showed the presence of many phytochemical constituets which may have a role in the antifungal activityies of the studied plant extracts

VI.4.3.1. GC-MS analysis of *Rosmarinus officinalis*

Chemical analysis performed by GC/MS revealed that essential oil of *Rosmarinus officinalis* (**Fig. 24**, Table 20) is characterized by the presence of many major compounds, which are: Limonene (23.03%), Cis- Vaccenic acid (12.91%), Trans-4-Decadienal (10.67%), Octane, 2, 4, 6- trimethyl (9.14%), 9, 12 Octadecadienoic acid (8.77%)., Trans-3-*Nonene (7.65%),* 4-Heptenal (6.90%), Eucalyptol (3.98%) and Linalool(3.50%)

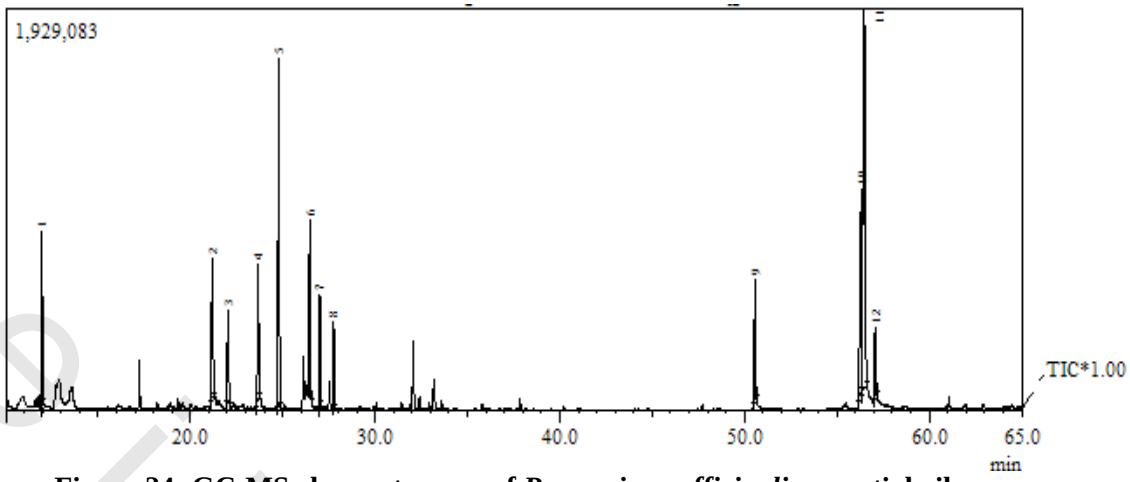


Figure 24: GC-MS chromatogram of *Rosmarinus officinalis* essential oils

Table 20: Essential oil composion of *Rosmarinus officinalis* by GC-MS analysis

Quatitative ID	Component identified	Retention time (min)	Area (%)
1	Eucalyptol	11.97	3.98
2	Trans-3-*Nonene*	21.20	7.65
3	2-Decenal	22.04	2.96
4	Trans-4- Decadienal.	23.67	10.67
5	.Limonene	24.79	23.03
6	Octane, 2, 4, 6- trimethyl	26.47	9.14
7	2-Undecenal	27.01	2.49
8	4-Heptenal	27.75	6.90
9	Linalool	50.75	3.50
10	9, 12 Octadecadienoic acid	56.33	8.77
11	Cis- Vaccenic acid	56.49	12.91
12	Octadecanoic acid	57.07	1.61

VI.4.3.2. GC-MS analysis of *Zingiber officinale*

Chemical analysis performed by GC/MS revealed that essential oil of *Zingiber officinale* (**Figure 25**, Table 21) is characterized by the presence of many major compounds, which are: Zingiberene (29.72%), Ca rveol (15.05%), Cyclohexene.3-(1, 5-dimethyl-4-hexenyl)-6-methylene (10.40%) and 9, 12 Octadecadienoic acid (9.20%)

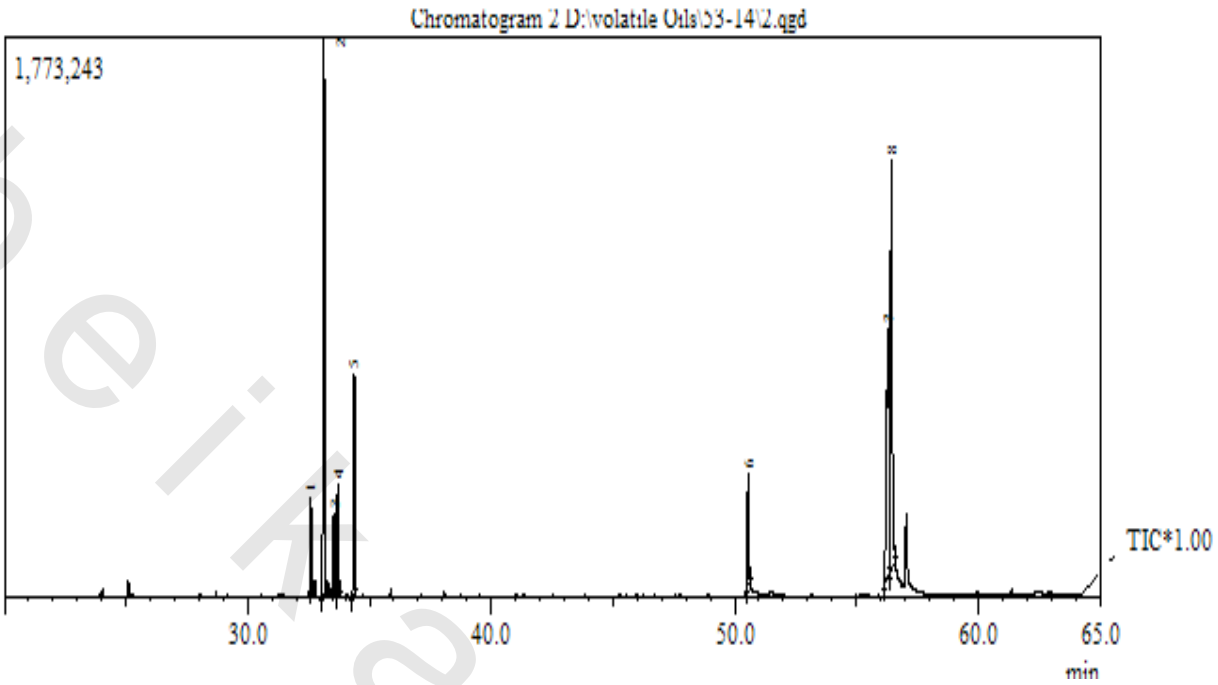


Figure 25: GC-MS chromatogram of *Zingiber officinale* essential oils

Table 21: Essential oil composion of *Zingiber officinale* by GC-MS analysis

Quatitative ID	Component identified	Retention time (min)	Area (%)
1	Zingiberene	33.14	29.74
2	Alpha- Farnesene	33.54	2.88
3	Cis-alpha- Bisabolene	33.70	5.04
4	Cyclohexene.3-(1, 5-dimethyl-4-hexenyl)-6-methylene	34.38	10.40
5	n-Hexadecanoic acid	50.56	3.93
6	9, 12 Octadecadienoic acid	56.30	9.20
7	Carveol	56.45	15.05

VI.4.3.3. GC-MS analysis of *Alium sativum*

Chemical analysis performed by GC/MS revealed that essential oil of *Alium sativum* (Fig. 26, Table 22) is characterized by the presence of many major compounds, which are: 2-Chloroethyl linoleate (42.01%), Allyl methyl trisulfide (28.21%), Diallyl disulfide (10.05%) and Cis-9-Hexadecenal (6.46%)

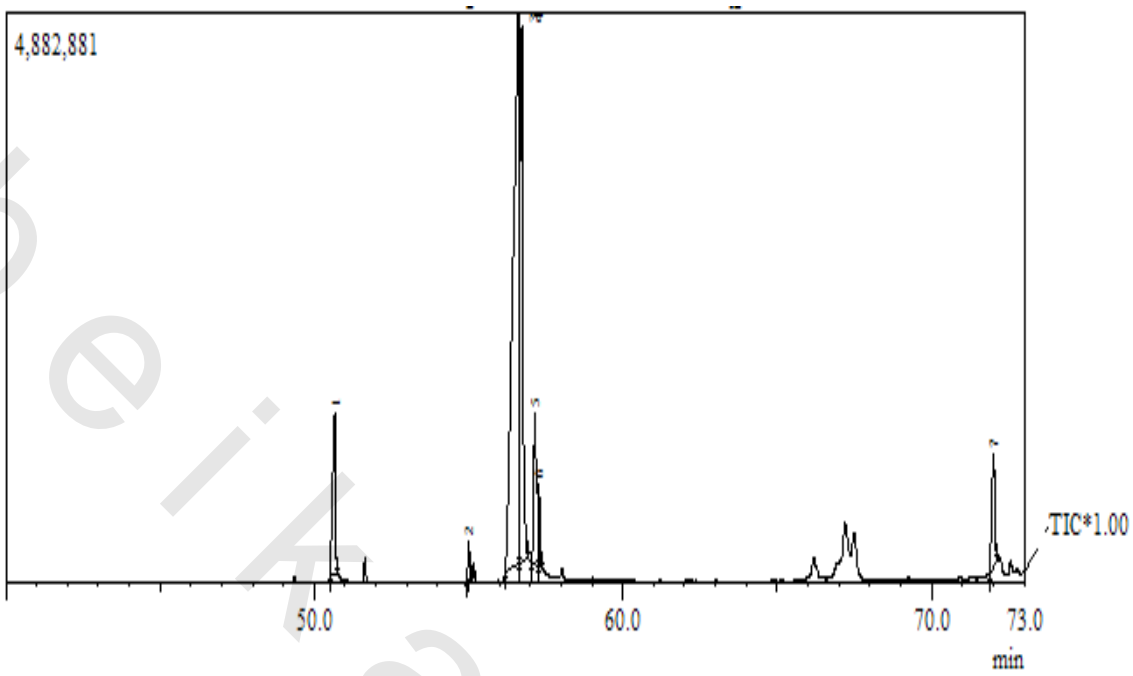


Figure 26: GC-MS chromatogram of *Alium sativum* essential oils

Table 22: Essential oil composion of *Alium sativum* by GC-MS analysis

Quatitative ID	Component identified	Retention time (min)	Area (%)
1	Diallyl disulfide	50.66	10.05
2	9, 12 Octadecadienoic acid	55.01	1.63
3	2-Chloroethyl linoleate	56.62	42.01
4	Allyl methyl trisulfide	56.74	28.21
5	Linoleic acid ethyl ester	57.14	5.67
6	Ethyl Oleate	57.28	2.56
7	Cis-9-Hexadecenal	72.02	6.46

VI.4.3.4. GC-MS analysis of *Alium cepa*

Chemical analysis performed by GC/MS revealed that essential oil of *Alium cepa* (**Fig.** 27 Table 23) is characterized by the presence of many major compounds, which are: propyl trisulphide (32.56%), 9, 12 Octadecadienoic acid (22.53%). n-Hexadecanoic acid (9.52%) and methyl propyl trisulphide (7.46%)

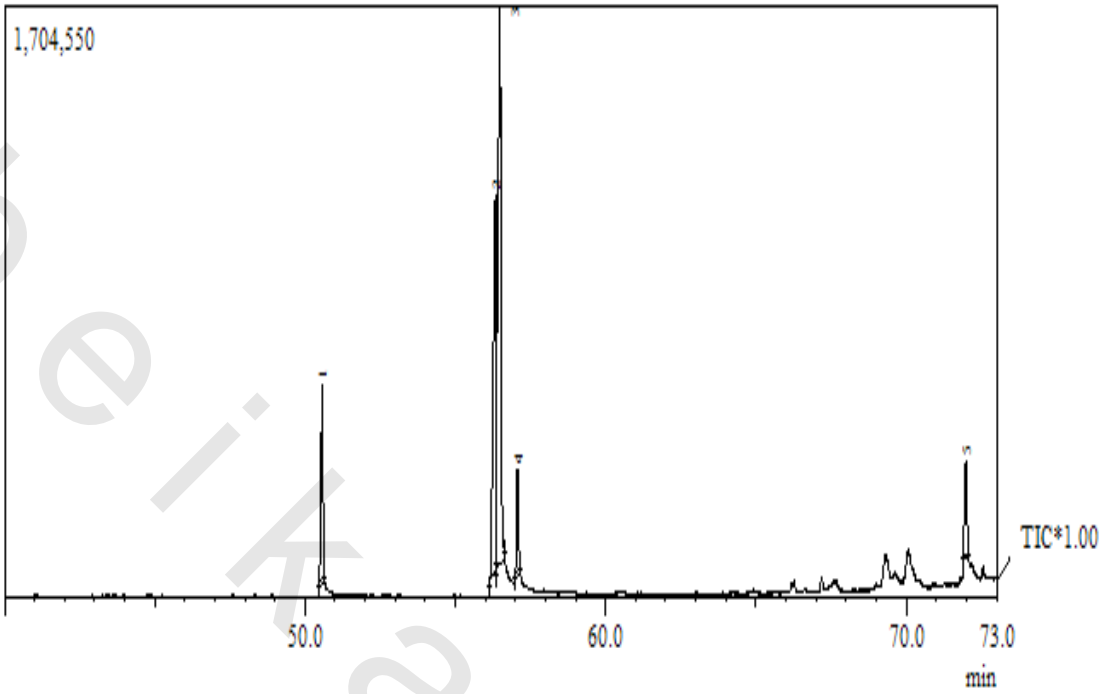


Figure 23: GC-MS chromatogram of *Alium cepa* essential oils

Table 27: Essential oil composion of *Alium cepa* by GC-MS analysis

Quatitative ID	Component identified	Retention time (min)	Area (%)
1	n-Hexadecanoic acid	50.57	9.52
2	9, 12 Octadecadienoic acid	56.33	22.53
3	propyl trisulphide	56.48	32.56
4	Octadecanoic acid	57.08	4.56
5	methyl propyl trisulphide	71.99	7.46

VI.4.3.5. GC-MS analysis of *Foeniculum vulgare*

Chemical analysis performed by GC/MS revealed that essential oil of *Foeniculum vulgare* (Fig. 28, Table 24) is characterized by the presence of many major compounds, which are: Cis- Vaccenic acid (3.23%), 9, 12 Octadecadienoic acid (29.49%), Pentadecanoic acid (7.51%), Estragole (4.39%) and D-Limonene (2.93%)

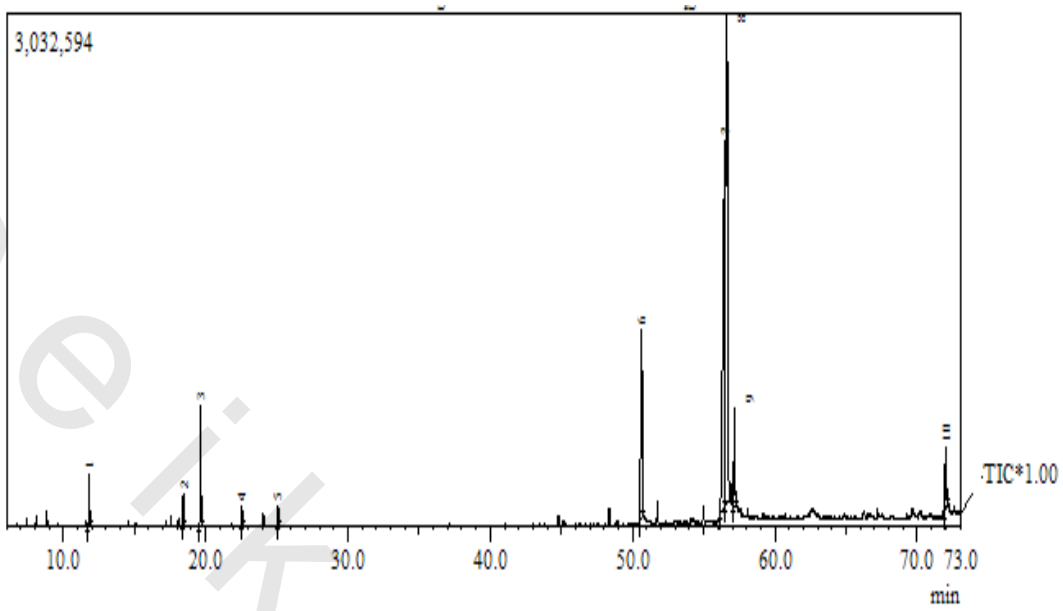


Figure 28: GC-MS chromatogram of *Foeniculum vulgare* essential oils

Table 24: Essential oil composion of *Foeniculum vulgare* by GC-MS analysis

Quatitative ID	Component identified	Retention time (min)	Area (%)
1	D-Limonene	11.81	2.93
2	Menthol	18.44	1.89
3	Estragole	19.66	4.39
4	2-Decenal.	22.51	1.58
5	2, 4-Decadienal.	25.10	1.68
6	Pentadecanoic acid	50.64	7.51
7	9, 12 Octadecadienoic acid	56.45	29.49
8	Cis- Vaccenic acid	56.63	31.23
9	Octadecadienoic acid	57.15	3.92
10	9-Octadecadienoic acid	72.00	3.75

VI.4.3.6. GC-MS analysis of *Nigella Sativa*

Chemical analysis performed by GC/MS revealed that essential oil of *Nigella Sativa* (Fig. 29, Table 25) is characterized by the presence of many major compounds, which are: Limonene (75.27%), 9, 12 Octadecadienoic acid (13.18%), n-Hexadecanoic acid (2.92%), Alpha- Phellandrene (0.74%) and Linalool, methyl ether (0.06%)

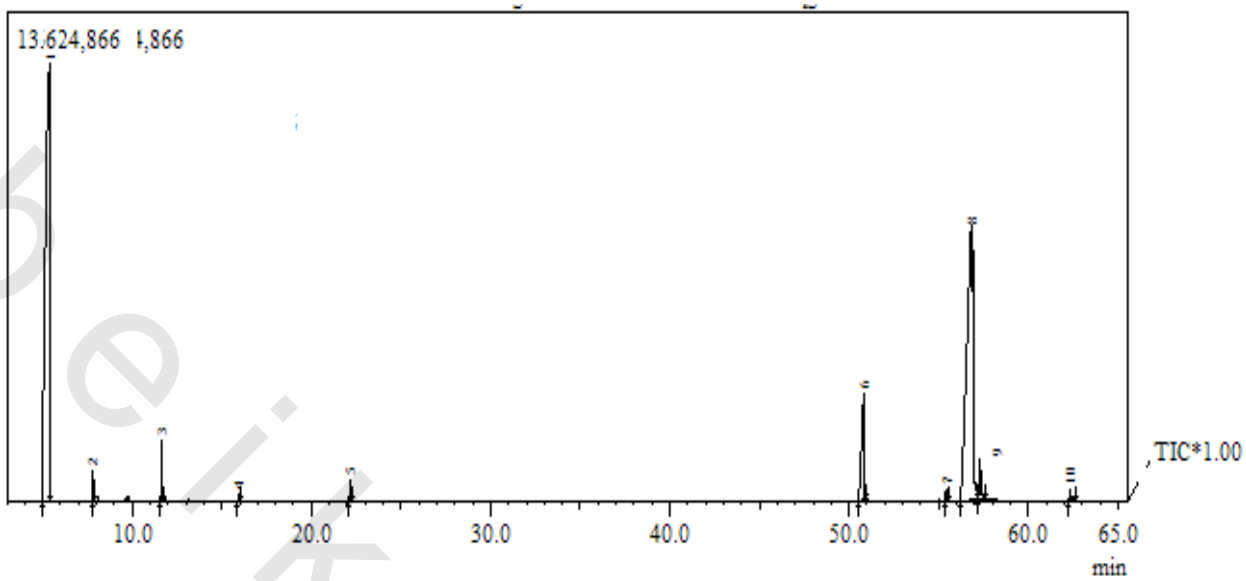


Figure 29: GC-MS chromatogram of *Nigella Sativa* essential oils

Table 25: Essential oil composion of *Nigella Sativa* by GC-MS analysis

Quatitative ID	Component identified	Retention time (min)	Area (%)
1	Limonene	5.38	75.27
2	Alpha- Phellandrene	7.81	0.74
3	Benzene, 1-methyl-3(1-methylethyl)	11.64	2.05
4	Linalool, methyl ether	15.925	0.06
5	2, 5- Cyclohexadiene- 1,4-dione, 2-methyl-5-(1-methylethyl)	22.156	0.23
6	n-Hexadecanoic acid	50.82	2.96
7	Phytol	55.43	0.16
8	9, 12 Octadecadienoic acid	56.81	13.18
9	Octadecadienoic acid	57.32	0.41
10	Linoleic acid ethyl ester	62.35	0,23

VI.4.3.7. GC-MS analysis of *Thymus vulgaries*

Chemical analysis performed by GC/MS revealed that essential oil of *Thymus vulgaries* (Fig. 30 Table 26) is characterized by the presence of many major compounds, which are: 9, 12 Octadecadienoic acid (24.32%), Limonene (20.90%), Erucic acid (11.34%), 1,E-11 ,Z-13-Octadecatriene (9.29%), thymol (8.03%) and p-cymene (5.56%)

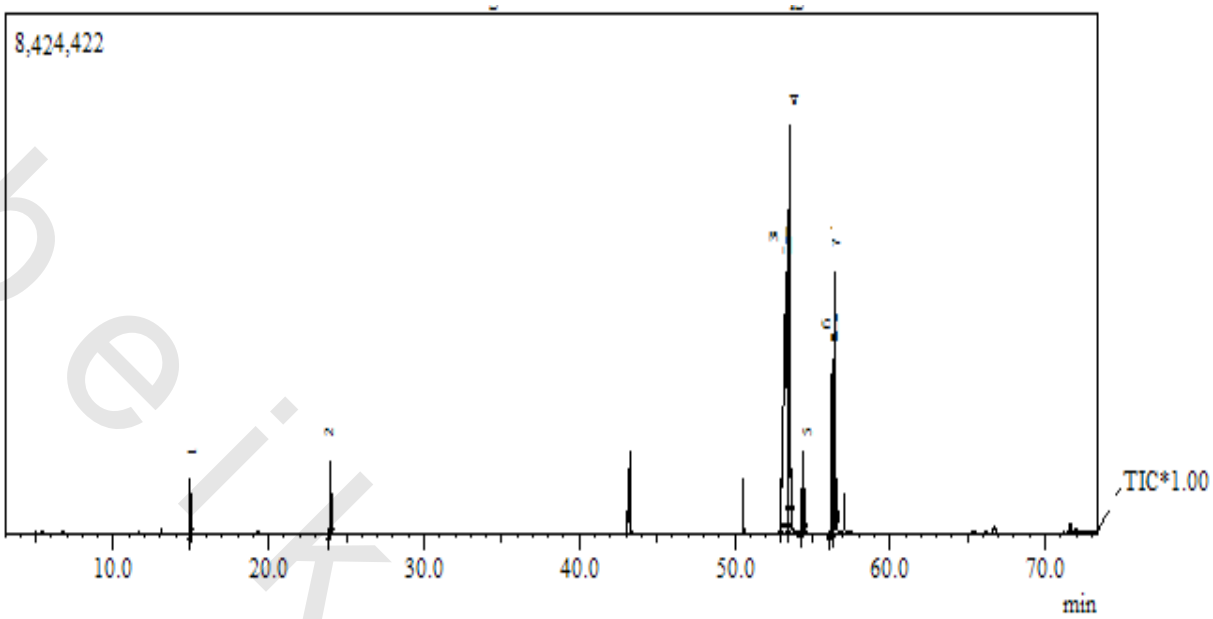


Figure 30: GC-MS chromatogram of *Thymus vulgaries* essential oils

Table 26: Essential oil composion of *Thymus vulgaries* by GC-MS analysis

Quatitative ID	Component identified	Retention time (min)	Area (%)
1	5 isoprc	14.95	2.25
2	thymol	23.95	8.03
3	9, 12 Octadecadienoic acid	53.30	24.32
4	Limonene	53.55	20.90
5	p-cymene	54.39	4.56
6	1,E-11 ,Z-13-Octadecatriene	56.30	9.29
7	Erucic acid	56.45	11.34

VI.4.3.8. GC-MS analysis of *Cassia Senna*

Chemical analysis performed by GC/MS revealed that essential oil of *Cassia Senna* (Fig. 31, Table 27) is characterized by the presence of many major compounds, which are: Tetratetriacontane (19.33%), Eugenol (17.65%), Cis- Vaccenic acid (14.29%) and Rhein (12.37%)

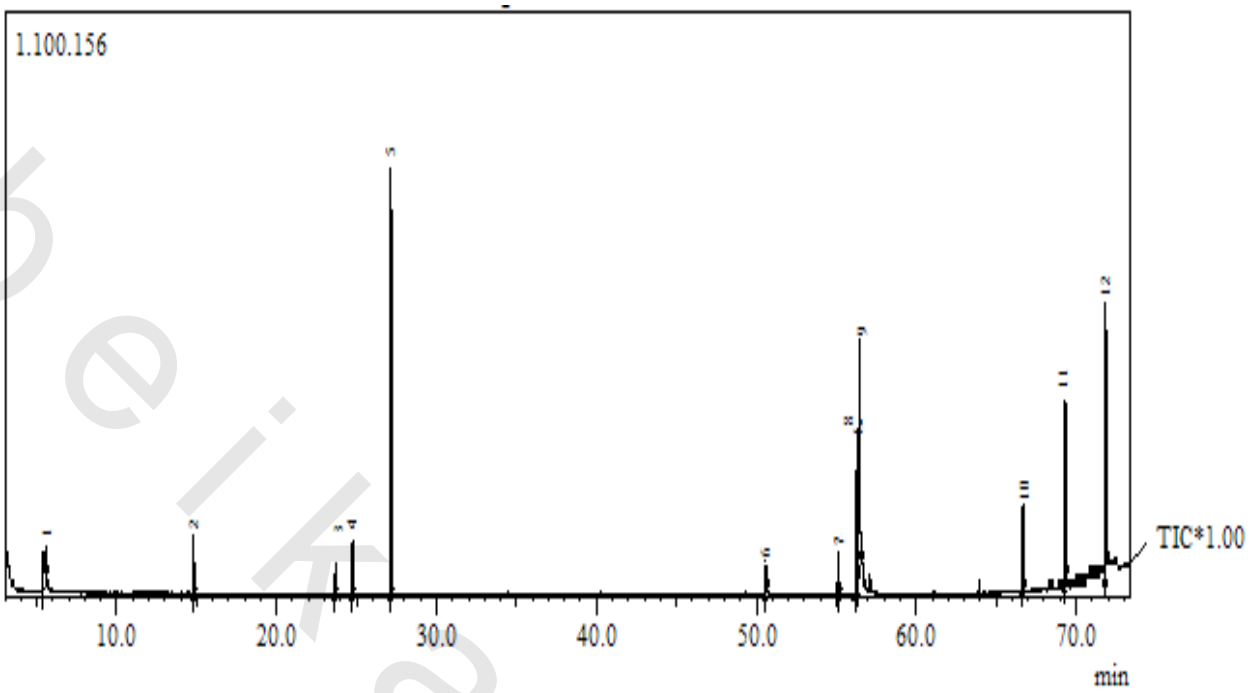


Figure 31: GC-MS chromatogram of *Cassia Senna* essential oils

Table 27: Essential oil composion of *Cassia Senna* by GC-MS analysis

Quatitative ID	Component identified	Retention time (min)	Area (%)
1	Dimethyl Sulfoxide (DMSO)	5.49	1.27
2	Undecane	14.79	4.37
3	2, 4-Nonoadienal	23.61	3.76
4	2, 4-Decadional	24.74	4.34
5	Eugenol	27.15	17.65
6	n-Decanoic acid	50.60	2.85
7	Methyl 2- hydroxyl dodecanoate	55.20	1.94
8	7-Tetradecyne	55.30	5.60
9	Cis- Vaccenic acid	55.44	14.29
10	2- Bromo dodecane	66.68	8.04
11	Rhein	69.32	12.37
12	Tetratetriacontane	71.56	19.33

VI.4.3.9. GC-MS analysis of *mentha varidis*

Chemical analysis performed by GC/MS revealed that essential oil of *mentha varidis* (Fig. 32, Table 28) is characterized by the presence of many major compounds, which are: piperitone (24.76%), Kalonic acid, ethyl 4-heptyl ester (17.80%), Alpha-Terpir (12.23%), Butanedioic acid (6.49%), Gamma- Terpineol (1.53%), Cis-P-Menthan (1.07%) and D-Limonene (0.64%)

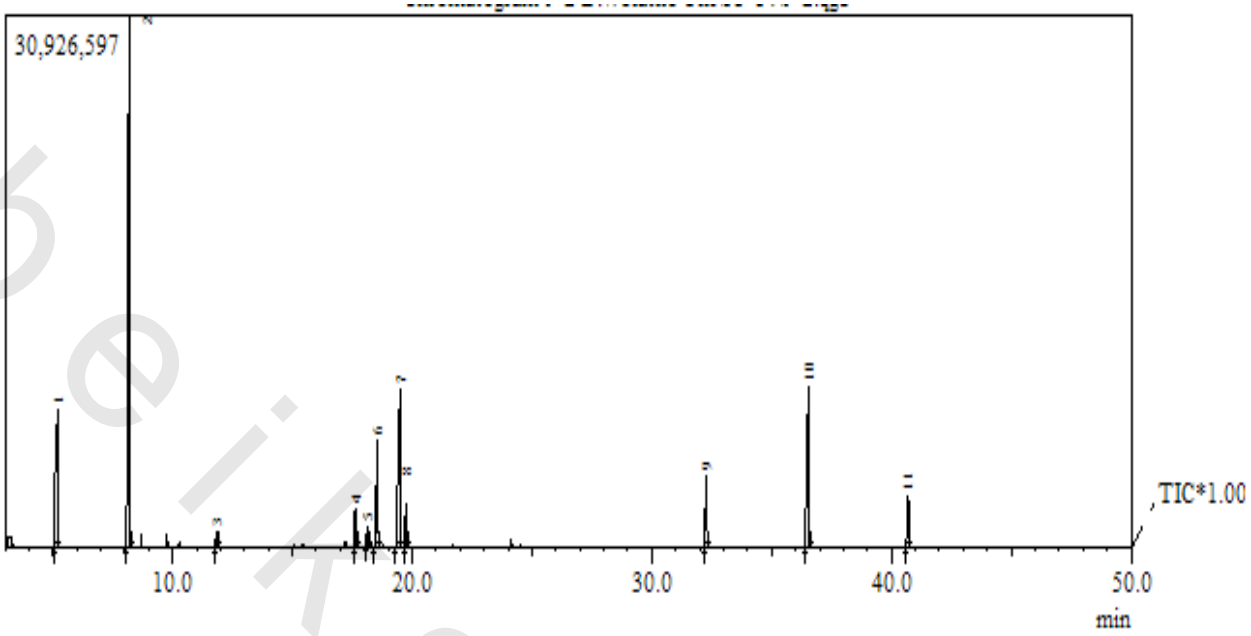


Figure 32: GC-MS chromatogram of *mentha varidis* essential oils

Table 28: Essential oil composion of *mentha varidis* by GC-MS analysis

Quatitative ID	Component identified	Retention time (min)	Area (%)
1	piperitone	5.20	24.76
2	IR-alpha-Pinene	7.82	0.09
3	D-Limonene	11.83	0.64
4	Cis-P-Menthan	17.63	1.07
5	Menthone	18.13	0.63
6	P-Menthan-3-ol alcohol	18.55	4.14
7	Alpha- Terpir	19.49	12.23
8	Gamma- Terpineol	19.73	1.53
9	Butanedioic acid	32.28	6.49
10	Kalonic acid, ethyl 4-heptyl ester	36.54	17.80
11	Adipic acid, diisobutyl ester	40.70	25.62

VI.4.3.10. GC-MS analysis of *Syzygium aromaticum*

Chemical analysis performed by GC/MS revealed that essential oil of *Syzygium aromaticum* (Fig. 33, Table 29) is characterized by the presence of many major compounds, which are: Eugenal (15.90%), camphor (15.76%), Octacosane (15.72%) and Tetratetracontane (9.56%)

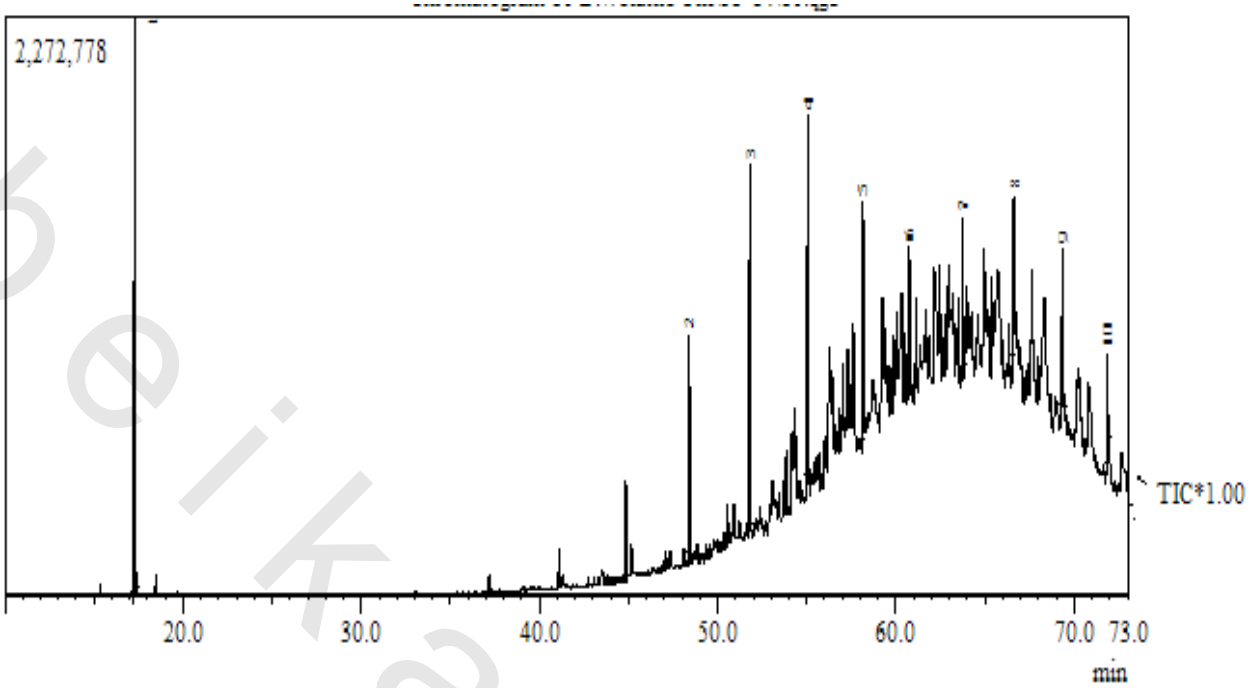


Figure 33: GC-MS chromatogram of *Syzygium aromaticum* essential oils

Table 29: Essential oil composion of *Syzygium aromaticum* by GC-MS analysis

Quatitative ID	Component identified	Retention time (min)	Area (%)
1	camphor	17.28	15.76
2	Eicosane	48.40	9.31
3	Octacosane	51.80	15.72
4	Eugenal	55.06	15.90
5	tetratetracontane	58.16	9.56
6	Dodocylclohexane	60.76	7.22
7	n-Heptadecyl cyclohexane	63.74	6.70
8	n- Pentadecylcyclohexane	66.60	6.17
9	thymol	69.35	5.57
10	Pentacosane	71.88	4.71

VI.4.3.11. GC-MS analysis of *Anethum graveolens*

Chemical analysis performed by GC/MS revealed that essential oil of *Anethum graveolens* (Fig. 34, Table 30) is characterized by the presence of many major compounds, which are: Estragole (14.11%), D-Limonene (10.72%), 9, 12 Octadecadienoic acid (10.49%), Tetratriacontane (10.42%) and p- Mentha-6,8- diene-2-one (7.97%)

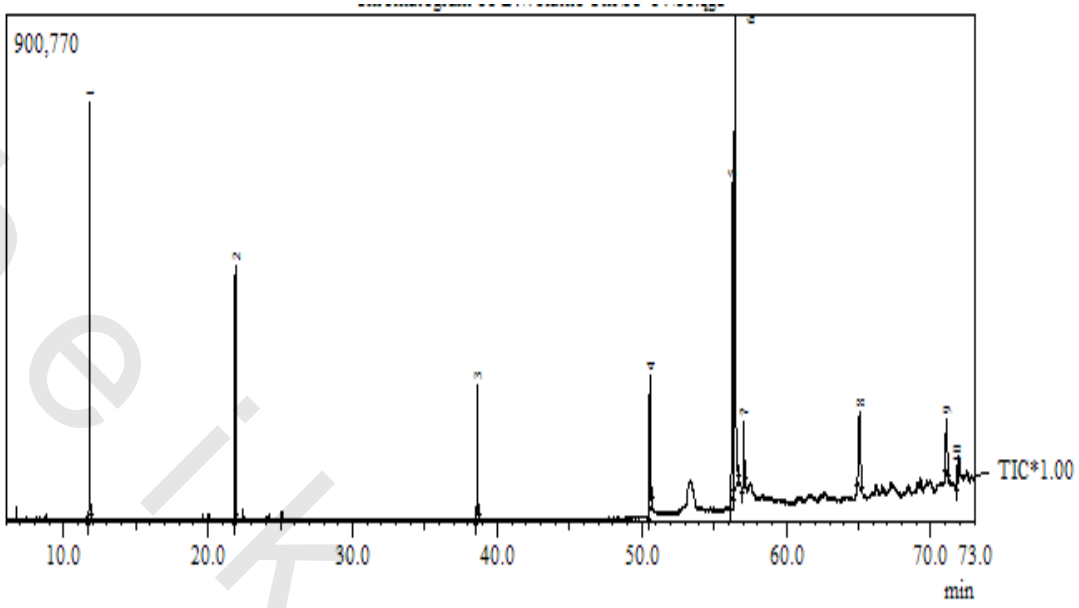


Figure 34: GC-MS chromatogram of *Anethum graveolens* essential oils

Table 30: Essential oil composion of *Anethum graveolens* by GC-MS analysis

Quatitative ID	Component identified	Retention time (min)	Area (%)
1	D-Limonene	11.81	10.72
2	p- Mentha-6,8- diene-2-one, (s)-(+)-	21.89	7.97
3	Apiol	38.63	3.49
4	n-Hexandecanoic acid	50.56	3.36
5	9, 12 Octadecadienoic acid	56.30	10.49
6	Estragole	56.45	14.11
7	Octadecadienoic acid	57.07	2.63
8	Tetratriacontane	65.08	10.42
9	Hentriacontane	71.07	5.53
10	Sulfurous acid	71.82	1.39

VI.4.3.12. GC-MS analysis of *Eucalyptus globules*

Chemical analysis performed by GC/MS revealed that essential oil of *Eucalyptus globules* (Fig. 35, Table 31) is characterized by the presence of many major compounds, which are: 1,8-eucalypol (23.08%), 1, E-11,Z-13- Octadecatriene (15.57%), Pentacosane (12.18%), Palmitic acid (11.25%) and linalool (10.14%)

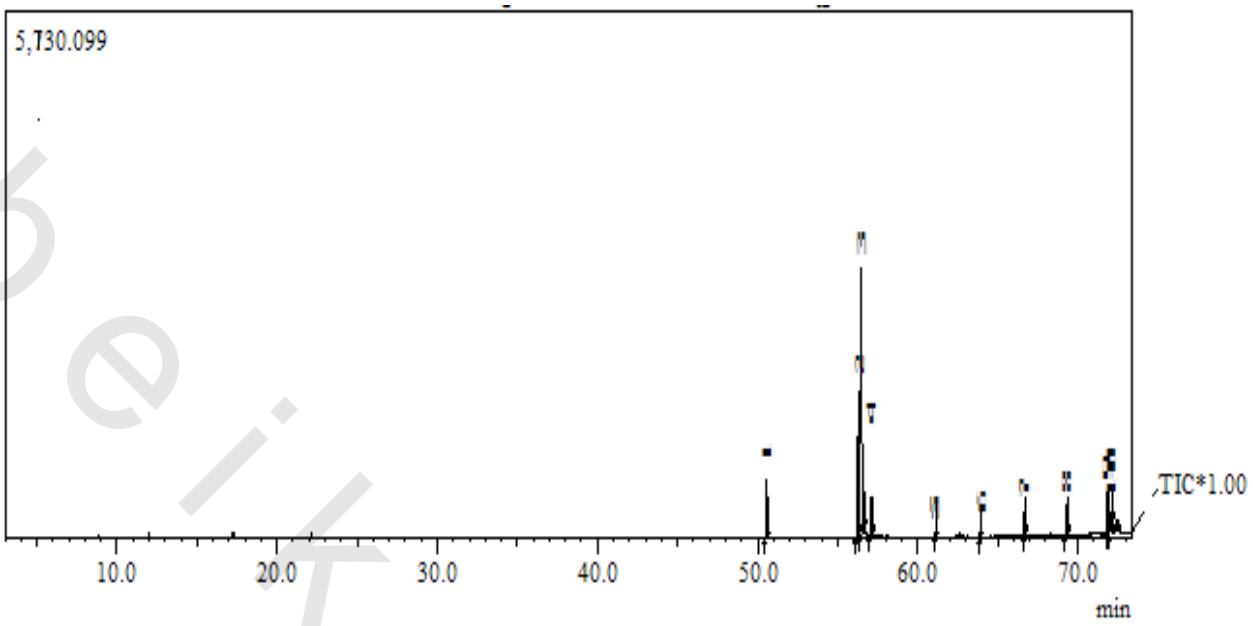


Figure 35: GC-MS chromatogram of *Eucalyptus globules* essential oils

Table 31: Essential oil composion of *Eucalyptus globules* by GC-MS analysis

Quatitative ID	Component identified	Retention time (min)	Area (%)
1	Palmitic acid	50.58	11.25
2	1, E-11,Z-13- Octadecatriene	56.32	15.57
3	1,8-eucalyptol	56.47	23.08
4	Octadecadienoic acid	57.08	6.89
5	2- Bromo dodecane	61.06	3.54
6	Tetratriacontane	63.91	6.84
7	Hentriacontane	66.65	9.15
8	linalool	69.29	10.14
9	Pentacosane	71.83	12.18
10	7- Tetradecenal, (Z)-	72.01	4.66

VI.4.3.13. GC-MS analysis of *Lavandula angustifolia*

Chemical analysis performed by GC/MS revealed that essential oil of *Lavandula angustifolia* Fig. 36, Table 32) is characterized by the presence of many major compounds, which are: Lavandulyl acetate (19.24%), Octacosane (18.50%), Eugenol (9.62%), Pentacosane (7.03%), Caryophyllene (5.92%), Hentriacontane (5.55%) and 9, 12 Octadecadienoic acid (5.43%)

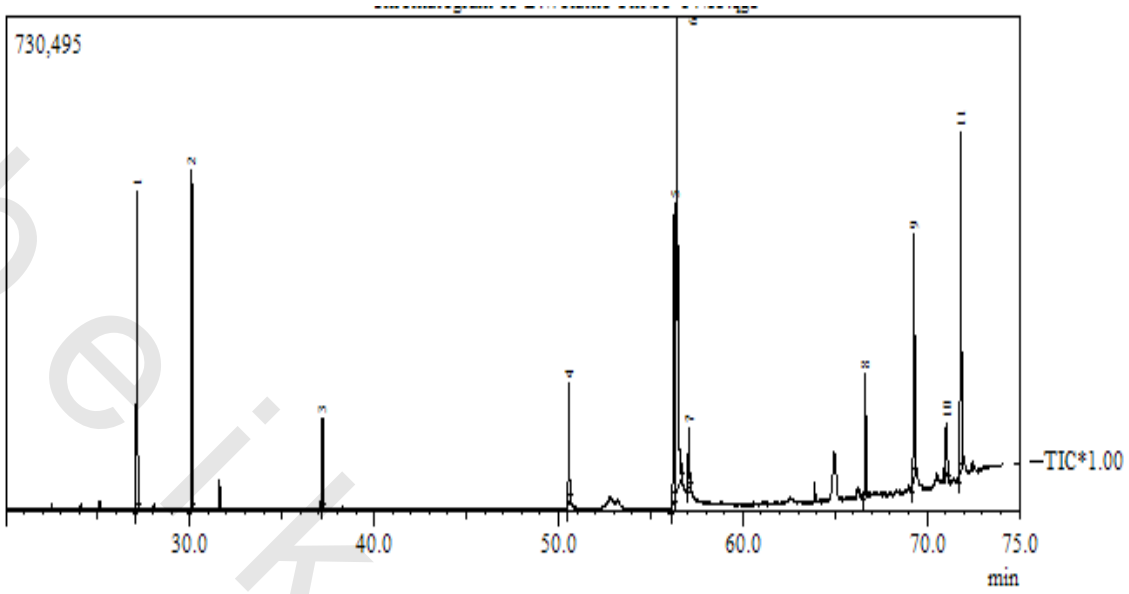


Figure 36: GC-MS chromatogram of *Lavandula angustifolia* essential oils

Table 32: Essential oil composion of *Lavandula angustifolia* by GC-MS analysis

Quatitative ID	Component identified	Retention time (min)	Area (%)
1	Eugenol	27.13	9.62
2	Caryophyllene	30.10	5.92
3	Caryophyllene oxide	37.19	0.37
4	Palmitic acid	50.57	1.68
5	9, 12 Octadecadienoic acid	56.29	5.43
6	Lavandulyl acetate	56.44	19.24
7	Octadecadienoic acid	57.07	0.69
8	Tetratriacontane	66.66	2.98
9	Hentriacontane	69.29	5.55
10	Pentacosane	71.05	7.03
11	Octacosane	71.84	18.50

VI.4.3.14. GC-MS analysis of *Olea eurpaea*

Chemical analysis performed by GC/MS revealed that essential oil of *Olea eurpaea* (Fig. 37, Table 33) is characterized by the presence of many major compounds, which are: Oleuropein (64.17%), n-Hexandecanoic acid (13.63%), 9- Octadecadienoic acid ethyl ester (4.65%), Octadecadienoic acid, ethyl ester (2.73%) and Verbascoside (1.66%)

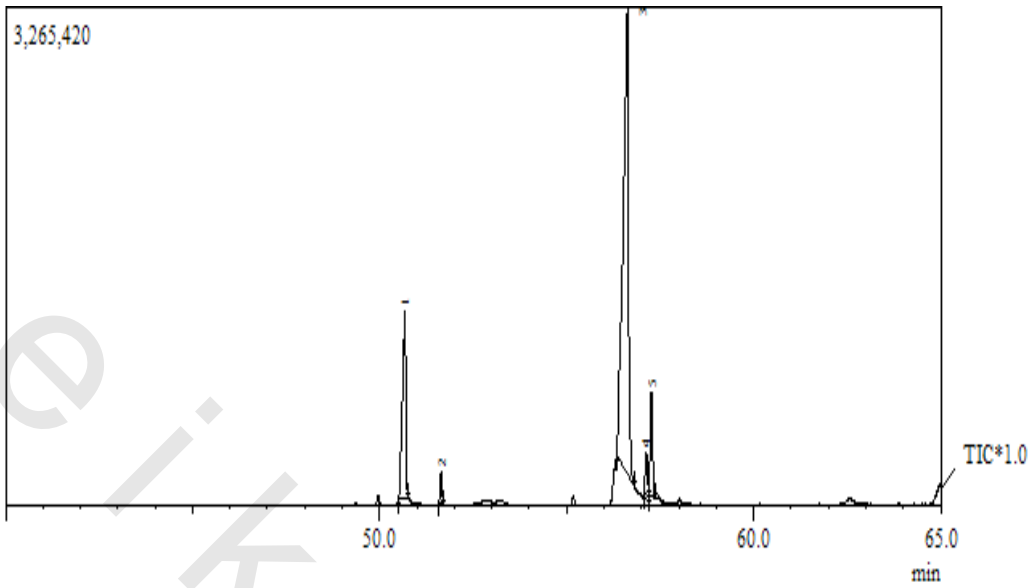


Figure 37: GC-MS chromatogram of *Olea eurpaea* essential oils

Table 33: Essential oil composion of *Olea eurpaea* by GC-MS analysis

Quatitative ID	Component identified	Retention time (min)	Area (%)
1	n-Hexandecanoic acid	50.67	13.63
2	Octadecadienoic acid, ethyl ester	51.64	2.73
3	Oleuropein	56.62	64.17
4	Verbascoside	57.13	1.66
5	9- Octadecadienoic acid ethyl ester	57.27	4.65

VI.4.3.15. GC-MS analysis of *ziziphus spina-csisti*

Chemical analysis performed by GC/MS revealed that essential oil of *ziziphus spina-csisti* (Fig.38, Table 34) is characterized by the presence of many major compounds, which are: Geranyl acetone (20.62%), 1, E-11,Z-13- Octadecatriene (18.34%), Methyl hexadecanoate (9.85%), Eicosane (8.94%), Heptandecane (7.20%) and n-Hexandecanoic acid (6.39%)

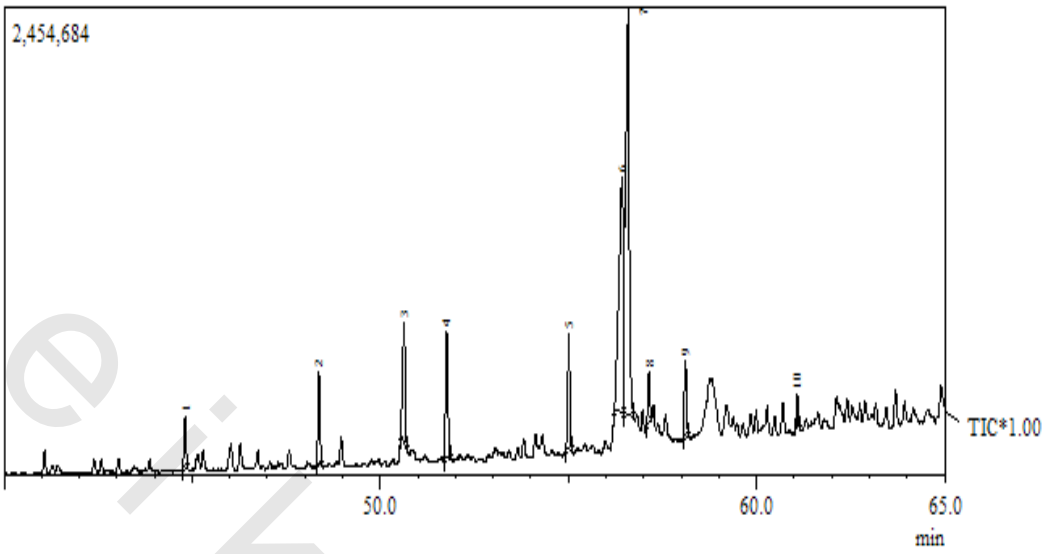


Figure 38: GC-MS chromatogram of *ziziphus spina-csisti* essential oils

Table 34: Essential oil composion of *ziziphus spina-csisti* by GC-MS analysis

Quatitative ID	Component identified	Retention time (min)	Area (%)
1	Hexandecane	44.81	3.92
2	Heptandecane	48.37	7.20
3	n-Hexandecanoic acid	50.63	6.39
4	Eicosane	51.77	8.94
5	Methyl hexadecanoate	55.01	9.85
6	1, E-11,Z-13- Octadecatriene	56.42	18.34
7	Geranyl acetone	56.59	20.62
8	Octadecadienoic acid	57.14	2.16
9	Tetracosane	58.11	5.13
10	Docosane	61.08	2.94

The essential oil was effective against wide spectrum of microbes. These may be due to the presence of multiple components in the volatile oil. It can be optimistically concluded that the essential oil may be used in variety of effective pharmaceutical formulations to treat various chronic diseases.