EFFICACY OF COMMON EDIBLE OILS TO CONTROL ASCOCHYTA BLIGHT IN LEGUMES

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EFFICACY OF COMMON EDIBLE OILS TO CONTROL ASCOCHYTA BLIGHT IN LEGUMES

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MOLECULAR PLANT PATHOLOGY

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So, which of the bounties of your Lord will you Deny?

Dedicated to

My Family

Mother, Father, Uncle, siblings and Grandparents for their encouragement, teachings and support.

My Teachers

A great Journey with many of cooperative resource persons for their assistance, guideline and supervison.

My Friends

Sweet and Caring fellows without their moral support and help this journey would be impossible.

DECLARATION

I hereby declare that the work presented in the following thesis is my own effort and the material contained in this thesis is original. I have not previously presented any part of this work elsewhere for any other degree.

Qurat ul Ain

PLAGIRISM CERTIFICATE

It is certified that **Ms. Qurat ul Ain** (Registration No. **02041613013**) has submitted her M.Phil. dissertation entitled **"Efficacy of Common Edible Oils to Control Ascochyta Blight in Legumes"** and it has been checked on Turnitin for similarity index (plagiarism). Thesis plagiarism has been found to be 14 % that lies in the limit provided by HEC (19%).

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LIST OF ABBREVIATIONS

%	Percentage
MEGA 7	Molecular evolutionary genetics analysis 7
PDA	Potato dextrose agar
G	Gram
Ml	Milliliter
µl	Microliter
PCR	Polymerase chain reaction
CTAB	Cetyltrimethyl ammonium bromide
DNA	Deoxyribonucleic acid

dNTP	Deoxynucleotide triphosphate
Taq Pol	Thermus aquaticus polymerase
°C	Centigrade
NARC	National agriculture research centre
Mm	Millimeter
Na_2CO_3	Sodium carbonate
NaOH	Sodium hydroxide
Na-K Tartrate	Sodium potassium tartrate
µg/g	Microgram per gram
mg/g	Milligram per gram
Cm	Centimeter
ITS	Internal transcribed spacer
sp.	Species
NCBI	National centre for biotechnology information
BLAST	Basic local alignment search tool
FTIR	Fourier Transform Infra Red Spectroscopy
USA	United States of America
f.sp	Forma specialis
BCMV	Bean Common Mosaic Virus
CMV	Cuccumber Mosaic Virus

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ABSTRACT

ABSTRACT

Kidney Bean, chickpea and pea are the most cultivated legumes worldwide. Their yield loss factors include fungal attack along with other biotic and abiotic issues. In this study, we have evaluated 15 different edible oils to control Ascochyta blight disease of these crops. Our results revealed different potential of edible oils to control and suppress disease and this activity was related to the functional groups present in them. Fourier Transform Infra-Red Spectroscopy (FTIR) was performed to evaluate the functional groups present in 15 different edible oils, used to check antifungal activity against *Aschochyta rabiei*. FTIR results demonstrated that the resistant oils contain nitrogen containing compounds (Amides, Alkaloids) in greater proportion than susceptible oils and these are responsible for their antifungal activity. Edible oils were tested both *in vitro* and *in vivo* for antifungal activity and the investigation showed that *Ricinus communis, Triticum aestivum, Sesamum indicum* and *Papaver somniferm* oils were efficiently controlling and suppressing disease. Remaining oils did not show antifungal activity to that extent, both *in vivo* and *in vitro*. Analyses of biochemical and physiological parameters helped us to evaluate the antifungal activity *in vivo*. Application of useful oils increased sugar content, chlorophyll content and relative water content in plants. This increase might have helped plants to alleviate stress level of the plants and they survived better than control. Our study proves edible oils to be one of the best environment friendly solutions of Ascochyta blight disease in pulses.

INTRODUCTION

INTRODUCTION

1.1. Kidney Bean

Kidney bean (*Phaseolus vulgaris*); a plant with terrestrial habitat is one of the grain legumes which are cultivated worldwide. There are different species of *Phaseolus* genus and among those *P vulgaris* makes up to 90% of the cultivated crops (SP Singh 1989). Botanically, the Kidney bean is included in legumes. All the legumes have nodules in their roots which contain nitrogen fixing bacteria. Plant is diploid with 22 chromosomes (2n). The Kidney bean belongs to the genus *Phaseolus* of Fabaceae family; a well-known genus including 150 species. Fabaceae family includes 19,400 species, including plants of economic importance such as chickpea, lentils, pea, *Acacia nilotica* and peanuts. The complete classification of Kidney bean is given in Table 1.

1.1.1. Morphology

The stems of kidney bean plants are branched, erect or twining. Color of stem and leaves varies from dark green to green. Leaves are compound, stipulate and arrangement of leaves is alternate and edges of leaf blade are entire without any teeth or lobes. Flowers are few to many, bilaterally symmetrical with racemose inflorescence and flower color varies from purple to blue or white (RMA Nassar *et al.,* 2010). There are four or five tapels or petals. Stamens are 10 in number. Fruit is dry but does not split open when ripe. It is a self-pollinating crop plant. However, they can hybridize with each other and produce fertile off springs (Singh *et al.,* 1991).

1.1.2. Economic and health importance of Kidney Bean:

Approximately, 10 million tons of kidney beans are cultivated worldwide over 13 million hectares (Honduras 1995). Kidney beans are highly nutritious containing high carbohydrate content, ash content and crude fiber content. Protein extraction studies show that out of total protein content in kidney beans, globulin polypeptides are the major polypeptides (Sai-Ut *et al.,* 2009). Kidney bean seeds also have lectin protein with significant antifungal and antiviral characteristics (Ye *et al.,* 2001). Kidney bean seeds also contain protease inhibitors that inhibit

the proteases of pathogens and play a significant role in plant defense. One such protein named *Colletotrichum lindemuthianum* protease inhibitor has been isolated and extracted from seeds of kidney beans (Mosolov *et al.,* 1979). It has been found that kidney beans are rich in manganese which have antioxidant properties and help to lower the chances of Cancer.

Beans are a great source of vitamin K that helps in brain nervous functioning by strengthening the neurons. Fibers present in kidney beans regularize the bowel movements and increase the healthy bacteria in our intestine. This reduces the chances of Colon cancer and reduces the cholesterol level and in this way the risk of cardiovascular disorders is minimized. Iron content is very high in kidney beans and this iron content helps to boost up energy levels and increases metabolism of body. Manganese and Calcium also help in bone strengthening and healthy body. Kidney beans also contain Fibernya and it helps prevent cholesterol formation in Liver. In this way bad cholesterol is prevented. Kidney beans are rich sources of antioxidants having anti-aging effects.

1.1.3. Pathogens of Kidney Beans:

Pathogens of Kidney Bean include Viruses, Bacteria, Fungi and Parasitic nematodes.

a. Bacterial diseases of Kidney Bean:

Several pathogenic bacteria affect the growth and vigor of this crop thereby reducing the yield worldwide. Among the significant diseases, bacterial brown spot is one of the devastating diseases caused by *Pseudomonas syringae pv. Syringae*. Main symptoms of this disease include small oval necrotic lesions on leaves and puckering of leaf tissue along the lesion. Pods also show necrotic lesions. The pathogen survives in the bean soil till April (Hoitink *et al.,* 1968). Pathogen survives as an epiphyte on leaf surfaces. Bacterial wilt is also a disease but it is of less importance and uncommon. It is caused by Bacteria *Xanthomonas campestris pv phaseoli* and is seed borne and overwinter in plant debris. This bacteria is gram negative and aerobic in nature having motile flagella (Saettler, 1989). Halo blight is another disease caused by bacteria *Pseudomonas syringae pv. phaseolicola.* This disease has done several economic losses and symptoms includes appearance of water soaked spots on lower surface of leaves and later on, yellow halo develops around the lesion. Pod lesions are also seen. Contaminated seeds are the source of spread of this bacterium (Grogan and Kimble, 1967).

b. Fungal diseases of Kidney Bean:

Fungi cause different diseases in kidney Beans and several species of fungi are responsible for yield losses in kidney beans. One of them is Alternaria leaf spot caused by fungus *Alternaria alternata.* This fungal disease is prevalent in USA and UK causing severe destruction of crops (Kareem, 2007). Symptoms appear in the form of lesions that are brown in color and don't cross over major veins. Different lesions collapse and large area of leaf become necrotic.

Another very important disease of tropics and subtropics is angular leaf spot that can cause up to 80% yield loss. This disease is caused by *Isariopsis griseola* which requires abundant but fluctuating moisture conditions along with moderate temperature to induce this disease (Schwartz *et al.,* 1982). This disease is prevalent in Brazil which is larger consumer and producer of Kidney Beans but it also occurs in Ethiopia, Kenya, Uganda, Central America and Mexico (Pastor- Corrales *et al.,* 1989). Plants are characterized by angular spots on the leaves. These lesions are initially tannish- grey but later on they become brownish black.

Powdery Mildew is disease which is caused by fungal pathogen named *Erysphie polygoni*. All above ground parts may be affected. White powdery spots are observed over the leaves and leaves may become dwarf followed by yellowing and abscission. Pathogenic fungus is responsible for another disease named Anthracnose in Kidney beans. Causative agent of this disease is *Colletotrichum lindemuthianum*. Up to 100% yield losses have been reported due to this disease (Schwartz *et al.,* 1982). The species from Colletotrichum genus are responsible for causing different diseases on a wide variety of plants (Bailey *et al.,* 1990). Fungus is distributed worldwide causing diseases in many tropical and temperate regions (). There are 41 races of this fungus (Mosolov *et al.,* 1979). Brick red to dark brown lesion is seen on the affected area. Fungus source of survival are debris of infected plants and seeds. Another devastating disease causing losses up to 100% is Rust; occurring and infecting worldwide. Rains having pH 3.2 have effects on the development of Rust causing fungus; hence disease cycle is slightly delayed (Shriner, 1978). Rust is caused by fungus *Uromyces phaseoli*.

Fusarium root rot of kidney beans is caused by *Fusarium solani f.sp phaseoli*. Pathogen is spread by irrigation water and transferred from field to field. Reported yield loses are up to 50 % (Dar *et al.,* 1997). Environmental conditions have a great effect on disease severity and it increases by

stress (KA Cichy *et al.*, 2007). Another fungal disease is white mold and is caused by *Sclerotinia sclerotium*. White mycelial growth and black sclerotia are seen on the infected part. This may be a soil borne or air borne fungus depending upon species. Infection starts by means of ascospores (Natti, 1971). Crop yield reduces up to 100% by this fungus (Mortan *et al.*, 1989). Root rot is also caused by fungus named Pythium and called as Pythium root rot. This specie is distributed worldwide and reduce yield (Nazungize and Lymugabe, 2012). Aschochyta blight is a disease caused by fungus *Aschochyta rabiei*. This disease also accounts for yield losses.

c. Viral diseases of Kidney bean:

There are several viral species that cause different ailments in plants resulting in yield loss. Common mosaic is a disease that is responsible for the yield loss up to 98 %. Virus responsible for this disease is Bean Common Mosaic Virus (BCMV). This virus is transmitted through infected seeds and by aphids. In seeds virus is confined to embryo (Fajardo, 1930). Early infected plants are yellow and dwarfed. Curly top disease is a viral disease prevalent in western United States, British Columbia and Canada. Downward curling and death of leaves are the typical symptoms of this disease. Curly top disease is caused by *Ruga verrucosous* virus. Stunted pods are also included in symptoms. Golden mosaic is a viral disease of kidney bean caused by Bean Golden Mosaic Virus which belongs to the genus Begomovirus and family Geminiviridae (Bonfim *et al.*, 2007). Yield loses are reported up to 100% by this disease. Mode of transmission of this virus is sweet potato white fly and silver fly (Valez *et al.*, 1998). Crop rotation is one of the prevention methods.

d. Diseases caused by Nematodes:

Nematodes also infect the bean and account for yield losses. Root rot nematodes attack the beans and up to 90% yield losses are reported. Symptoms include yellowing, stunting, wilting and leaf edge necrosis. Diseased roots are shortened and thickened and root system is reduced. Root rot is caused by nematodes species belonging to the genus Meloidogyne. These species are distributed worldwide. Knots and galls are formed on the root.

1.2. Pea

Pea (*Pisum sativum*) is one of the oldest and important crop grown worldwide. About 84 countries are growing pea, at present (Roy *et al.,* 2010). Botanically, pea is included in legumes. All the legumes have nodules in their roots which contain nitrogen fixing bacteria. Plant is diploid with 14 chromosomes (2n). The chickpea belongs to the Fabaceae family, which includes 19,400 species, including plants of economic importance such as beans, lentils, chickpea, *Acacia nilotica* and peanuts. The complete classification of pea is given in table 3.

1.2.1. Morphology

Pisum sativum is herbaceous annual plant. The stems of pea plants are low growing or vining. Color of stem and leaves varies from dark green to green. Leaves are pinnately compound, stipulate and edges of leaf blades are entire or dentate. Flowers are few to many, flower color varies from white to reddish purple and peduncle is ½ to twice the length of stipules. Flowers are 1-3 in number with calyx 8-15 mm long. Fruit is pod and split open when ripe. Stamens are diadelphous, 9 united, 1 free. It is a self-pollinating plant.

1.2.2. Economic and Health importance of pea

Pea is a cultivated legume and in terms of annual production, it is ranked fifth and accounts for 35-40 % of total trade in pulses (Ratnayake *et al.*, 2001). There is a variation in the production of this crop in different regions of the world and India is contributing 27% of the world pulses (Kelley *et al.,* 1997). Pea seeds are a source of carbohydrates, proteins, vitamins and minerals. Carbohydrates are mainly present in the form of starch (Dahl *et al.,* 2012). Green pea is low in sodium and this is good for health. A great health benefit of pulses including pea for diabetic patients is to reduce glycemic index which adds to good health. Pea seeds are good source of soluble fibers and high intake of soluble fibers results in low cholesterol level. This in turn reduces the risk of colon cancer and heart attack (Sharma and Kawatra, 1995). Pea is included in low cost health substitute for different ailments (Marinangeli *et al.,* 2009). The risk of CVD and diabetes type 2 is reduced by whole pea flour (Marinangeli *et al.,* 2011). High nutritional values of legumes including pea plays vital role to decrease macronutrient malnutrition (Parihar *et al.,* 2016). Human carbohydrate metabolism is less affected by intake of leguminous seeds (Seewi *et al.,* 1999). Pea seeds contain high level of poly phenols which exhibit strong antioxidant

properties (Azarpazhooh & Boye, 2012). Cooked peas contain glacto oligosaccharides (Brummer *et al.,* 2015).

1.2.3. Diseases of pea

There are different diseases of pea which can be divided into two categories.

- Diseases caused by abiotic factors
- Diseases caused by biotic factors/ pathogens.

1.2.3.1. Diseases caused by abiotic factors

Abiotic factors such as heavy metal contamination, freezing and hailing can cause injuries and help to affect the vigor of the plant. Intra cellular ice formation is responsible for freeze injury (Vertucci, 1989). Freezing injury is predominant in northern latitudes and responsible for yield loss. Cold temperatures below $0^{\circ}C$ are responsible for freeze injury. Hail injury also adds to the yield loss to some extent in quality loss. Purple blight in pea plant is caused mainly due to manganese toxicity. Manganese is a micronutrient and required in small quantities for proper functioning of enzymes and act as a cofactor. Its excess quantity results in low relative growth rate (RGR) as it has major effect on photosynthesis (Doncheva *et al.,* 2005). Chlorosis is one of major symptom of this disease (Warington, 1954).

1.2.3.2. Diseases caused by pathogen

Pathogens of pea include bacteria, fungi and viruses.

a. Diseases caused by bacteria:

Bacteria is the causative agent of different diseases among them the most important is bacterial blight caused by *Pseudomonas syringae pv. Pisi*. This disease was first found in USA in early 20^{th} century (Wallace & MM Wallace, 1951). Plants carry this bacteria in dormant form when exposed to low temperatures, naturally or artificially, show the symptoms of blight (Wark, 1954). Common symptoms include lesions on all above ground parts with water soaked appearance, infecting leaves stipules and pods etc.

b. Diseases caused by Fungi:

Fungi are responsible for different diseases of Pea because of airborne spores and easy spread. Anthracnose is a common disease of pea and it is caused by *Colletotrichum pisi*. Its common symptoms are necrotic lesions having depression at centre that appear on first stem, branches and then appear on pods and leaves. This fungus reproduces by Conidia (Tivoli *et al,* 2006). Anthracnose was first appeared on pea crops in 1972 (Hadjedorn, 1974). The fungus responsible for this disease belongs to Colletotrichum genus and mostly the specie of this genus produce appresoria (Podila *et al.,* 1993). Most of the species have airborne dispersal (Buchwaldt *et al.,* 1996). This pathogen overwinters in plant debris (Gibson, 1994). Fungus responsible for another disease Septoria Blotch is Septoria sp. This fungus sporulates heavily on the residue after tillage (Bailey *et al.,* 2001). Yellow disease lesions are the common symptoms. These lesions are different in size and have no specific boundary.

Downy mildew of pea is caused by a fungus specie *Peronospora pisi* (Hickey and MD Coffey, 1977). When flowering occurs, the infection rate becomes high in upper leaves and this is the major yield reducing factor. Downy mildew is a fungal disease of pea in cool regions (Ivanovic *et al.,* 2000). Mildew and Anrthracnose are yield reducing diseases (Ramya, 2017). Gray mold is caused by fungal specie *Botrytis cinerea* (Adsule, 1998). This fungus affects aerial parts of the plants (Nelson and Powelso, 1988). Fungicides are used to control this disease. Important fungicide is Vinclozolin (Szeto *et al.,* 1989). This disease is included in regionally important diseases (Redden *et al.,* 2005). Pythium tip blight is caused by *Pythium* spp. and affects axil or bud of young leaf (Hare 1949). Zoospores are formed by this fungus (Royle *et al.,* 1964). Overhead irrigation is also a factor triggering the disease and increment in disease rate (Adegbola and Hadgedom-Red 1970). Depending upon the severity of infection, death of plant may occur (Schroeder, 1953). The major symptoms are blight which further results in necrosis.

Another significant disease of pea is Aphanomyces root rot and some species of Brassica family are used as soil amendments to reduce disease incidence (Muhlchen *et al.,* 1990). Fusarium root rot is another disease caused by *Fusarium solani* fsp pissi (Sen and Majumdar 1970). At conditions when root growth is decreased, the Fusarium root rot is increased (Kraft 1996). Stress conditions also add to the increased disease incidence. (Leath and Byers, 1977). Resistant cultivars are not found against this disease (Grunwald *et al.,* 2003). Wilting is one of the

important symptoms of root rot but Pythium species are responsible for wilting and affect the yield collectively (Kerr, 1963). Aschochyta blight is another disease and this also accounts for the yield loss in different regions of the world (Timmerman-Vaughan *et al.,* 2002). Aschochyta blight is caused by fungus which belongs to the Aschochyta genus and is a serious disease worldwide (Skoglund, 2011). The fungus is soil borne and survives in soil, infected seeds and debris and these all are the sources of inoculums (Bretag *et al.,* 2006).

c. Diseases caused by virus:

Another important pathogen and responsible for yield loss in pea crop is virus. Enation mosaic virus (EMV) causes the disease called Enation Mosaic and this virus transmits either mechanically or by aphids (De Zoeten *et al.,* 1972). The virus is hexagonal in shape and multiplies in nucleus (Hull and Lane, 1973). Blister like outgrowths are found on underside of foliage and pods as a symptom of diseased plant and premature death occurs. Mosaic disease caused by pea common mosaic virus is mostly transmitted through aphids (Doolittle and Jones, 1925). Mottling of leaves and vein clearing is a specific symptom (Hull, 1965). Streak disease of pea is caused by some strains of Alfalfa Mosaic Virus (AMV) and Wisconsin streak virus (Zaumeyer 1938). Histological studies show that the virus is located near the nucleus and cell wall in the infected cell (Boss and Huertoes, 1972). Symptoms include necrotic streaking of stem and petioles and browning of nodes. A very rough condition of leaves and nodes is observed as a symptom (Hadgedorn and Walker 1949).

1.3. Chickpea

Chickpea (*Cicer arietinum*) is one of the most important grain legume and its seed is a major source of plant based dietary protein for humans (Y.T.Gan *et al.,*2005). Botanically, chickpea is included in legumes. All the legumes have nodules in their roots which contain nitrogen fixing bacteria. Plant is diploid with 16 chromosomes (2n). The chickpea belongs to Fabaceae family, which includes 19,400 species , including plants of economic importance such as beans, lentils, pea, *Acacia nilotica* and peanuts. The complete classification of chickpea is given in Table 1.

1.3.1. Morphology

The stems of chickpea are branched, erect or spreading, sometimes the stems are much shrubby, branched and 0.2-1m tall. Colour of stem and leaves varies from green to bluish green. Root system of chickpea is robust, up to 2m deep but major portion may be up to 60 cm. Leaves are imparipinnate, glandular and pubescent with 3-8 pairs of leaflets, which are ovate to elliptic. Flowers are solitary, 2 in one inflorescence and axillary in nature, peduncles 0.6-3 cm long, pedicels 0.5-1.3 cm long, bracteate. The staminal column is diadelphous and the ovary is sessile, inflated and pubescent (Duke, 1981; Cubero, 1987; Vander Maesen 1987). There are 3 maximum seeds per pod and seed colour varies from cream yellow to black. It is a self-pollinated crop plant.

1.3.2. Economic and health importance of chickpea

Chickpea ranks third among the pulse crops accounting for 10.1 million tons annual production, worldwide (Muehlbauer and Ashutosh Sarker, 2017). The major producers include North America, Canada and areas within the Asia (India, Pakistan) and Middle East. It is believed to have originated from Middle East, approximately 7450 years ago (Maitte & Wesche- Ebiling, 2001). In 2003 India was both the leading producer (123 thousand metric tons) and importer (83 thousand metric tons) of chickpea.

There are two distinct types of chickpea, Desi and Kabuli type. Chickpea is a highly nutritious and an inexpensive source of protein that is estimated at 24% and ranges from 15 to 30% (Hulse 1994). Chickpea has 60–65% carbohydrates, 6% fat and is a good source of minerals and essential B vitamins (Muehlbauer and Sarker, 2017). Chickpea seeds are used in the preparation of different dishes, salads and fermented foods, worldwide. Chickpea ranks third among the pulse crops and accounts for 10.1 million tons yield, annually. This ranking places chickpea behind beans (21.5 million tons) and peas (10.4 million tons). (Muehlbauer and Ashutosh Sarker, 2017). Chickpea cannot be considered as an oilseed crop since its oil content is relatively low (3·8–10%) (24- 60 %) in comparison with other important oilseed pulses such as soyabean or groundnut. However, chickpea oil has medicinal and nutritionally important tocopherols, sterols and tocotrienols (Jukanti *et al.,* 2012).

1.3.3. Pathogens of chickpea

Pathogens of chickpea include Fungi, viruses and bacteria.

a. Bacterial diseases of chickpea

There are number of factors that affect the yield of chickpea, annually. Among those are diseases caused by different pathogens and one of them is bacteria causing bacterial blight, leaf spots and stem cankers. These diseases are caused by two bacterial species, *Pseudomonas syringae* and *Xanthomonas campestris pv cajani* (Nene *etal*., 1996). These bacteria play a role in yield loses of this crop.

b. Fungal diseases of chickpea

Many fungal pathogens attack chickpea in different seasons and destroy crop vigor and result in yield losses. Botrytis Grey Mold is one of the important fungal diseases and causes up to 100% yield loses. This disease has been reported in Bangladesh, India, Nepal, Pakistan, Australia and Argentina. Botrytis Grey Mold is also found in Canada, Chile, Colombia, Hungary, Mexico, Myanmar, Spain, Turkey, the USA and Vietnam. Fungal specie *Botrytis cinerea Pers. ex Fr*. is responsible for this drastic disease and is seed borne in nature having wide host range and affects stem, leaves and pods (Nene *et al.,* 2012). Another fungal disease occurring and affecting pods and flowers is Alternaria blight caused by fungal pathogen *Alternaria alternata*. Infected flowers die and infected pods become dirty black in colour (Nene *et al.,* 2012). This blight is mostly present in some parts of Bangladesh and India.

Another soil and seed borne disease is Colletotrichum blight and it is caused by *Colletotrichum dematium*. This disease kills the plants at any stage depending upon weather and amount of inoculum, Phoma blight is caused by *Phoma medicaginis* and its symptoms are irregular, light brown lesions on the leaves, while stem and pods have dark margins. Pycnidia are dark, minute and submerged irregularly scattered in the infected tissue. Discolored seeds are present in infected pods (Nene *et al.,* 2012).

Sclerotinia stem rot has been reported from most of the chickpea growing regions of the world and is caused by *Sclerotinia sclerotiorum*. This fungus has a wide host range and affects 500 different plant species (Sarma *et al.,* 2006). *Fusarium oxysporum f.sp. ciceri* affects chickpea and causes disease named as wilting and causes 70-100 % yield loses in different regions of the world. This fungus is soil borne and survives in soil in the form of chlamydospores and affects the host by spreading through soil and infected seeds (Haware *et al.,* 1978). Disease incidence

varies from 14-32 % in India and up to 100% yield loses have also been recorded (Gopalakrishnan *et al.,* 2011).

Verticellum wilt is caused by fungal species *Verticillium albo-atrum Reinke & Berthier* and is similar to Fusarium wilt in symptoms and weather conditions. Wet root rot is caused by *Rhizoctonia solani* and its control mainly lies on fungicides (Dubey *et al.,* 2011). Ascochyta blight is a major disease in west Asia, northern Africa, and southern Europe. Disease incidence takes place in February and March in Pakistan and northern India because at that time, the temperatures are low and favorable for pathogen growth and crop canopy is dense. This disease is caused by *Aschochyta rabiei* and causes up to 100% grain loss (Nene *et al.,* 2012).

c. Viral diseases of chickpea

Viruses are also responsible for different diseases and yield loses of Chickpea across the world. Stunting is one of the most devastating diseases in Chickpea and is caused by leaf roll virus. This virus is transmitted through aphids which may be migrating in nature. The major symptoms are reduced growth and reddening of leaves (Horne 1994). Systemic infection occurs in phloem and browning is observed (Nene *et al.,* 2012). Mosaic is another important disease of Chickpea and is caused by Alfalfa Mosaic Virus (AMV). This virus has a wide geographical distribution and host range infecting 305 species in 47 plant families (Hull 1969). Major hosts are Tobacco, Alfalfa, Chickpea and French bean. Symptoms vary from specie to specie. Proliferation is another viral disease of Chickpea caused by Cucumber Mosaic Virus which belongs to the family Bromoviridae. CMV is transmitted by more than 80 different species of Aphids. This virus is seed borne and is able to survive in dry summers in dormant seeds (Jones and Coutts 1996). Characteristic symptoms are bushy and stunted plants and these are similar to Poty virus (Nene *et al.,* 2012). Narrow leaf (a viral disease of Chickpea) is caused by Bean yellow mosaic Virus and is reported in India, Iran and USA. At present, some of Chickpea varieties are resistant to this disease (MicKirdy *et al.,* 2000).

1.4. *Ascochyta rabiei:*

Ascochyta rabiei occurs worldwide and belongs to Ascomycetes. This fungus reproduces by ascospores and high moisture and low temperature is required for the growth and proliferation of this fungus. This fungus is heterothallic (Reddy and Kabbabeh, 1985). A foliar pathogen,

A.rabiei grows at optimal temperature of 15- 20 ^{0}C with upper limit of temperature at 30^{0}C (Kaiser, 1973). Solanopyrones are produced by this fungus which are phytotoxic in nature (Höhl *et al.,* 1991). Symptoms include spots on leaves and stem and then these spots develop into necrotic spots because of cell death (Höhl *et al.,* 1990).

1.4.1. Host species of *Ascochyta rabiei:*

This fungus is responsible for disease on different legumes including chickpea, kidney beans, lentils, Garden pea and many other food legumes. Disease caused by this fungus is called blight (Kaiser *et al,* 1997). This pathogen is highly variable and undergoes recombination at sexual level so it plays a key role in reducing yield loss (Gan *et al.,* 2006).

1.4.2. Blight disesase:

Blight disease is responsible for yield losses in different crops, particularly Chickpea. Pathogen is distributed worldwide and survives in infected chickpea (Trapero-casas and Kaiser 1992). Temperature and relative humidity affects growth rate of fungal spores and spread blight. High relative humidity (86-100%) retards the growth of spores (Navas-Cortés *et al.,* 1998).

1.5. Biofungicides

Fungicides from biological origin are termed as biofungicides e.g. microorganisms, plant extracts and secondary metabolites (Alabouvette et al., 2006). This approach is realistic with minimum adverse effects and many scientists are focusing on phyto-extracts and microbes, now a day (Mansour and El-Sharkawy, 2014). Alternative way is a chemical control characterized as best control as it works by using synthetic pesticides (Kishore and Pande, 2005). Side effects of synthetic chemical pesticides are very adverse to environment by affecting food chain and human health by causing serious ailments (Ho et al., 2007). Therefore, an alternative method to control the diseases with little or no side effects is need of the hour. After sometime pathogen become resistant to chemical pesticides. Fungicides from biological origin are biodegradable in nature, non-target species are not affected, economically feasible compared with chemicals,

toxicity level is low and less time consuming. Numerous biological materials are used as fungicides including plant extracts, microbes and plant oils.

1.5.1. Fungicidal properties of plant oils:

Essential oils extracted from plants have shown remarkable fungicidal properties (Daferera et al., 2000). Antimicrobial activity is associated with many essential oils (Maruzzella, 1963). Many fungal and bacterial species can be controlled by essential oils. A rapid assay has been evidenced to determine antifungal activity in both plant extracts and essential oils (Golan, 2001). Thyme oil is responsible for hyphal growth of fungus (Arras and Piga, 1994). Previous work of various researchers have shown that plants oils and extracts have antagonistic effect against fungal diseases (Kandasamy et al., 1974; Hale & Mathers, 1977; Rahber-Bhatti, 1988; Kalo and Taniguchi, 1987). Essential oils are organic fungicides but they also have some side effects but less than synthetic chemicals. If misused they can also kill beneficial bugs and skin or eye irritation may be caused. So, one should be cautious enough while using these products (Calvert and Chalker Scott, 2014).

1.5.2. Health importance of Fungicides from Biological origin:

To cope with the hazardous effects of chemicals, bio-fungicides serve as the best substitutes. Ecosystem and food chain is badly affected by chemical pesticides and fungicides and bio fungicides are ecofriendly, in this regard. Biofungicides are biodegradable and they are very less contributor of environmental pollution (Patil *et al*., 2010).

1.6. Edible oils:

Aromatic oils are of considerable importance for their antimicrobial properties from many past decades. Carvacol and thymol are the phenolic compounds responsible for the antimicrobial activity of Origanum oil, used commonly as a food flavouring agent. (Knobloch *et al.,* 1989). This oil has been effective against some of the fungal strains spoiling food and some other strains like Aspergillus (Barrata et al., 1998; Tantaoui-Elaraki and Beraoud, 1994). Antimicrobial agents are proved effective against food spoilage but there are some exceptions too. Chemicals are being shifted to natural and biological additives to food as they have many advantages (Avila-

Sosa et al., 2009). Volatile compounds are present in oreganum oil and antimicrobial activity is due to these phenols (Skandamis and Nychas, 2000).

1.6.1. Advantages of Edible oils as fungicides:

Pesticides are synthetic chemicals and they pose serious problems on economy, health, biodiversity and environment (Paster and Bullerman, 1988). Due to all these drawbacks of synthetic chemicals, scientists are working to find safer alternatives. Natural pesticides having biological rather than synthetic origin formed and preferred this way because of some positive points. These protective agents have low toxicity rates and can produce best results without causing environmental pollution (Don- Pedro, 1996; Hamilton-Kemp et al., 2000; Liu and Ho, 1999; Paranagama et al., 2003; Paster et al., 1995). Complex volatile compounds called essential oils produced in different plant parts have many essential functions and they have antimicrobial activity (Goubran and Holmes, 1993). Antimicrobial potential of essential oils and complicated nature is due to terpenehydrocarbons as well as their oxygenated derivatives, such as alcohols, aldehydes, ketones, acids and esters (Wijesekara et al., 1997).

1.7. Fourier Transform Infra-Red spectroscopy:

Over the past decade, the application of this technique expanded in food research and particularly has become a powerful analytical instrument in the field of edible oils and fatty tissues. FT-IR spectroscopy is a rapid, non-destructive technique with the minimum sample preparation requirement. It uses the quality of the compound and vibrations of atoms present in sample to evaluate functional groups.

1.8. Aims and Objectives of study:

The aims and objectives of this study are following;

- Analyses of different edible oils to explore their anti-fungal activity potential.
- Environment friendly control of epidemic diseases of economically important legume crops.
- Characterization of different edible oils on the basis of their functional group profiling.
- Comparative disease control analysis in three different crops.
- Recommendation of best disease control method to legume growers.

MATERIALS AND METHODS

MATERIALS AND METHODS

This study was conducted to evaluate the effects of different edible oils for the control of fungal infection of three legume crops. The experiment was accomplished by the following way;

2.1. *In vivo* antifungal activity analysis

Following methodology was adopted to see the antifungal effects of different edible oils in three legume crops.

2.1.1. Seed collection and sterilization

Healthy seeds of susceptible varieties of chickpea, pea and kidney bean were obtained from National Agricultural Research Centre, Islamabad Pakistan. For all crop plants namely Chickpea, Kidney bean and Pea, 150 seeds of each crop were sterilized with 0.1% sodium hypochlorite solution with continuous shaking. After surface sterilization the seeds were soaked overnight to make their seed coat softer.

2.1.2. Soil preparation

Soil and sand was collected, autoclaved and mixed in 3:1 ratio after sieving. This soil was used for the sowing, germination and plant growth purposes. Each pot was filled with approximately 200 g of soil.

2.1.3. Seed sowing and germination

Sterilized seeds were placed for germination in pots filled with soil. About 3 seeds per pot were placed and total 50 pots per crop were prepared. Three plants were assigned to each of 15 edible oils and remaining 5 plants were selected as control (2 with no treatment and 3 with fungus inoculation).

2.1.4. Czapek media formation

Czapek media was preapared by adding sucrose (30 g), sodium nitrate (2 g), disodium phosphate (1 g), magnesium sulphate (0.5 g), potassium chloride (0.5 g) and ferrous sulphate (0.01 g). Fungal spores were added and broth culture was placed in shaker for 3 days, at 30°C and 120 rpm. Spores were filtered by using Muslin fabric. For spore confirmation, culture was examined under light microscope. Concentration of spores was determined using hemocytometer.

Table 1.1. Treatments of edible oils

Sr. No.	Treatment	Name
1.	FC	Fungus control
2.	HC	Healthy control
3.	T1	*Cucurbita pepo* oil (field pumpkin)
4.	T2	*Phyllanthus emblica oil* (gooseberry)
5.	T3	Fish oil
6.	T4	Juglan regia oil (walnut oil)
7.	T5	*Papaver somniferum oil* (opium)
8.	T6	*Sesamum indicum oil* (sesame)
9.	T7	*Nigella sativa oil* (black cumin)
10.	T8	*Olea europaea oil* (olive oil)
11.	T9	*Triticum aestivum oil* (wheat oil)
12.	T10	*Prunus dulcis oil* (sweet Almond oil)
13.	T11	*Prunus arminiaca oil* (apricot oil)
14.	T12	*Linum usitatissimum oil* (flax oil)
15.	T13	*Ricinus communis oil* (castor oil)
16.	T14	*Raphanus sativus oil* (raddish oil)
17.	T15	*Cocos mucifera oil* (coconut oil)

2.1.5. Collection and dilution of edible oils

Fifteen different edible oils were purchased from Murree Karyana Store, Aabpara, Islamabad (Fig2.3). Emulsification of oils were done by mixing 0.5 ml oil and 49.5 ml of distilled water and centrifuged at 5000 rpm for 1 minute. The mixture was stored at room temperature and diluted further to desired concentration with distilled water before use.

2.1.6. Fungal inoculation on plants and oils application

Czepak media containing *A. rabei* spores was sprayed over plants and after 2 to 4 hours, oils were applied on all plants except fungus control and healthy control.(table 1.1) Visible symptoms were noticed after 6-7 days.

2.1.7. Physiological and Biochemical Parameters

After the emergence of true leaves, following biochemical and physiological parameters were studied. (Fig 2.1)

2.1.7.1 Sugar Content of Leaves

Sugar content of leaf was determined by following the method of Dube *et al*., (1956), with little modification (Johnsan *et al*., 1966). Fresh plant material of 0.1 g was homogenized in 2 ml of distilled water. Homogenized material was centrifuged at 3000 rpm for 5 minutes and 0.1 ml of supernatant was taken. In supernatant, 1 ml of phenol (80 % w/v) was added. The mixture was incubated for 1 hour at room temperature. After this, 5 ml of conc. sulfuric acid was added and samples were incubated for 4 hours. Absorbance of samples was measured at 420 nm using spectrophotometer. Distilled water was used as blank. Sugar contents were determined by using following formula:

Sugar content = k value x dilution factor x absorbance/ sample weight

k = 20

2.1.7.2. Protein Contents of Leaves

Leaves were used for determining protein contents, following the method of Ulmer *et al*., (1951). Leaf sample (0.1 g) was taken and ground in 1 ml of phosphate buffer with the help of mortar and pestle. Phosphate buffer was prepared by dissolving 2.76 g of dibasic sodium phosphate in 100 ml distilled water. This dibasic sodium phosphate (84 ml) was mixed with monobasic sodium phosphate (16 ml) and pH was adjusted to 7.5.

After grinding the leaf material with phosphate buffer, the mixture was centrifuged at 3000 rpm for 10 minutes and the supernatant (0.1 ml) was taken in a test tube. This step was followed by the addition of distilled water (0.9 ml), so as to make total volume of 1ml.

Other reagents were prepared as:

Reagent A: 2g of sodium carbonate (Na_2CO_3), 0.4 g of NaOH (0.1 N) and 1 g of Na-K. Tartrate was dissolved in 100 ml of distilled water.

Reagent B: $CuSO_4.5H_2O$ (0.5 g) was dissolved in 100 ml of distilled water in 1:1 ratio.

Reagent C: Reagent A (50 ml) and Reagent B (1 ml) were mixed together.

Reagent D: Folin phenol reagent was diluted with distilled water in 1:1 ratio.

Reagent C (1 ml) was added and shaken for 10 minutes, followed by the addition of Reagent D (0.1 ml). Mixture was incubated at room temperature for 30 minutes. The absorbance of each sample was recorded at 650 nm. The concentration of protein content was determined. Protein contents were calculated using the following formula:

$$\text{Protein content} = k \times \text{dilution factor} \times \text{absorbance/ sample weight}$$

k = 17.52

2.1.7.3. Proline Content of Leaves

Estimation of proline contents of leaves was carried out by the procedure of Bates *et al*., (1973). Fresh plant material (0.1 g) was grinded using 4 ml aqueous solution of sulfosalicylic acid (3 %). Samples were centrifuged at 3000 rpm for 5 minutes and 2 ml supernatant was taken. Then, 2 ml of acidic ninhydrin solution was added which was prepared by dissolving 1.25 g of ninhydrin in 30 ml of glacial acetic acid and 20 ml of 6 M phosphoric acid with continuous stirring until dissolved. Samples were placed in water bath for 1 hour at 100 °C and allowed to cool. Mixture was extracted with 4 ml of toluene with vigorous mixing. Absorbance was observed at 520 nm

wavelength, using toluene as a blank. Proline contents were determined by the following formula:

$$\text{Proline contents} = k \times \text{dilution factor} \times \text{absorbance/ sample weight}$$

k = 19.6

2.1.7.4. Determination of Chlorophyll *a, b* and Carotenoid Contents of Leaves

Method of Arnon, (1949) was used for the determination of chlorophyll and carotenoid contents of leaves. For this purpose, 0.3 g of fresh plant material was used and homogenized in 1 ml of 80% acetone and acetone level was raised to 5 ml. Then the mixture was centrifuged at 3000 rpm for 10 minutes. Absorbance of the supernatant was measured at 645 nm (chlorophyll a), 663 nm (chlorophyll b) and 480 nm (carotenoids) on spectrophotometer. As a blank, 80% acetone was used. Total chlorophyll content was determined using the following;

Chl *a* (mg/g) = [12.7 (OD663)-2.69(OD645)] ×V/1000×W

Chl *b* (mg/g) = [22.9 (OD645)-4.68(OD663)] ×V/1000×W

where V = Volume of the extract (ml), W = Weight of fresh leaf tissue (g)

Carotenoids (mg/g): (OD at 480) x 4

2.1.7.5. Shoot Length

Lengths of freshly harvested shoots were measured using measuring tape.

2.1.7.6. Root Length

Lengths of freshly harvested roots were measured using measuring tape.

2.1.7.7. Fresh and Dry Weight of Shoots

Fresh weights of shoots were recorded by using weighing balance. Then the samples were kept in an oven at 70ºC for 24 hours. Dry weight of shoot samples were taken after 72 hours.

2.1.7.8. Fresh and Dry Weight of Roots

Fresh weights of roots were recorded by using weighing balance. Then the samples were kept in an oven at 70ºC for 24 hours. Dry weight of root samples were taken after 72 hours.

2.1.7.9. Relative Water Contents of Leaves

Leaf relative water content (LRWC) was estimated for fresh leaves by determining the turgid weight of fresh leaf samples, followed by drying in hot air oven till constant weight (Whetherley, 1950). About 0.5 g fresh leaf (FW) was taken and placed in petri plate filled with distilled water, overnight in dark. The leaf turgid weight (TW) was determined by using sensitive weighing balance. The leaf was placed in an oven at 72^{o}C for overnight and the dry weight (DW) was determined.

LRWC was calculated by the following formula;

$$RWC\ (\%) = [(FW-DW) / (TW-DW)] \times 100$$

2.2. *In vitro* antifungal activity analysis

In vitro, antifungal profiling of different edible oils was performed by the following way;

2.2.1. Collection and growth of fungus

The identified fungus strain *Ascochyta rabiei* was obtained from National Agriculture Research Centre, Islamabad, Pakistan

Fungus was grown on PDA media which was prepared by boiling 200g of potatoes in one liter of water until the starch solution becomes ready. Then it was filtered through muslin cloth. The filtrate was subjected to the addition of dextrose (20g) and agar (15g). This mixture was autoclaved for 15 min at 121ºC. The resultant molten media was poured into autoclaved Petri plates under sterilized conditions in Laminar flow hood and allowed to solidify. Streptomycin was added as an antibacterial agent. The fungus spores were cultured on media by using

sterilized spatula or forceps and plates are covered with Parafilm and incubated at 25ºC for 2 days.

2.2.2. Identification of fungus

Fungal strain was identified by morphology, microscopy and further by sequencing.

2.2.2.1. Morphological Identification of fungus

Morphological identification was done by observing fungal growth on PDA media. The petri plate was observed from both sides and characteristic features were noted.

2.2.2.2. Microscopic Identification of Fungus

Clean slide was taken and a drop of lactic acid was placed. An inoculating needle was used to gently remove small portion of the fungal culture. The small portion of fungal culture was placed on the slide having a drop of lactic acid. Then a drop of lactophenol blue was placed. In order to thinly spread the fungus, two sterile dissecting needles were used and gentle teasing of fungus was done. Then a drop of lactic acid was place and covered with a cover slip in such a way that there were no air bubbles. Slides were examined under microscope.

2.2.2.3. Molecular Identification of Fungus:

For the molecular identification of fungus, fungal DNA was extracted using CTAB method (Lee and Taylor, 1990). PCR was performed to amplify 18S ribosomal RNA gene (White *et al*., 1990). PCR reaction was prepared using 6 µL of dNTP, 1.5 µL of Taq polymerase, 1 µL of genomic DNA, 5 µL of 10 × polymerase buffers and 1 µL of each fuse. The PCR amplification was performed at 94 °C for 4 minutes followed by 35 rounds of 94 °C for 1 minute, 58 °C for 1 minute, 72 °C for 1 minute and a cycle of 72 °C for 10 minute. The resultant product was sequenced and explored in the database of NCBI (http://www.ncbi.nlm.nih.gov).

2.2.2.4. Phylogenetic Analysis using MEGA 7.0

MEGA 7.0 was used for phylogenetic analysis of *Ascochyta rabiei* by constructing Phylogenetic tree from FASTA sequence .

2.2.3. Poisoned Food Method for *in vitro* testing

In this technique, PDA media was poured into autoclaved Petri plates and 15 oils (0.5 ml each) were mixed in respective petri plate. Three control plates were not amended with oil for the growth of fungus. These plates were allowed to cool and incubated overnight. Then fungus was inoculated by mycelia disc ranging from 2-5 mm and incubated at 25°C. Diameters of fungal growth in control plates and in treatment plates were measured. Antifungal activity was done by comparing the diameter difference of control plate and treatment plate. Growth inhibition was described in percentage by using the following formula,

$$\% \text{ age} = D_C - D_S/D_C \times 100$$

Where,

D_C = Diameter of growth in control plate

D_S= Diameter of growth in plates containing oils.

2.2.4 Agar well diffusion method

PDA media was prepared and poured into petri plates in sterile medium in Laminar flow hood. When media solidified, inoculum was applied over the entire surface of media. By means of sterile cork borer, wells were made in solidified media. In each well, oil treatment was introduced and plates were incubated at 25°C. After some days, zone of inhibition of different oils was recorded.

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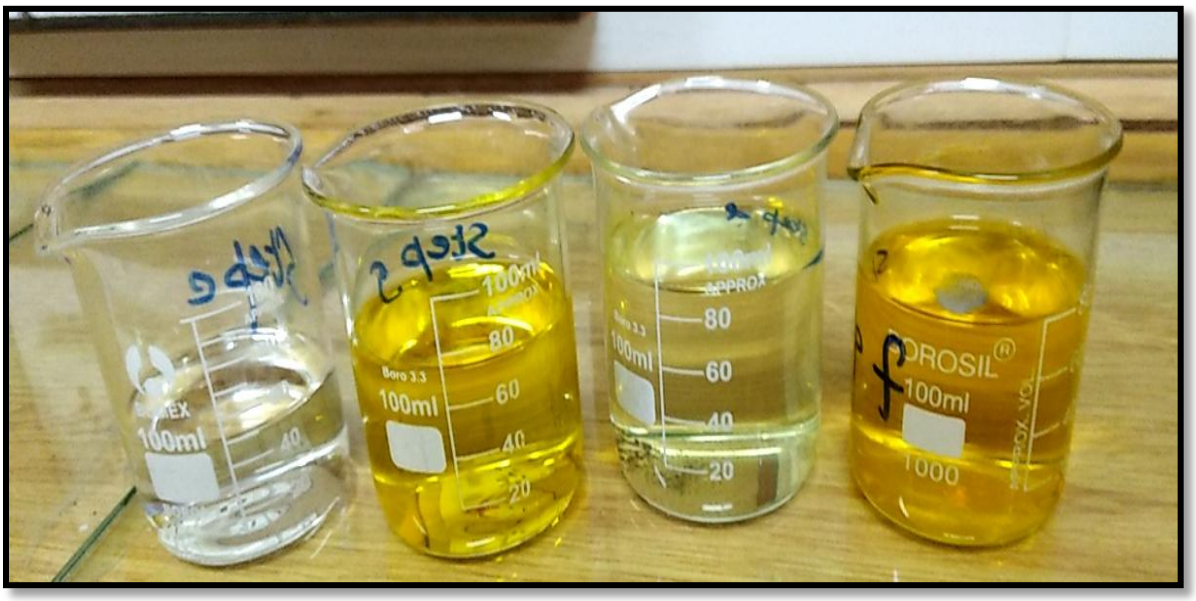


Fig 2.1. Solutions for biochemical parameters

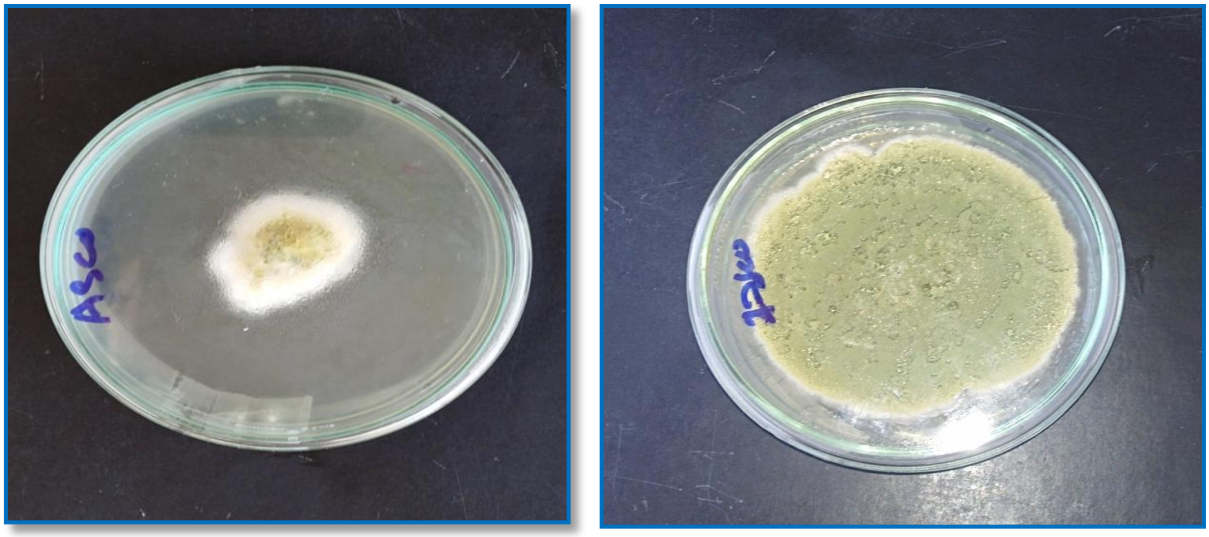


Fig 2.2 (Left to Right) Growth of *Ascochyta rabiei* after 24 hours and after 4 days.

Fig 2.3: Essential oils used in the experiment

RESULTS

RESULTS

3.1. Symptoms of Ascochyta blight on leaves:

The symptoms of Ascochyta blight of Chickpea, Kidney bean and Pea leaves were in the form of small patches of blight on leaves and stem and these patches got enlarged in humid and wet conditions. Small black spots (pycnidia), less than 1mm in diameter, could be seen in the affected areas (Fig.3.1- 3.6).

3.2. Evaluation of Edible oils by detached leaf assay, in vitro

Altogether, 15 emulsified edible oils were tested by detached leaf assay to determine the disease incidence evaluation. Proficiency of different edible oils was checked by this experiment. The strength of oils showed variation and best results were shown by castor oil, sesame oil, opium oil and wheat oil. Fish oil controlled the fungus *in vitro* and disease severity was also low but it caused wilting and stress to plants (Fig. 3.7-3.12)

3.3. Mechanism of disease suppression by oils

Spores of *Aschochyta rabiei* were incubated on glass slides treated with 15 oils and results were recorded. It was found that fish oil, sesame oil, opium oil, wheat oil and castor oils showed the maximum reduction of spores of the tested fungal strain.(Fig 3.13)

3.4. Measurement of disease severity by scale method

In this method, diseased area was examined in control and other treatments. After 2 week of foliar inoculation of fungus, disease percentage in control was highest which shows it to be highly susceptible. Highly significant resistance was observed in different treatments of edible oils and it was found that some oils have common effects on Chickpea, Pea and Kidney beans, thereby reducing the disease severity and best results were found having low severity percentages by fish oil, sesame oil, opium oil, wheat oil and castor oils.(Table 3.4, Fig 3.17-3.19)

3.5. Biochemical and physiological parameters:

3.5.1. Shoot length:

In Chickpea, the values of shoot length ranged from 5.5-24cm and highest value of shoot length was found in healthy control and least in plants treated with Walnut oil. Different oils treatments also showed different shoot length in plants. Plants treated with Fish oil, *Riccinus communis* and *Prunus armaniaca* oil showed highest values of Shoot length (Table 3.5, Fig. 3.20).

In Kidney Bean, different treatments showed different values . The values ranged from 19-40cm and highest value of shoot length was found in healthy control and least in plants treated with Apricot oil. Different oils treatments also showed different shoot length in plants. Plants treated with Fish oil, *Papaver somniferum* oil, *Triticum aestivum* and *Riccinus communis* oils showed high values of Shoot length (Table 3.5, Fig. 3.21).

In Pea, there was a significant difference in the values of control and different treatments of edible oils. The values range from 11-21.6 cm and highest value of shoot length was found in healthy control and least in plants treated with Coconut oil. Different oils treatments also showed different shoot length in plants. Plants treated with Fish oil, *Sesamum indicum* oil, *Triticum aestivum* and *Riccinus communis* oils showed high values of shoot length (Table 3.5, Fig 3.22).

3.5.2. Root length:

In Chickpea, edible oils varied the plants root length values. The values range from 9-29 cm and highest value of root length was found in healthy control and least in plants treated with Indian gooseberry oil. Different oils treatments also showed different root length in plants. Plants treated with Fish oil, *Nigella sativa* oil and *Prunus armaniaca* oil showed high values of root length (Table 3.6, Fig 3.23).

In Kidney bean, the values ranged from 7.1-16.5 cm and highest value of root length was found in healthy control and least in plants treated with Raddish oil. Different oils treatments also showed different root length in plants. Plants treated with Fish oil, *Sesamum indicum* oil, *Triticum aestivum* and *Riccinus communis* oils showed high values of root length (Table 3.6, Fig. 3.24).

In Pea,the root length values ranged from 12-22 cm and highest value of root length was found in healthy control and least in plants treated with Sweet almond oil. Different oils treatments also showed different root length in plants. Plants treated with Fish oil, *Nigella sativa* oil and *Prunus armaniaca* oil showed high values of root length (Table 3.6, Fig 3.25).

3.5.3. Dry root/shoot ratio

In Chickpea, Plants treated with *Juglans regia* and *Riccinus communis* oils showed high values of root/shoot ratio The values ranged from 0.43-0.89 and highest value of root/shoot ratio was found in healthy control and least in plants treated with Sesame oil. Different oils treatments also showed different effects on root/shoot ratio in plants. (Table 3.7, Fig. 3.26).

In Kidney Bean,. the values ranged from 0.21-0.92 and highest value of root/shoot ratio was found in healthy control and least in Fungus control. Different oils treatments also showed different effects on root/shoot ratio in plants. Plants treated with *Olea europea* and *Riccinus communis* oils showed high values of root/shoot ratio (Table 3.7, Fig 3.27).

In Pea, highest value of root/shoot ratio was found in healthy control and least in fungus control The values ranged from 0.45-1.1 and highest value of root/shoot ratio was found in healthy control and least in Fungus control. Different oils treatments also showed different effects on root/shoot ratio in plants. Plants treated with Fish oil, *S. indicum* oil, *T. aestivum* and *R. communis* oils showed high values of root/shoot ratio. (Table 3.7, Fig 3.28).

3.5.4. Sugar content

In chickpea, there was not a significant difference in sugar content values of control and different treatments of edible oils. Highest value of sugar content was found in healthy control and plants treated with *Riccinus communis* oil (Table3.8 , Fig. 3.29).

In Kidney beans, there was a significant difference in sugar content values of control and different treatments of edible oils. The values ranged from 720-1150µg/g and highest value of sugar content was found in healthy control and least in fungus control. This describes that the plant might be in stress condition so its efficiency for the synthesis of sugar might be reduced. Different oils treatments also showed different sugar content in plants. Plants treated with

Papaver somniferum, Sesamum indicum, Nigella sativa and Olea europea oils showed high values of sugar content (Table 3.8, Fig 3.30).

In Garden pea, there was not a significant difference in the values of control and different treatments of edible oils. Highest value of sugar content was found in healthy control and plants treated with Fish oil, olive oil, wheat oil and *Riccinus communis* oil (Table 3.8, Fig 3.31).

3.5.5. Protein content:

In Chickpea, the values ranged from 150-315 µg/g and highest value of protein content was found in healthy control and least in plants treated with olive oil. This describes that the plant might be in stress condition so its efficiency for synthesis of protein might be reduced. Different oils treatments also showed different protein content in plants. Plants treated with *P. somniferum, R. communis, R. sativus and T. aestivum* oils showed high values of Protein content (Table 3.9, Fig 3.32).

In Kidney beans, no significant difference was found in the values. Highest value of protein content was found in healthy control. Plants treated with Fish oil and *R. communis* oil showed very close value to healthy control. Remaining treatments also have values with insignificant difference (Table 3.9, Fig 3.33).

In Pea, there was a significant difference in the values of control and different treatments of edible oils. The values was ranging from 56-119 µg/g and highest value of protein content was found in healthy control and least in plants treated with *C. pepo* oil. This describes that the plant might be in stress condition so its efficiency for the synthesis of protein might be reduced. Different oils treatments also showed different protein content in plants. Plants treated with Fish oil, *R. communis* and *T. aestivum* oils showed high values of Protein content (Table 3.9, Fig. 3.34).

3.5.6. Proline Content

In Chickpea, the values ranged from 220-1176 µg/g and highest value of Proline content was found in Fungus control and least in plants treated with *Riccinus communis* oil. This describes

that the plant might be in stress condition so it had elevated Proline level. Different oils treatments also showed different proline content in plants. Plants treated with Fish oil, *N. sativa, P. armaniaca* and *R. sativus* oils showed high values of Proline content (Table 3.10, Fig 3.35).

In Kidney Bean, *R. communis* oil treatment significantly lowered the Proline content. The values were ranging from 1100-1825 µg/g and the highest value of Proline content was found in Fungus control. This describes that the plant were might be in stress condition so it showed elevated Proline level. Different oils treatments also showed different proline content in plants. Plants treated with *C. pepo* oil, Fish oil, *Olea europea and P. armaniaca* oils showed highest values of Proline content (Table 3.10, Fig. 3.36).

In Pea, highest value of Proline content was found in Fungus control and least in plants treated with *Riccinus communis* oil. The values ranged from 123-1164 µg/g This describes that the plant might be in stress condition so it has elevated Proline level. Different oils treatments also showed different proline content in plants. Plants treated with *J. regia* oil, Fish oil, *R. sativus* and *C. nucifera* oils showed highest values of Proline content (Table 3.10, Fig. 3.37).

3.5.7. Chlorophyll content:

In Chickpea healthy control showed the highest value of chlorophyll content along with some oils. The values range from 25-42 µg/g and lowest value of Chlorophyll content was found in Fungus control and highest in plants treated with *Riccinus communis* oil. Plants treated with *J. regia* oil, Fish oil, *P. dulcis and C. nucifera* oils showed high values of Chlorophyll content (Table 3.11, Fig. 3.38).

In Kidney bean, different values are obtained from different edible oils. The values ranged from 29-46 µg/g and least value of Chlorophyll content was found in Fungus control and highest in healthy control and plants treated with *Riccinus communis* oil. Different oils treatments also showed different Chlorophyll content in plants. Plants treated with *O. europea* oil, *R. sativus* and *C. nucifera* oils showed high values of Chlorophyll content (Table 3.11, Fig. 3.39).

In Pea, the values ranged from 30-44µg/g and least value of Chlorophyll content was found in Fungus control and highest in healthy control and in plants treated with *Riccinus communis* oil.

Plants treated with *J. regia* oil, *T. aestivum* oil, *R. sativus* and *C. nucifera* oils showed high values of Chlorophyll content (Table 3.11, Fig. 3.20).

3.5.8. Relative water content

In Chickpea, there was a significant difference in the values of control and different edible oils treatments. The values was ranging from 44-77% and the highest value of relative water content was found in healthy control and least in plants treated with *Cucurbeta pepo* and Fish oil. This describes that the plant might be in stress condition so its turgor pressure might be decreased. Different oils treatments also showed different relative water content in plants. Plants treated with *Papaver somniferum* oil, *Triticum aestivum* and *Riccinus communis* oils showed high values of relative water content (Table 3.12, Fig. 3.41).

In Kidney bean, the values ranged from 50-88% and the highest value of relative water content was found in healthy control and least in plants treated with *Prunus dulcis* and Fish oil. This describes that the plant might be in stress condition so its turgor pressure might be decreased. Different oils treatments also showed different relative water content in plants. Plants treated with *Papaver somniferum* oil, *Triticum aestivum* and *Riccinus communis* oils showed high values of relative water content (Table 3.12, Fig. 3.42).

In Pea, Raphanus sativus and fish oil treated plants resulted in least water content. The values rangedfrom 33-85% and the highest value of relative water content was found in healthy control This describes that the plant might be in stress condition so its turgor pressure might be decreased. Different oils treatments also showed different relative water content in plants. Plants treated with *Sesamum indicum* oil, *Triticum aestivum* and *Riccinus communis* oils showed high values of relative water content (Table 3.12, Fig 3.43).

3.4.9. Fresh root/shoot ratio:

In Chickpea, treatments of different oils resulted in different root/ shoot ratio.The values ranged from 0.45-0.9 and highest value of root/shoot ratio was found in healthy control and least in plants treated with Sesame oil. Different oils treatments also showed different effects on

root/shoot ratio in plants. Plants treated with *J. regia* and *R. communis* oils showed high values of root/shoot ratio (Table 3.13, Fig 3.44).

In Kidney Bean, the highest value of root/shoot ratio was found in healthy control and leasr in fungus control. The values ranged from 0.23-0.95Plants treated with *Olea europea* and *Riccinus communis* oils showed high values of root/shoot ratio (Table 3.13, Fig 3.45.).

In Pea, the root/shoot ratio was significantly increased by Fish oil, *Sesamum indicum* oil, *Triticum aestivum* and *Riccinus communis* oils The values range from 0.5-1.2 and highest value of root/shoot ratio was found in healthy control and least in Fungus control. Different oils treatments also showed different effects on root/shoot ratio in plants. (Table 3.13, Fig 3.46).

3.6. *In vitro* evaluation of edible oils by Agar well diffusion method

Agar well diffusion method was also used to check the zone of inhibitions of different edible oils against *Aschochyta rabiei.* Zone of inhibitions were studied by measuring diameters using scale. It was recorded that fish oil, sesame oil, opium oil, wheat oil and castor oils showed the maximum zone of inhibitions against the tested fungal strain (Table 3.15, Fig. 3.48).

3.7. *In vitro* evaluation of edible oils by poisoned food method

Poisoned food method was used to check and compare the diameter of control fungal plate and oil treated media plate. It was recorded that fish oil, sesame oil, opium oil, wheat oil and castor oils showed the minimum growth diameters against the tested fungal strain (Table 3.16, Fig. 3.47).

3.8. Determination of the chemical Nature of oils using FTIR

Functional groups are important in phyto-chemisty as they provide sound basis for the compounds present in different oils. Functional groups study was done by using FTIR which elucidated the chemical basis for antifungal activity. The FTIR spectrum of different oils has

been presented in (Fig 3.59 – 3.61.). The information on the peak values, absorbance and the probable functional groups, present in the oils have been represented in Table (Table 1 to Table 15). FTIR spectra of various oil samples showed notable differences in peak values and absorbance. Secondary alcohols are present in many oils except walnut, sesame olive, castor, black cumin, apricot and linseed oil. In fish oil Carboxylic acids are also in more quantity as compared to others because its FTIR spectrum also contains 914 cm−1 wavelength peaks with 0.01 absorbance units. Aliphatic amine, alcohol, ether, ester and phenolic compounds are also present in more quantity in fish oil, as compared to others.

These differences in functional groups composition of Fish oil may be the reason of wilting of leaves and relative low water content respectively. Alcohols, phenols, 1˚, 2˚ amines amides and ethers were present in considerable amount in disease resistant oils (*Ricinus communis*, Fish, *Triticum aestivum*, *Papaver somniferum* and *Sesamum indicum* oils) which are the possible reason of their strong antifungal activity. Majority of Functional groups are same for different edible oils and they vary slightly in their absorbance units Table 3.14)

3.9. FTIR spectral data interpretation:

FTIR spectral peak values and functional groups obtained for sample 1

An FTIR spectrum was used to identify the important functional groups of oil sample 1. Alcoholic groups appeared at spectra 3009.53 cm^{-1}. Main peak in the range 2849.38-2917.48 cm^{-1} showed the presence of alkanes. The presence of carboxylic acid was detected at 2852.99 cm. The characteristic peak of esters was detected at 1743.08cm^{-1}. Alkenes showed their peak at 914.01-967.33cm^{-1} , while tertiary alcohol and secondary alcohol appeared with their peaks at 1159.55 and 1098.04cm^{-1}.Aryl ether appeared with its characteristic peak at 1236.01 cm^{-1}. Halogens showed their presence below 800cm^{-1}.

FTIR spectral peak values and functional groups obtained for sample 2

An FTIR spectrum was used to identify the important functional groups of oil sample 2. Alcoholic groups appeared at spectra 3007.58 cm^{-1}. Main peak in the range 2849.38-2930.48 cm^{-1} showed the presence of alkanes. The presence of carboxylic acid was detected at 2852.37 cm^{-1}.

The characteristic peak of esters was detected at 1743.08cm^{-1}. Alkenes showed their peak at 914.01-967.33cm^{-1}, while tertiary alcohol and secondary alcohol appeared with their peaks at 1159.55 and 1098.04cm^{-1}.Aliphatic ether appeared with its characteristic peak at 1098.70 cm^{-1}. Halogens showed their presence below 800cm^{-1}.

FTIR spectral peak values and functional groups obtained for sample 3

An FTIR spectrum was used to identify the important functional groups of oil sample 3. Alcoholic groups appeared at spectra 3007.58 cm^{-1}. Main peak at 1462.46 cm^{-1} showed the presence of alkanes. The presence of carboxylic acid was detected at 2921.73 cm^{-1}. The characteristic peak of esters was detected at 1743.98cm^{-1}. Alkenes showed their peak at 913.72 and 1653.77cm^{-1} , while tertiary alcohol and secondary alcohol appeared with their peaks at 1159.55 and 1118.79cm^{-1}.Aliphatic ether appeared with its characteristic peak at 1236.01 cm^{-1}.Halogens showed their presence below 800cm^{-1}.

FTIR spectral peak values and functional groups obtained for sample 4

An FTIR spectrum was used to identify the important functional groups of oil sample 4. Alcoholic groups appeared at spectra 3009.58 cm^{-1}. Main peak at 1461.53 cm^{-1} showed the presence of alkanes. The presence of amines was detected at 2853.00 cm^{-1}. Phenol with its peak appeared at 1376.38cm^{-1}. The characteristic peak of esters was detected at 1743.98cm^{-1}. Alkenes showed their peak at 914.01cm^{-1} , while tertiary alcohol and secondary alcohol appeared with their peaks at 1159.55 and 1118.79cm^{-1}.Aliphatic ether appeared with its characteristic peak at 1236.01 cm^{-1}.Halogens showed their presence below 800cm^{-1}.

FTIR spectral peak values and functional groups obtained for sample 5

An FTIR spectrum was used to identify the important functional groups of oil sample 5. Alcoholic groups appeared at spectra 2921.76-3007.50 cm^{-1}. Main peak at 1462.57 cm^{-1} showed the presence of alkanes. The presence of amines was detected at 2852.43 cm^{-1}. Phenol with its peak appeared at 1376.70cm^{-1}. The characteristic peak of esters was detected at 1743.65cm^{-1}., while tertiary alcohol and secondary alcohol appeared with their peaks at 1160.02 and 1118.64cm^{-1}.Aliphatic ether appeared with its characteristic peak at 1098.24cm^{-1}.Halogens showed their presence below 800cm^{-1}.

FTIR spectral peak values and functional groups obtained for sample 6

An FTIR spectrum was used to identify the important functional groups of oil sample 4. Alcoholic groups appeared at spectra 2922.75- 3009.52 cm^{-1}. Main peak at 1461.55 cm^{-1} showed the presence of alkanes. The presence of amines was detected at 2852.98 cm^{-1}. Phenol with its peak appeared at 1376.41cm^{-1}. The characteristic peak of esters was detected at 1743.06cm^{-1}. Alkenes showed their peaks at 967.67 and 1654.23cm^{-1} , while tertiary alcohol appeared with their peaks at 1159.49 cm^{-1} .Aliphatic ether appeared with its characteristic peak at 1098.07 cm^{-1}.Halogens showed their presence below 800cm^{-1}.

FTIR spectral peak values and functional groups obtained for sample 7

An FTIR spectrum was used to identify the important functional groups of oil sample 7. Alcoholic groups appeared at spectra 2922.75 - 3008.56 cm^{-1}. Main peak at 1461.57 cm^{-1} showed the presence of alkanes. The presence of amines was detected at 2853.22 cm^{-1}. Phenol with its peak appeared at 1376.91cm^{-1}. The characteristic peak of esters was detected at 1742.97cm^{-1}. Alkenes showed their peak at 913.36 and 1657.07cm^{-1} , while tertiary alcohol appeared with their peaks at 1159.93cm^{-1}.Aliphatic ether appeared with its characteristic peak at 1097.98 cm^{-1}.Halogens showed their presence below 800cm^{-1}.

FTIR spectral peak values and functional groups obtained for sample 8

An FTIR spectrum was used to identify the important functional groups of oil sample 8. Alcoholic groups appeared at spectra 2923.51 cm^{-1}. Main peak at 1458.84 cm^{-1} showed the presence of alkanes. The presence of amines was detected at 2853.37 cm^{-1}. The characteristic peak of esters was detected at 1742.61cm^{-1}, while tertiary alcohol appeared with their peaks at 1161.84. Halogens showed their presence below 800cm^{-1}.

FTIR spectral peak values and functional groups obtained for sample 9

An FTIR spectrum was used to identify the important functional groups of oil sample 9. Alcoholic groups appeared at spectra 2921.94- 3006.62 cm^{-1}. Main peak at 1462.33 cm^{-1} showed the presence of alkanes. The presence of amines was detected at 2852.59 cm^{-1}. Phenol with its

peak appeared at 1376.44cm^{-1}. The characteristic peak of esters was detected at 1743.41cm^{-1}. Alkenes showed their peak at 914.01cm^{-1} , while tertiary alcohol and secondary alcohol appeared with their peaks at 1159.95 and 1118.67cm^{-1}.Aliphatic ether appeared with its characteristic peak at 1096.97 cm^{-1}.Halogens showed their presence below 800cm^{-1}.

FTIR spectral peak values and functional groups obtained for sample 10

An FTIR spectrum was used to identify the important functional groups of oil sample 10. Alcoholic groups appeared at spectra 2921.97-3006.06 cm^{-1}. Main peak at 1462.18 cm^{-1} showed the presence of alkanes. The presence of amines was detected at 2852.73 cm^{-1}. Phenol with its peak appeared at 1376.61cm^{-1}. The characteristic peak of esters was detected at 1743.39cm^{-1}. Alkenes showed their peak at 1654.85 - 914.01cm^{-1} , while tertiary alcohol and secondary alcohol appeared with their peaks at 1159.76 and 1118.70cm^{-1}.Aliphatic ether appeared with its characteristic peak at 1095.36 cm^{-1}.the peak of vinyl ethers was detected at 1031.23cm $^{-1}$ .Halogens showed their presence below 800cm^{-1}.

FTIR spectral peak values and functional groups obtained for sample 11

An FTIR spectrum was used to identify the important functional groups of oil sample 11. Alcoholic groups appeared at spectra 2953.99-2920.96 cm^{-1}. Main peak at 1459.42 cm^{-1} showed the presence of alkanes. The presence of amines was detected at 2852.28 cm^{-1}. Phenol with its peak appeared at 1376.74cm^{-1}. The characteristic peak of esters was detected at 1747.74cm^{-1}. Alkenes showed their peak at 1654.85 - 914.01cm^{-1} , while tertiary alcohol appeared with their peaks at 1159.76 cm^{-1}.Aliphatic ketone appeared with its characteristic peak at 1712.61 cm^{-1}.the peak of vinyl ethers was detected at 1216.74cm $^{-1}$ .Halogens showed their presence below 800cm^{-1}.

FTIR spectral peak values and functional groups obtained for sample 12

An FTIR spectrum was used to identify the important functional groups of oil sample 12. Alcoholic groups appeared at spectra 2923.00-3006.84 cm^{-1}. Main peak at 1461.29 cm^{-1} showed

the presence of alkanes. The presence of amines was detected at 2853.06 cm^{-1}. Phenol with its peak appeared at 1376.14cm^{-1}. The characteristic peak of esters was detected at 1742.93cm^{-1}. Alkenes showed their peak at 1652.93 - 968.63cm^{-1}, while tertiary alcohol and secondary alcohol appeared with their peaks at 1159.51 and 1118.70cm^{-1}.Alkyl/aryl ether appeared with its characteristic peak at 1236.93 cm^{-1}. Halogens showed their presence below 800cm^{-1}.

FTIR spectral peak values and functional groups obtained for sample 13

An FTIR spectrum was used to identify the important functional groups of oil sample 13. Alcoholic groups appeared at spectra 2922.89 and 3009.41 cm^{-1}. Main peak at 1461.39 cm^{-1} showed the presence of alkanes. The presence of amines was detected at1031.62 and 2853.13 cm^{-1}. Phenol with its peak appeared at 1376.47cm^{-1}. The characteristic peak of esters was detected at 1743.08cm^{-1}. Alkenes showed their peaks at 1654.60 and 913.67.01cm^{-1} , while tertiary alcohol appeared with their peaks at 1159.39 cm^{-1}.Aliphatic ether appeared with its characteristic peak at 1097.68 cm^{-1}. .Halogens showed their presence below 800cm^{-1}.

FTIR spectral peak values and functional groups obtained for sample 14

An FTIR spectrum was used to identify the important functional groups of oil sample 14. Alcoholic groups appeared at spectra 2922.43-3008.28 cm^{-1}. Main peak at 1462.28 cm^{-1} showed the presence of alkanes. The presence of amines was detected at 2853.04 cm^{-1}. Phenol with its peak appeared at 1377.03cm^{-1}. The characteristic peak of esters was detected at 1743.35cm^{-1}. Alkenes showed their peak at 913.45cm^{-1} , while tertiary alcohol and secondary alcohol appeared with their peaks at 1159.46 and 1119.20cm^{-1}.Aliphatic ether appeared with its characteristic peak at 1097.44 cm^{-1}..Halogens showed their presence below 800cm^{-1}.

FTIR spectral peak values and functional groups obtained for sample 15

An FTIR spectrum was used to identify the important functional groups of oil sample 15. Alcoholic groups appeared at spectra 1417.89 and 2921.85cm^{-1}. Main peak at 1464.20 cm^{-1} showed the presence of alkanes. The presence of amines was detected at 2852.78 cm^{-1}. Phenol with its peak appeared at 1376.86cm^{-1}. The characteristic peak of esters was detected at 1742.45cm^{-1}. Alkenes showed their peak at 962.56 and 889.17cm^{-1} , while tertiary alcohol and secondary alcohol appeared with their peaks at 1152.15 and 1110.29cm^{-1}.Alkyl/aryl ether

appeared with its characteristic peak at 1228.59 cm^{-1}.Halogens showed their presence below 800cm^{-1}.

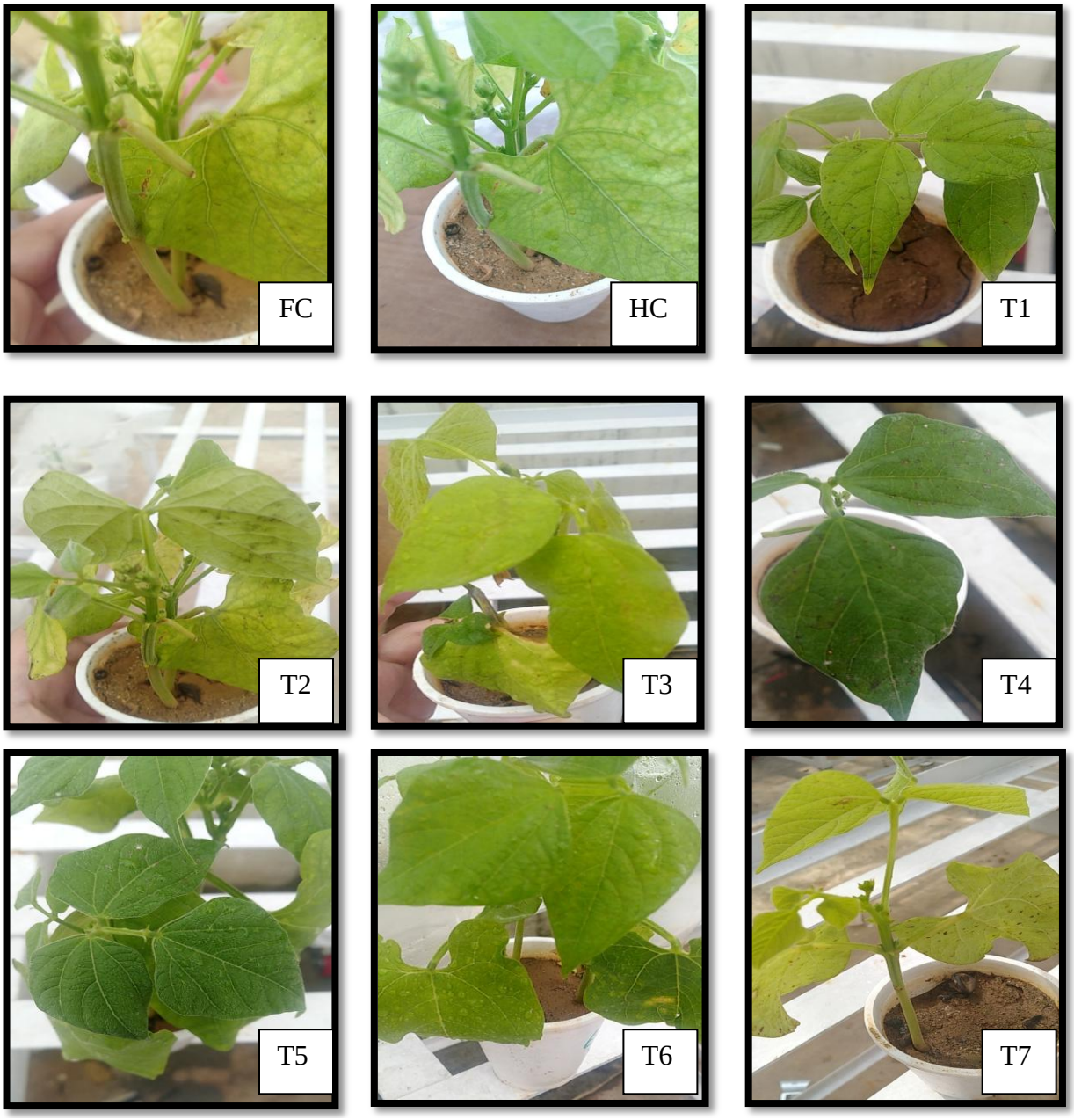


Fig 3.1: Visual symptoms of blight on kidney Bean after foliar inoculation of fungus and spraying with essential oils. (FC = Fungus Control, HC = Healthy Control, T1-T7 = treatment 1 to 7 as mentioned in table 1.1)

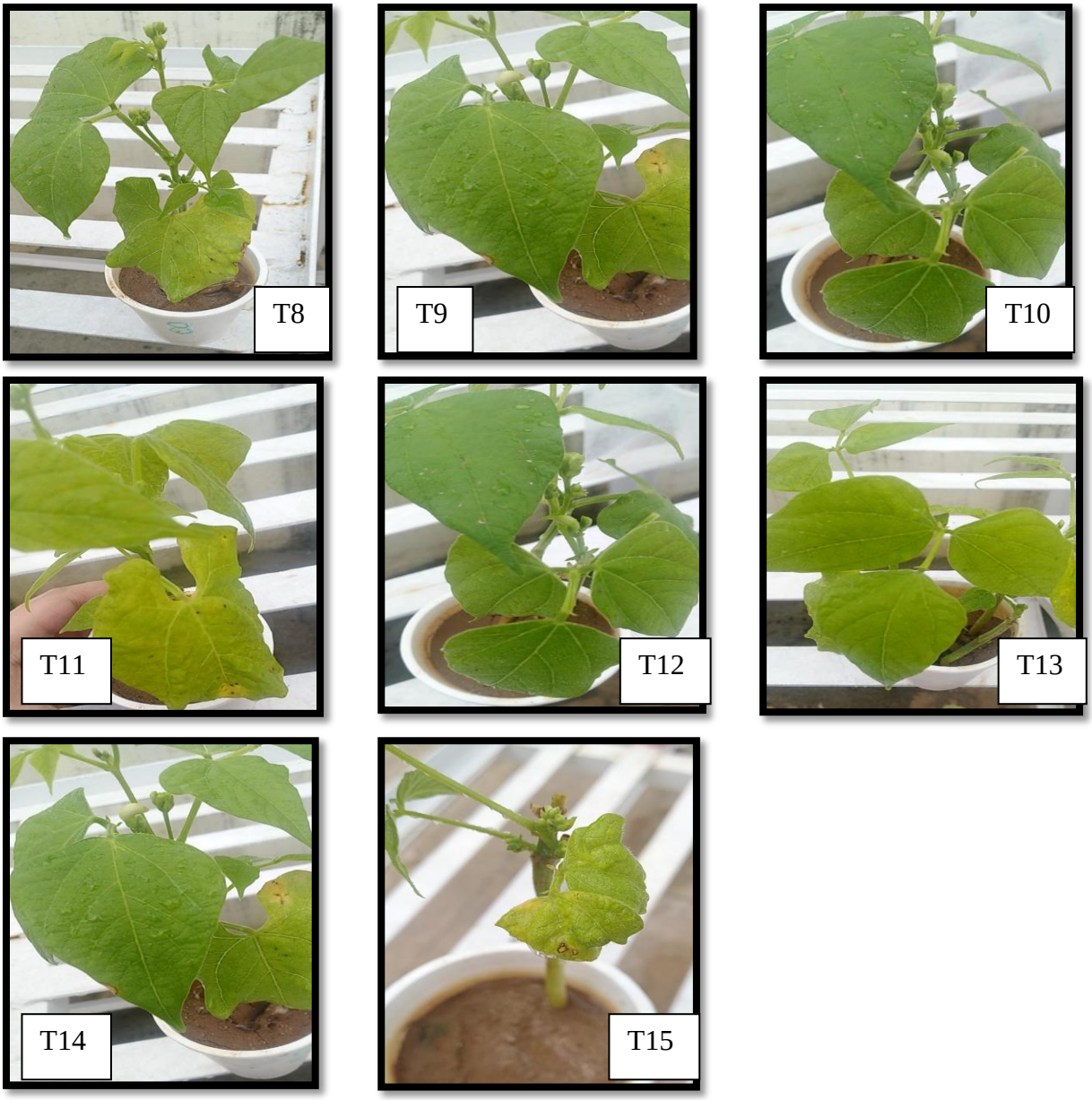


Fig. 3.2: Visual symptoms of blight on kidney Bean after foliar inoculation of fungus and spraying with essential oils. (T8 –T15 = treatment 8 to 15 as mentioned in table 1).

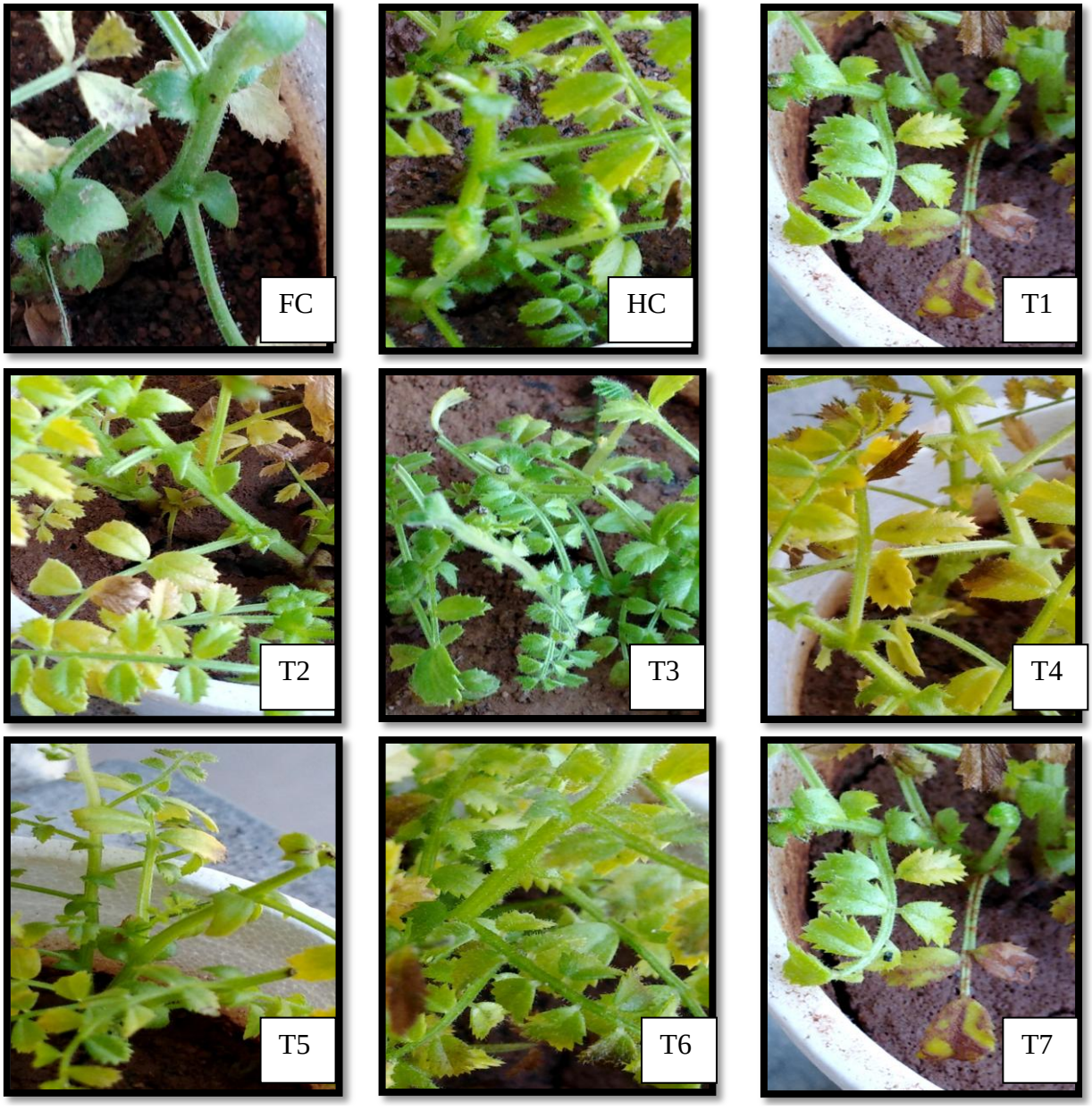


Fig. 3.3: Visual symptoms of blight on Chickpea after foliar inoculation of fungus and spraying with essential oils. (FC = Fungus Control, HC = Healthy Control, T1-T7 = treatment 1 to 7 as mentioned in table 1)

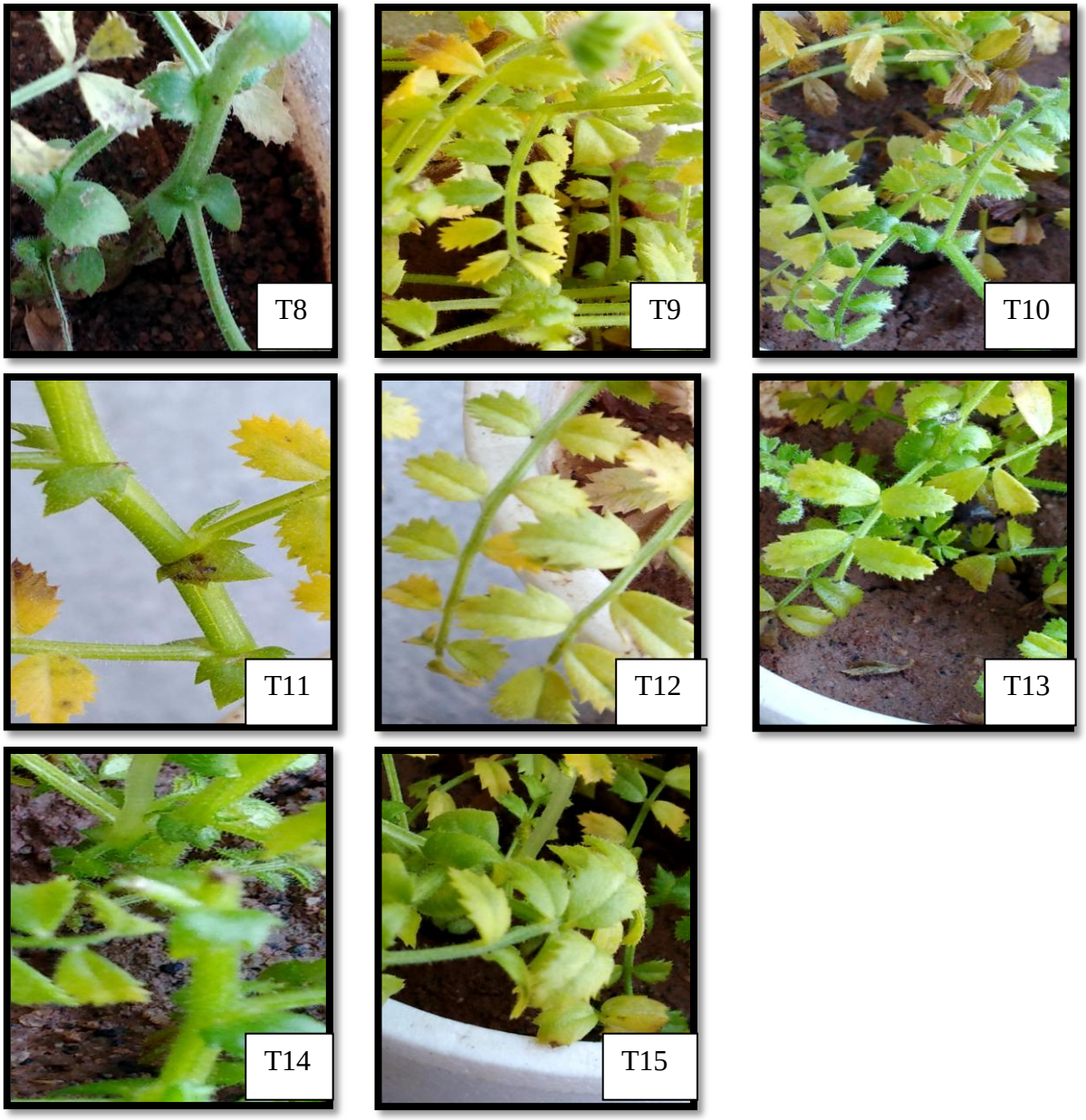


Fig. 3.4: Visual symptoms of blight on Chickpea after foliar inoculation of fungus and spraying with essential oils. (T8 –T15 = treatment 8 to 15 as mentioned in table 1).

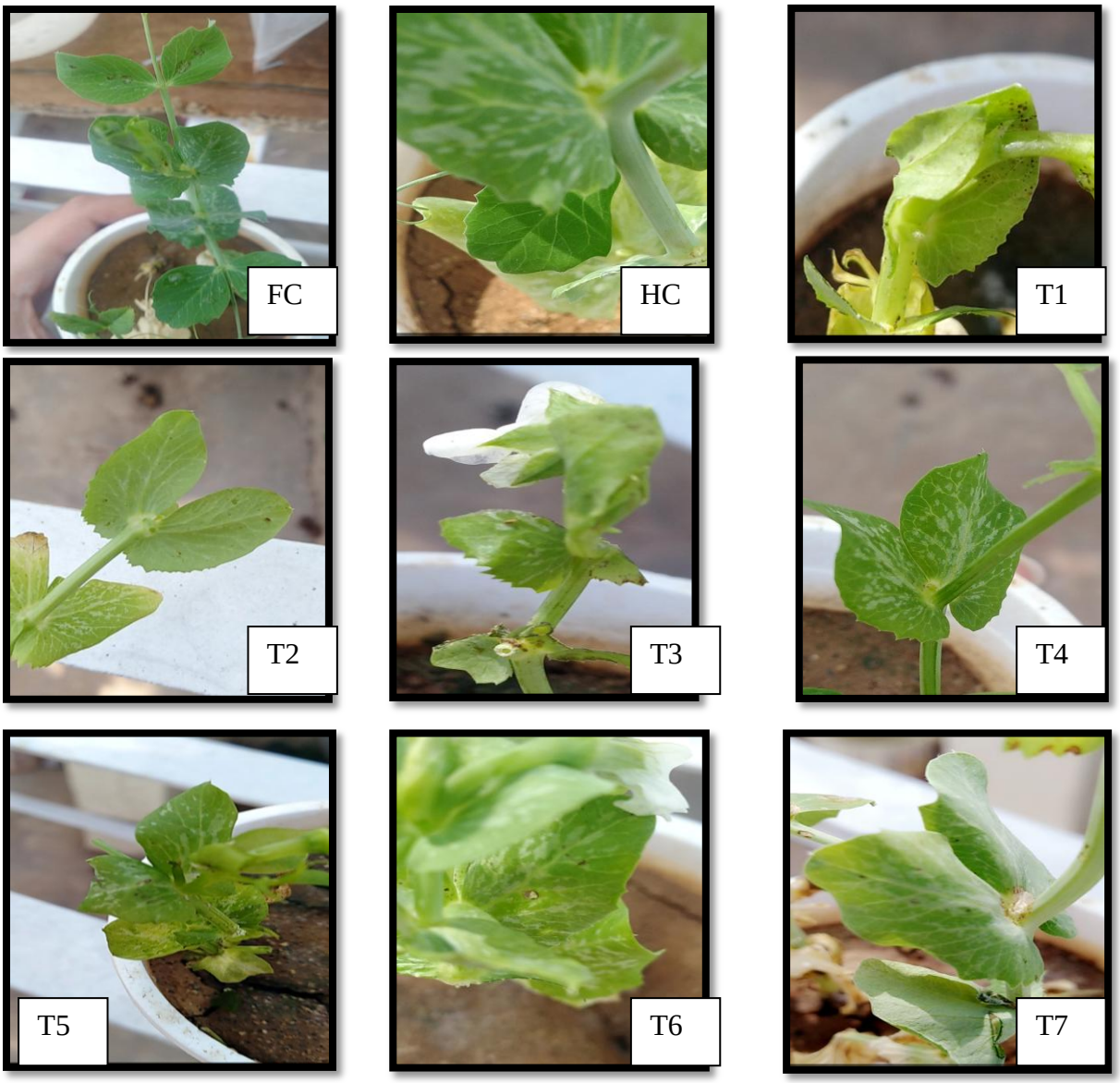


Fig. 3.5: Visual symptoms of blight on Pea after foliar inoculation of fungus and spray with essential oils. (FC = Fungus Control, HC = Healthy Control, T1-T7 = treatment 1 to 7 as mentioned in table 1.1)

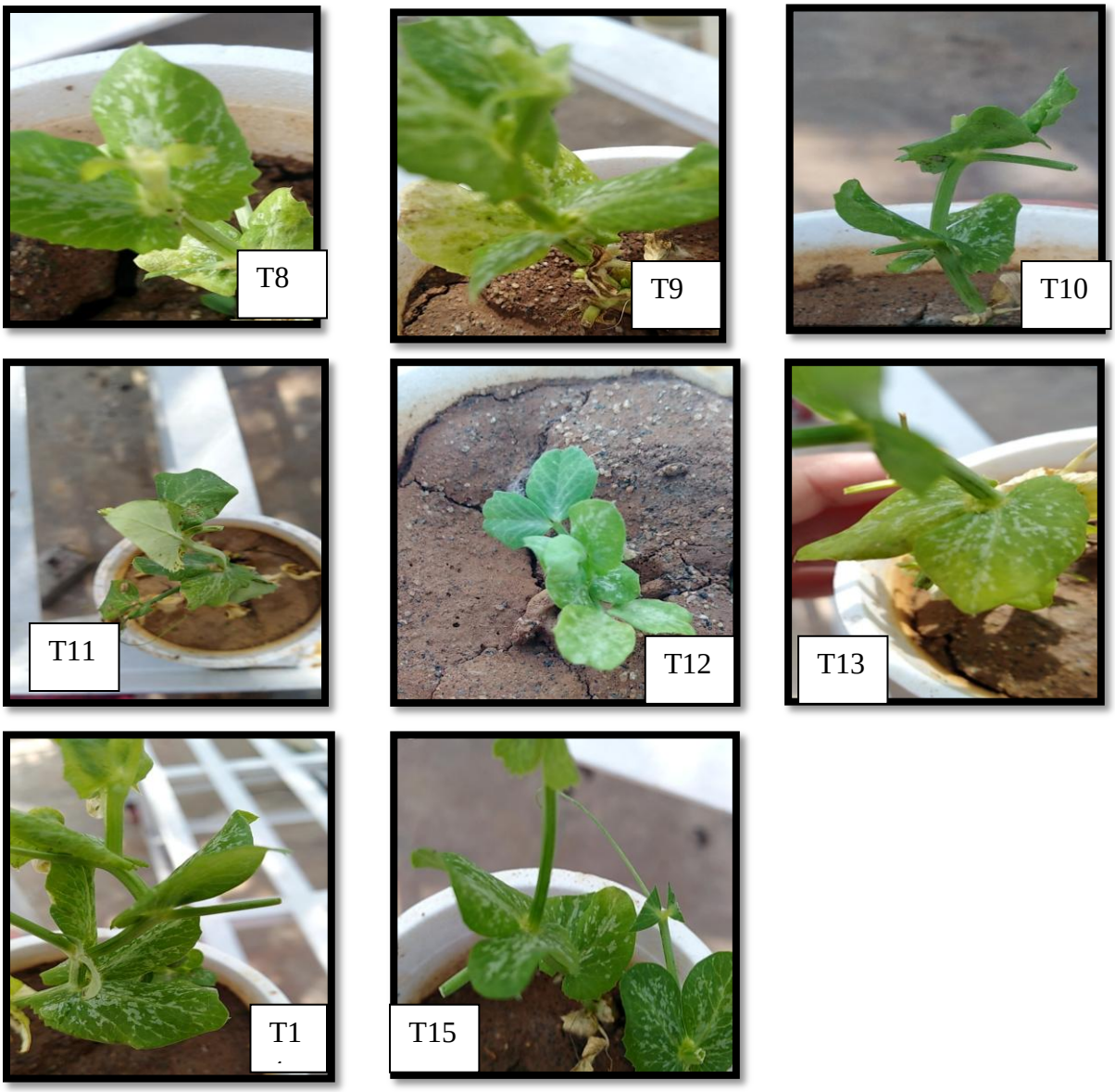


Fig. 3.6: Visual symptoms of blight on Pea after foliar inoculation of fungus and sprayiwith essential oils. (T8 –T15 = treatment 8 to 15 as mentioned in table 1.1).

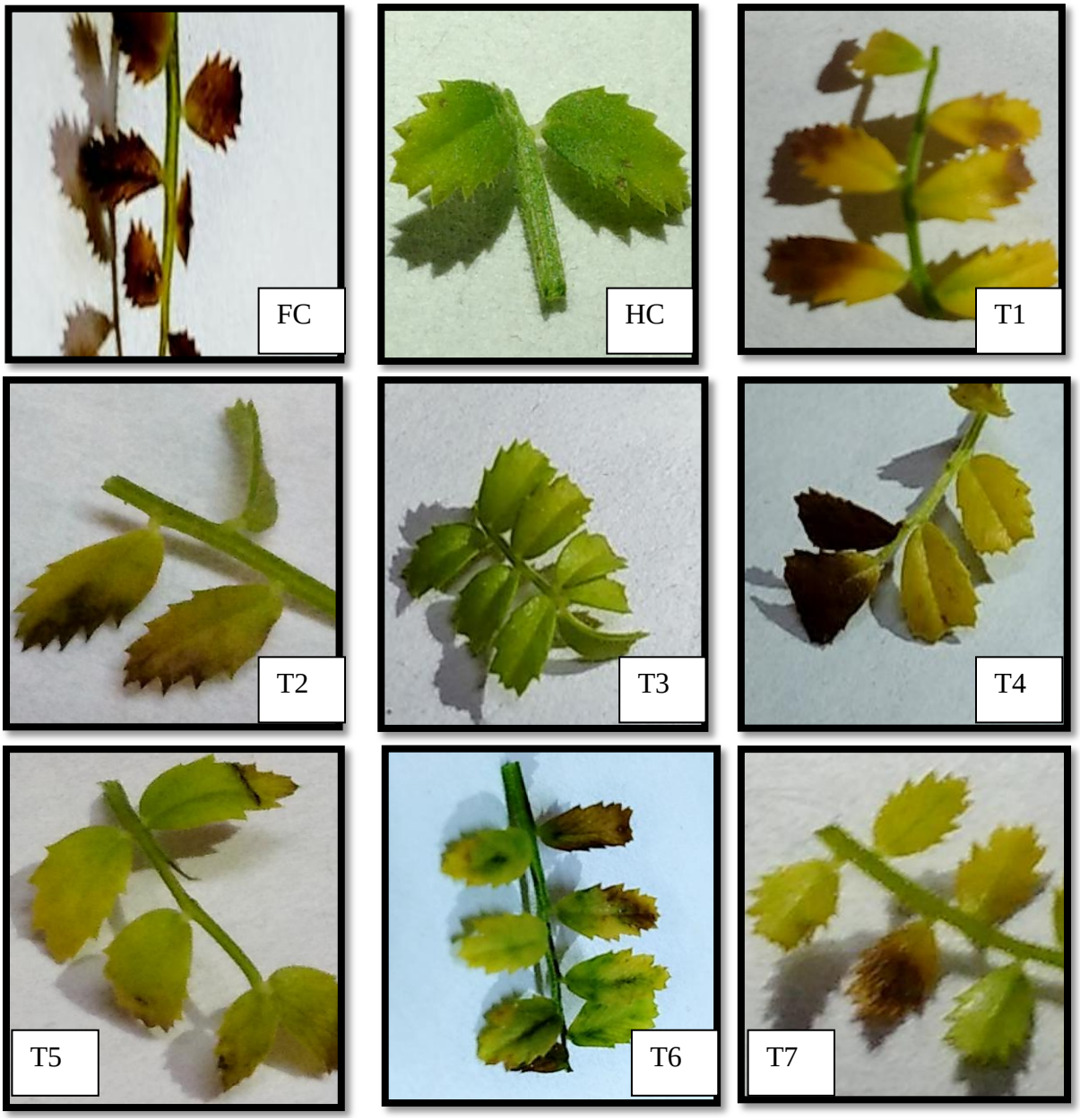


Fig. 3.7: Disease severity analysis of Chickpea after foliar inoculation of fungus and spraying with essential oils. (FC = Fungus Control, HC = Healthy Control, T1-T7 = treatment 1 to 7 as mentioned in table 1.1)

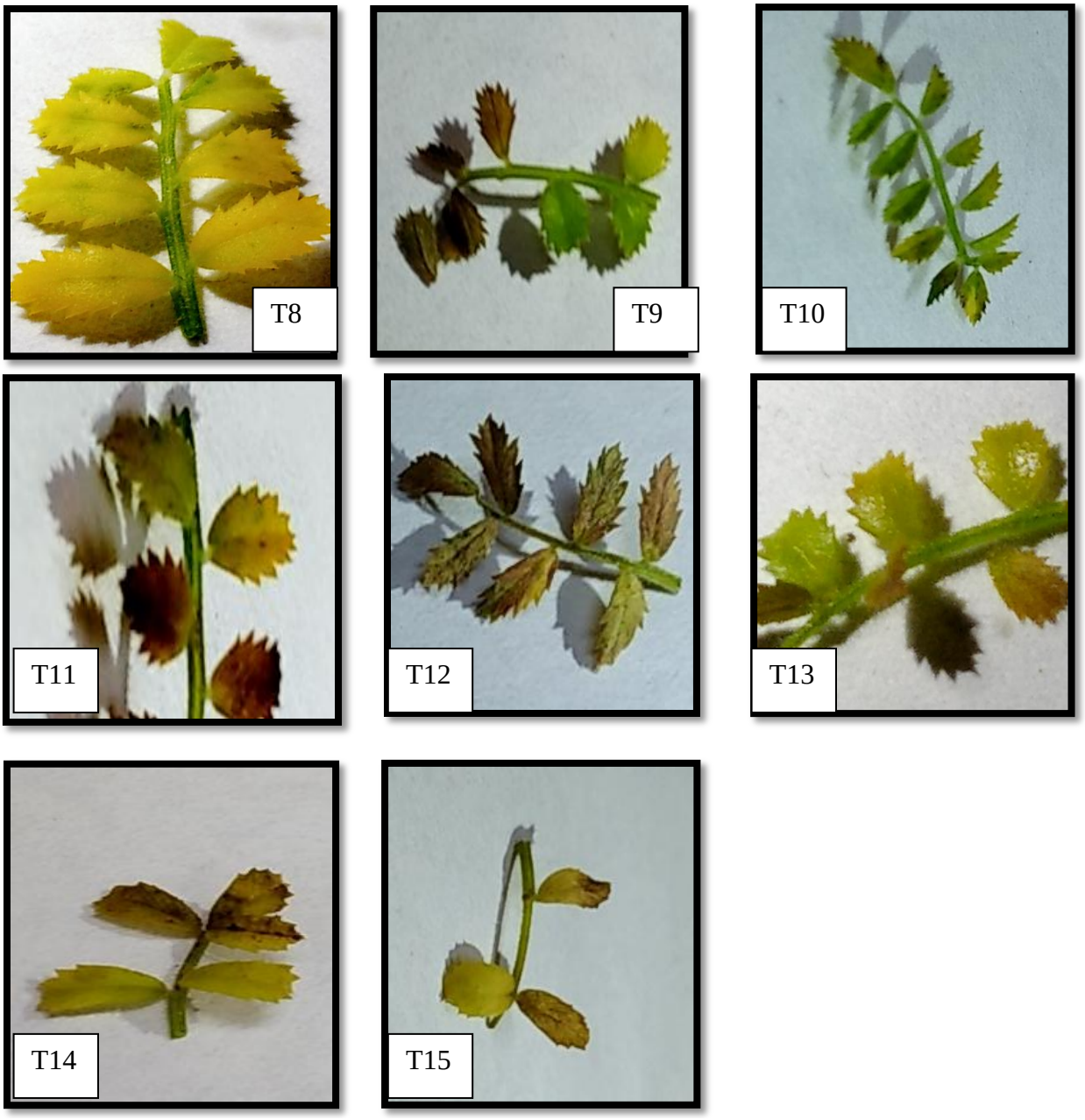


Fig. 3.8: Disease severity analysis of Chickpea after foliar inoculation of fungus and spraying with essential oils. (T8 –T15 = treatment 8 to 15 as mentioned in table 1.1).

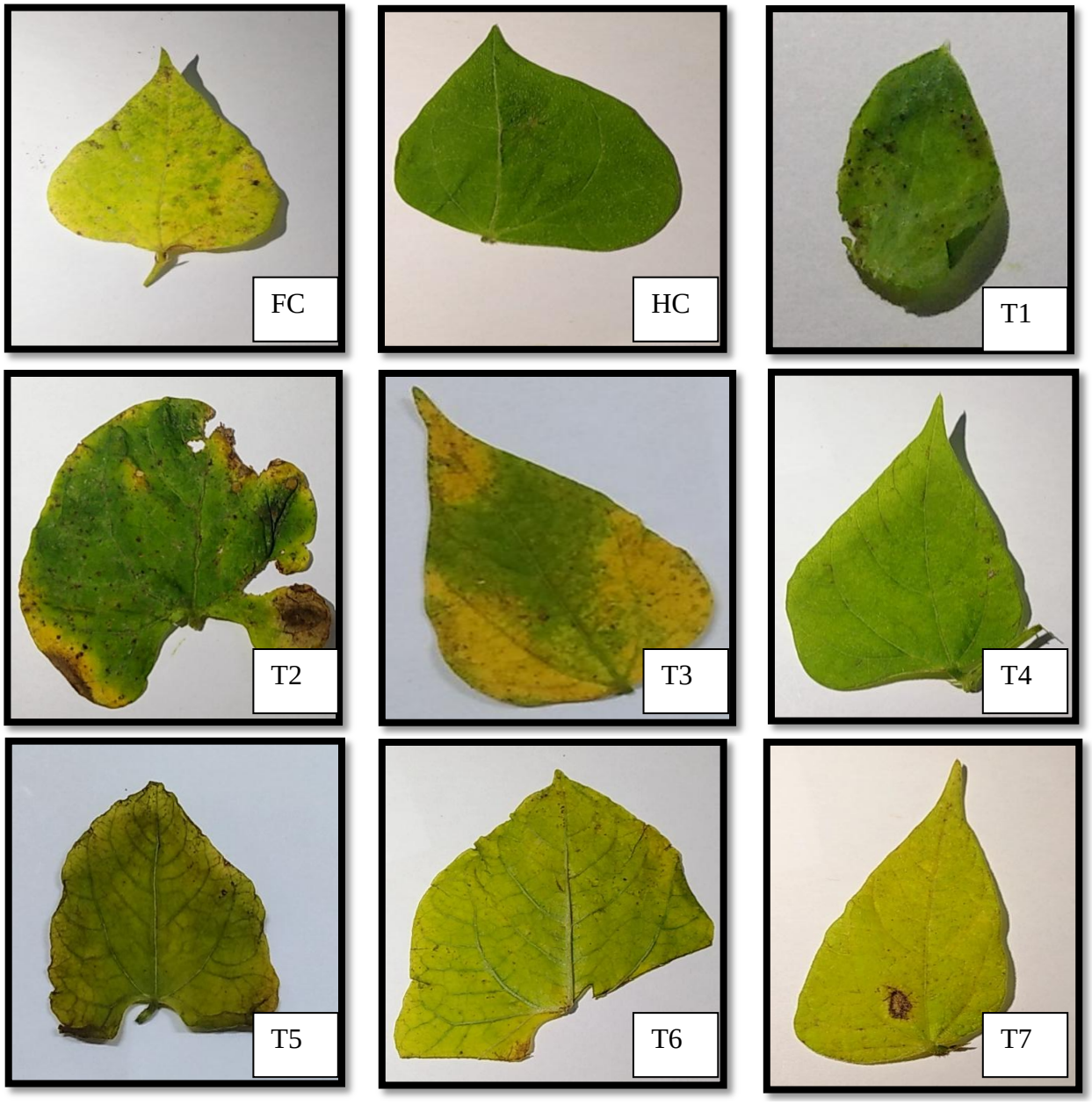


Fig. 3.9: Disease severity analysis of Kidney Bean after foliar inoculation of fungus and spraying with essential oils. (FC = Fungus Control, HC = Healthy Control, T1-T7 = treatment 1 to 7 as mentioned in table 1.1)

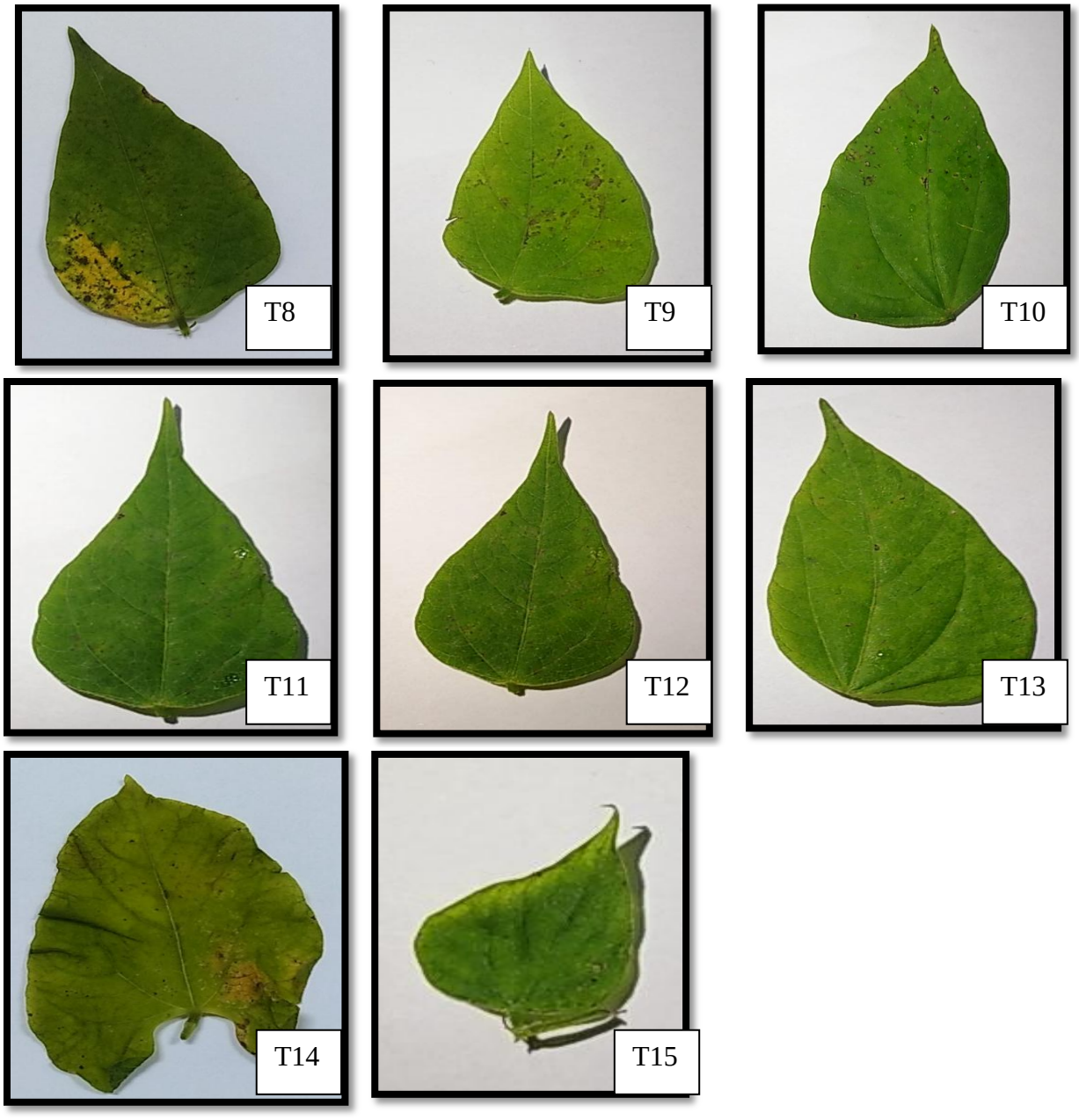


Fig. 3.10: Disease severity analysis of Kidney Bean after foliar inoculation of fungus andspraying with essential oils. (T8 –T15 = treatment 8 to 15 as mentioned in table 1.1).

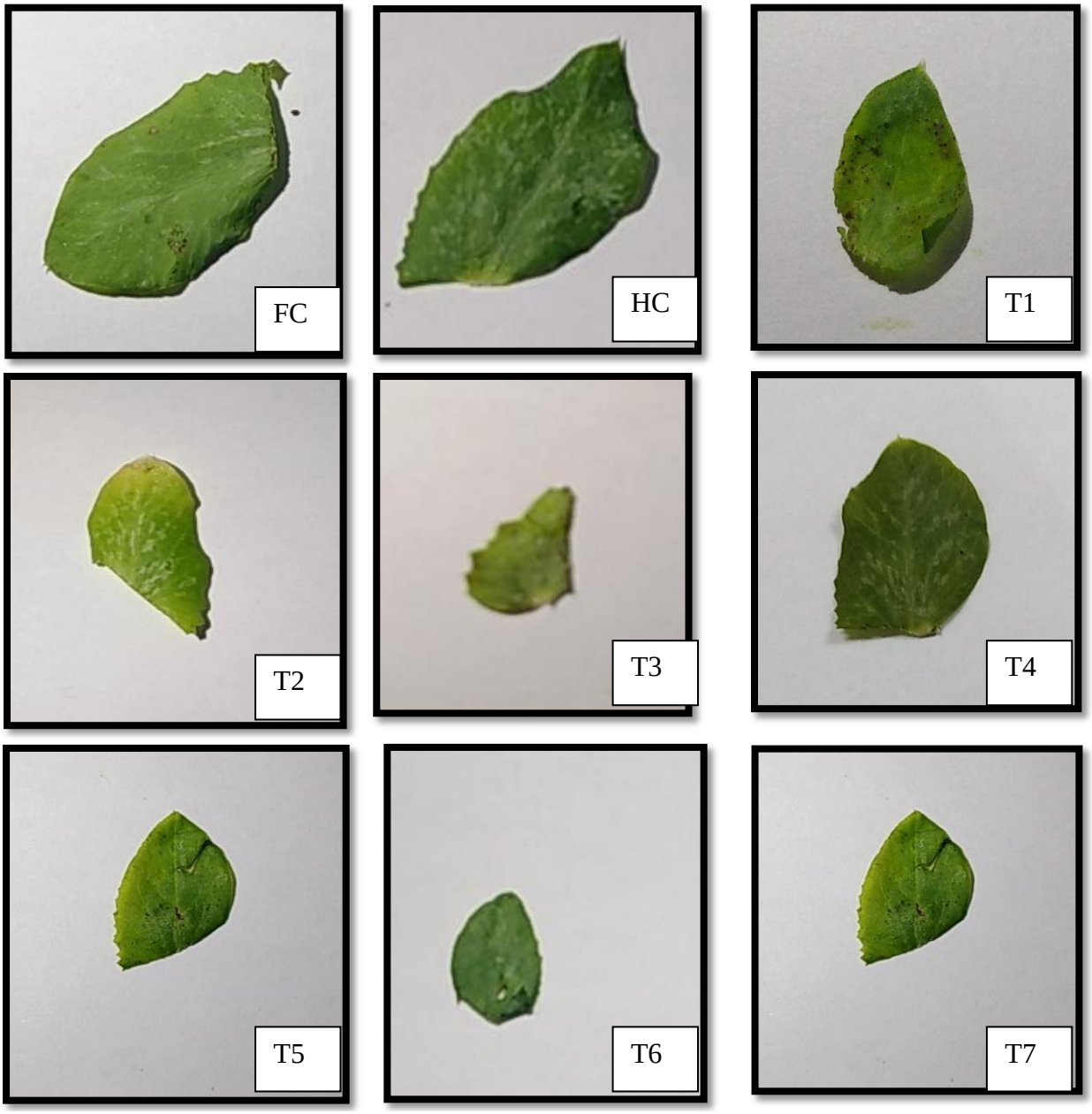


Fig. 3.11: Disease severity analysis of Pea after foliar inoculation of fungus and spraying with essential oils. (FC = Fungus Control, HC = Healthy Control, T1-T7 = treatment 1 to 7 as mentioned in table 1.1)

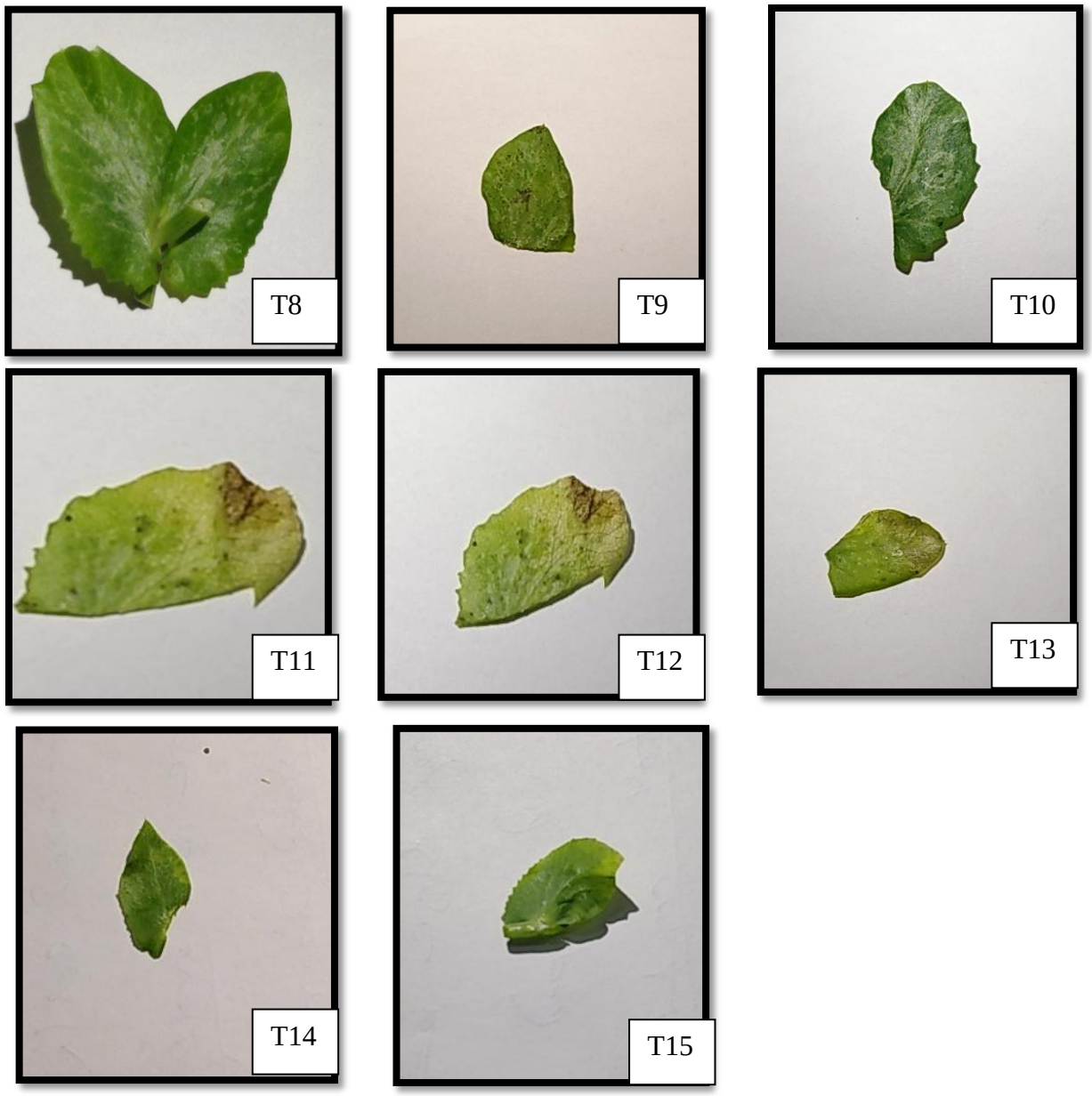


Fig. 3.12: Disease severity analysis of Pea after foliar inoculation of fungus and spraying withessential oils. (T8 –T15 = treatment 8 to 15 as mentioned in table 1.1).

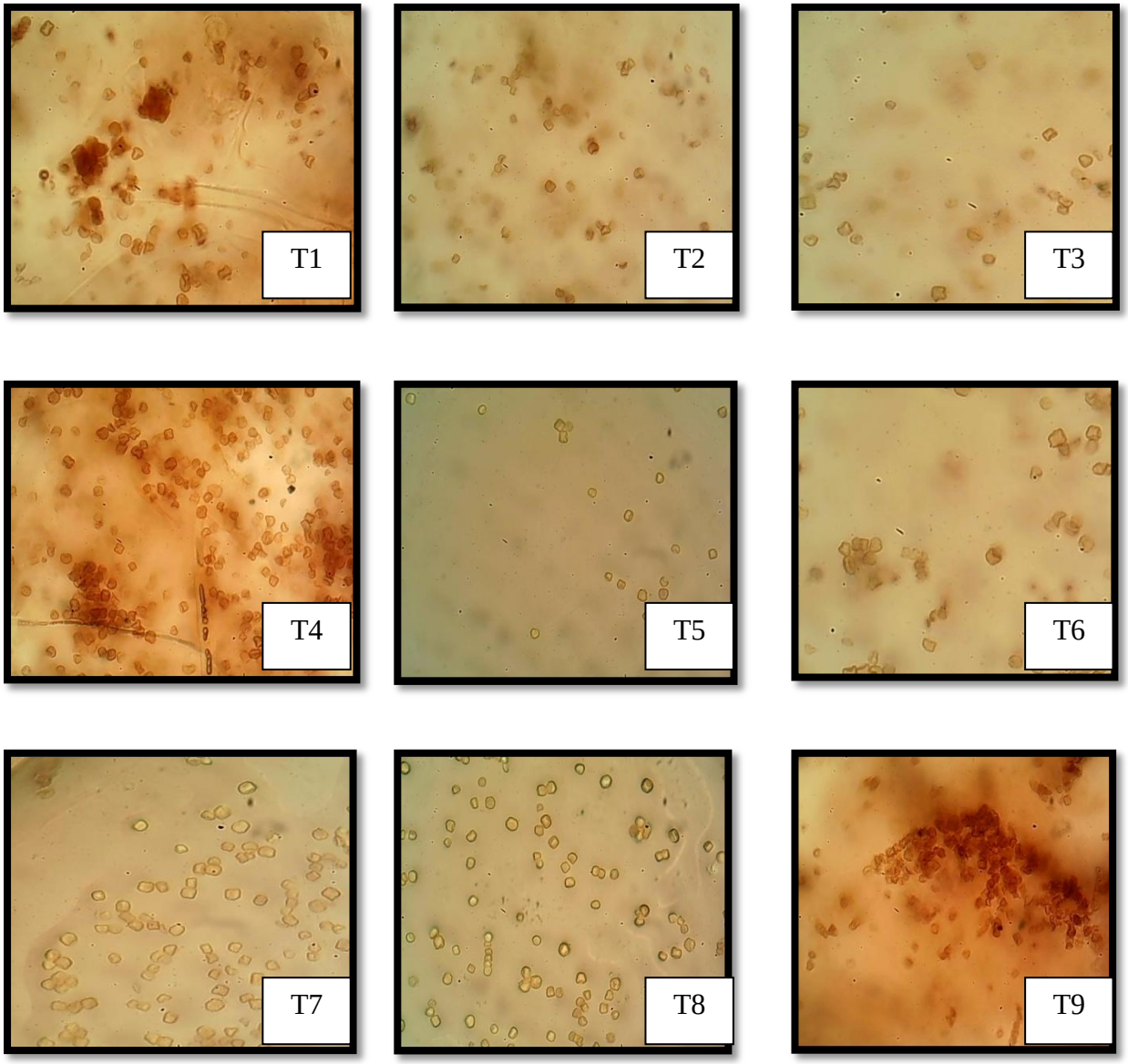


Fig 3.13: Comparative analysis of *Aschochyta rabiei* spores with application of different edible oils. (T1-T9 = Treatment 1-9 as mentioned in table 1.1).

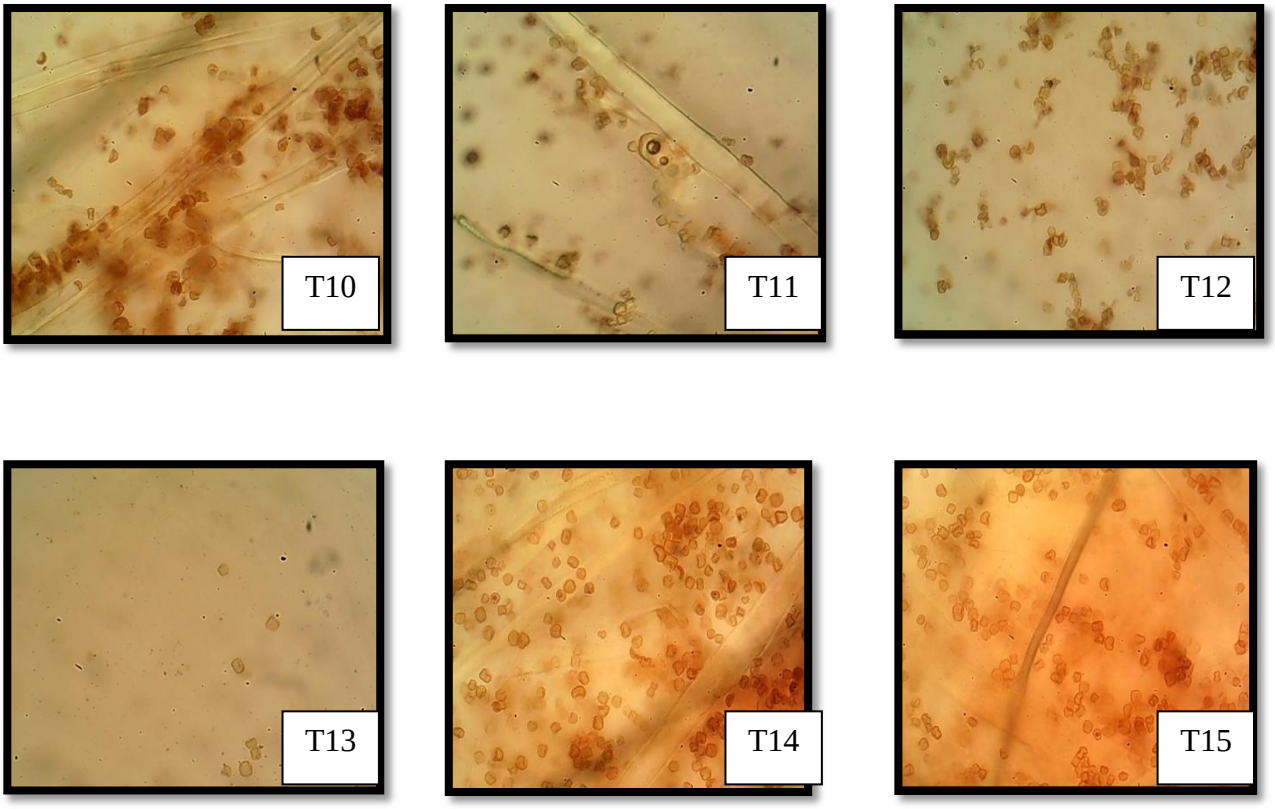


Fig 3.14: Comparative analysis of *Ascochyta rabiei* spores with application of different edible oils. (T10-T15 = Treatment 10-15 as mentioned in table 1.1).

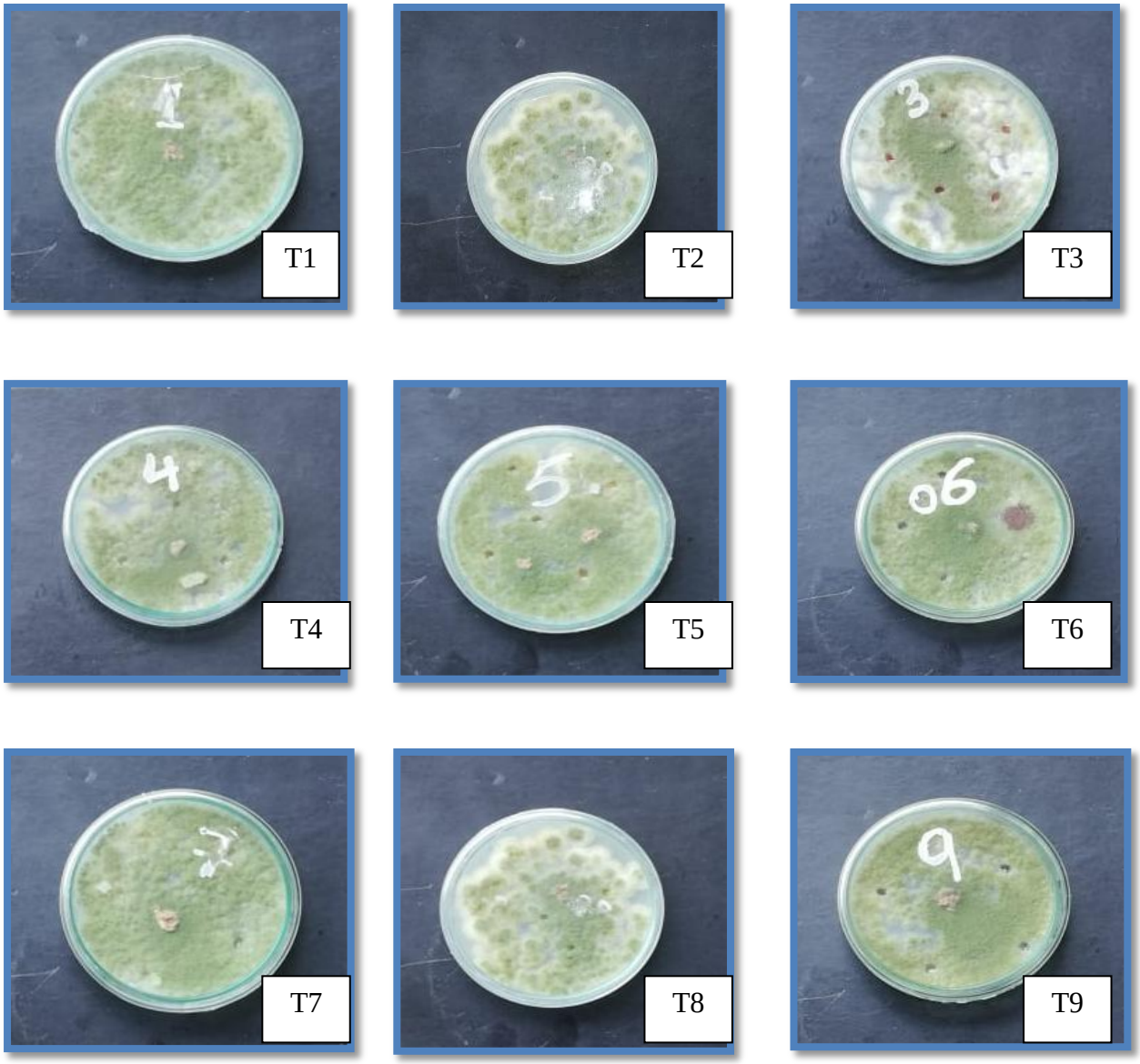


Fig 3.15: Comparative analysis of *Ascochyta rabiei* growth(mycelia and spores) with application of different edible oils by agar well diffusion method. (T1-T9 = Treatment 1-9 as mentioned in table 1.1).

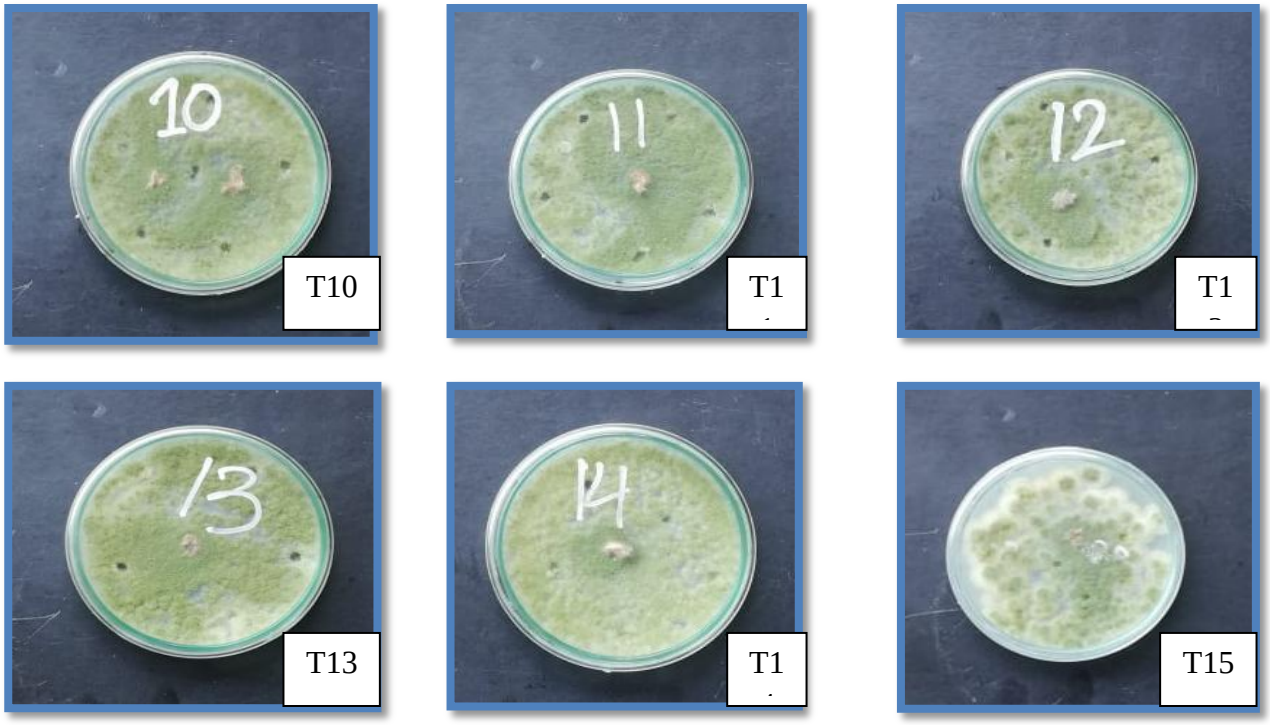


Fig 3.16: Comparative analysis of *Ascochyta rabiei* growth (mycelia and spores) with application of different edible oils by agar well diffusion method. (T10-T15 = Treatment 10-15 as mentioned in table 1.1).

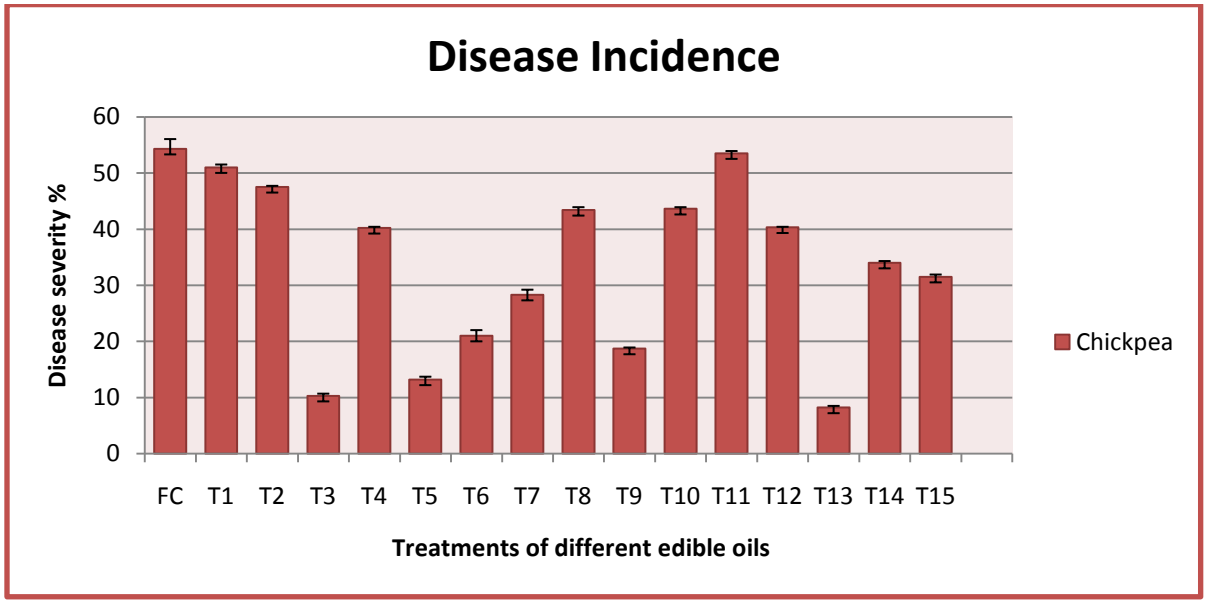


Fig 3.17: Disease severity analysis of Chickpea after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

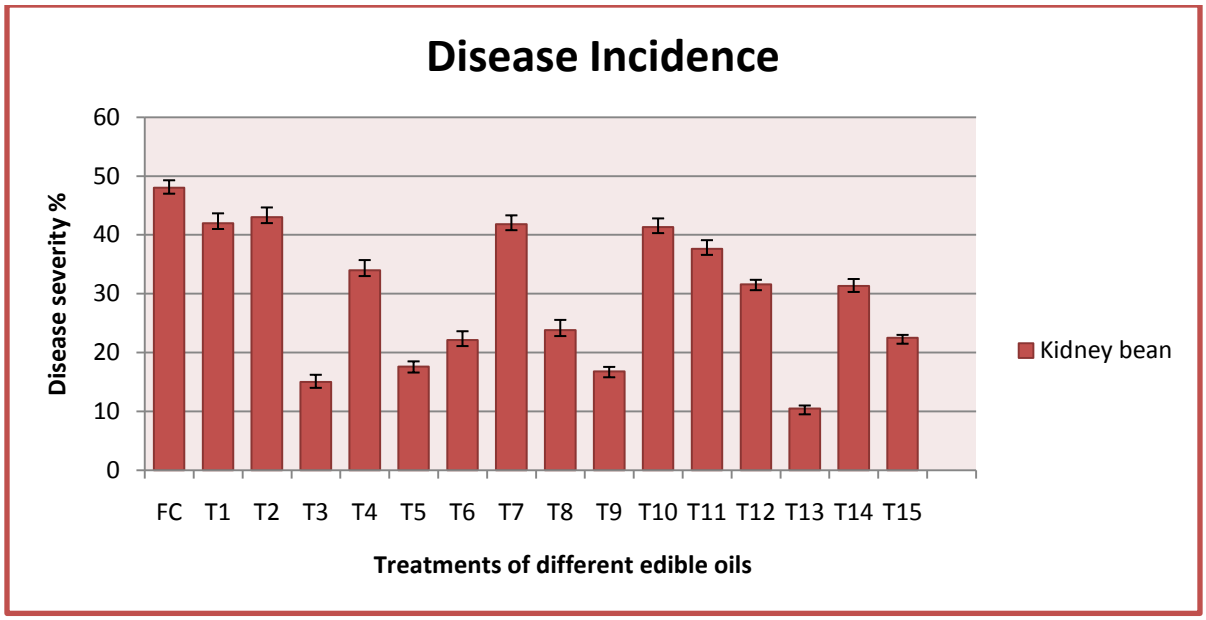


Fig 3.18: Disease severity analysis of Kidney Bean after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

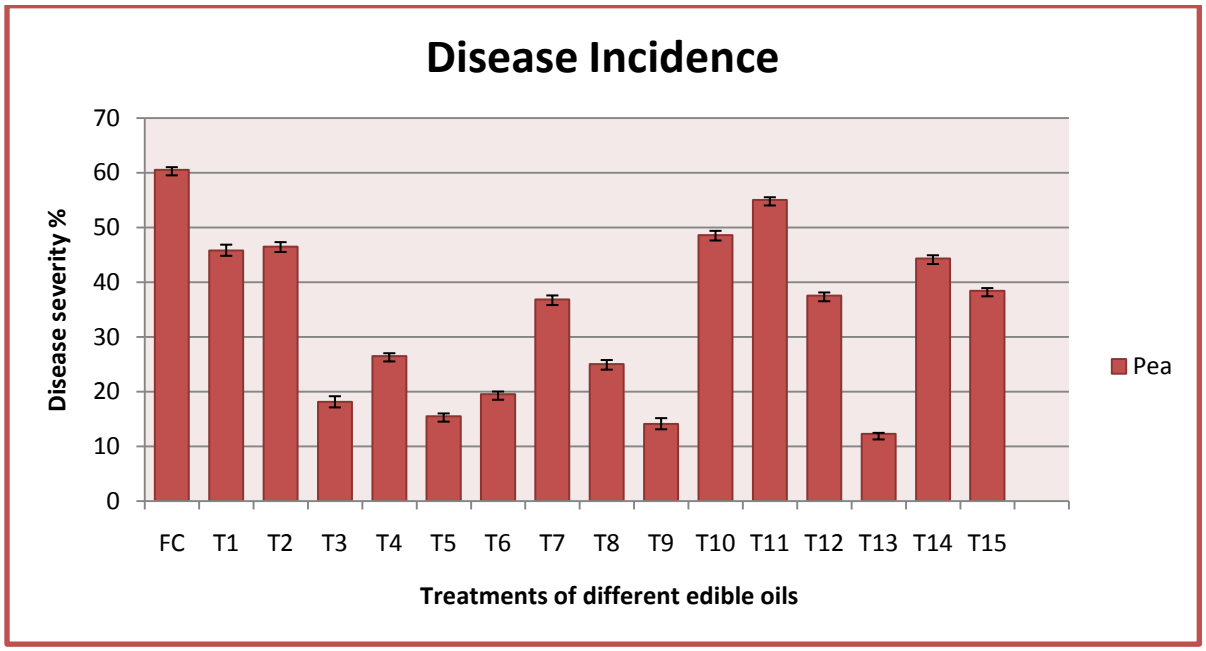


Fig 3.19: Disease severity analysis of Pea after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

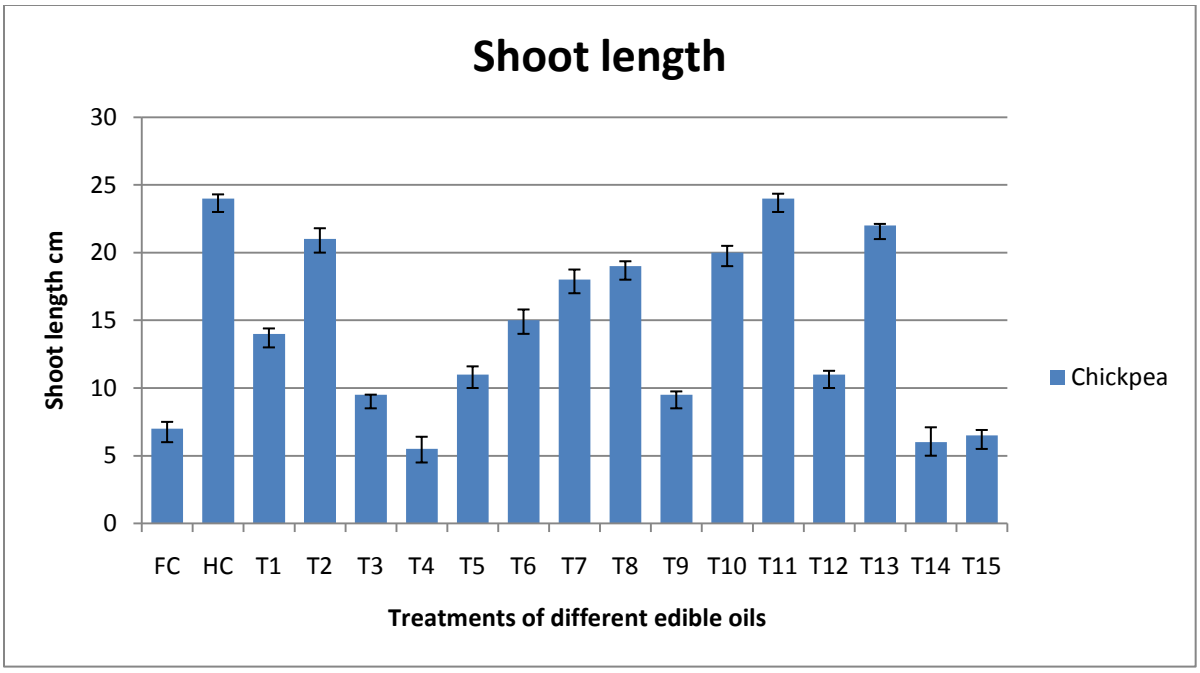


Fig 3.20: Shoot length of Chickpea after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

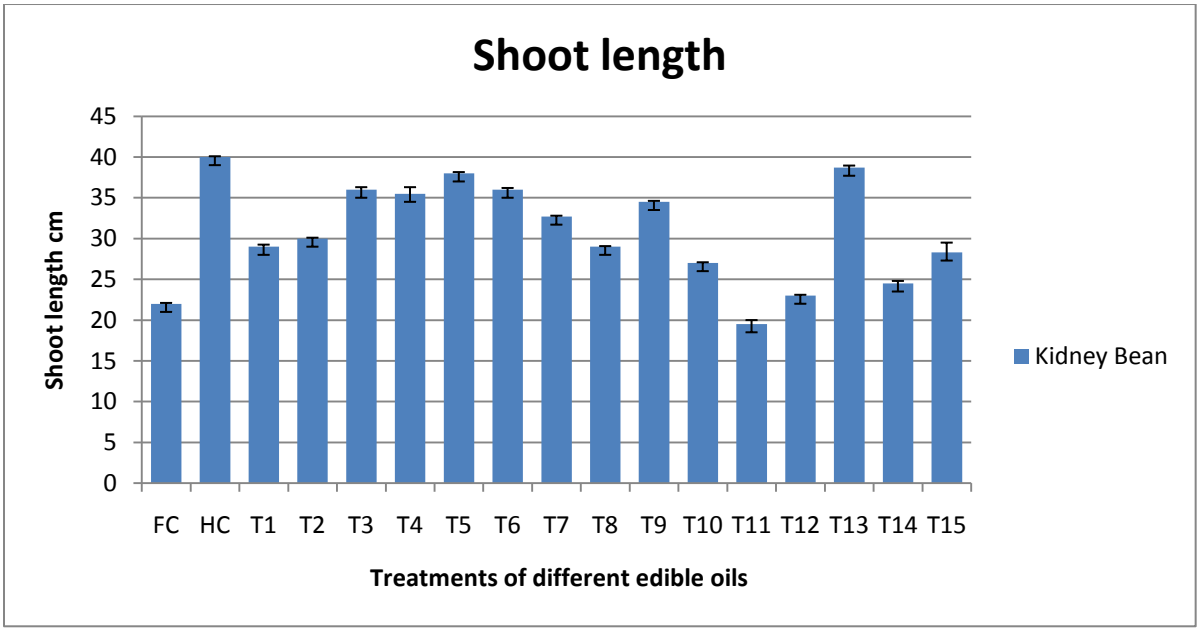


Fig 3.21: Shoot length of Kidney Bean after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

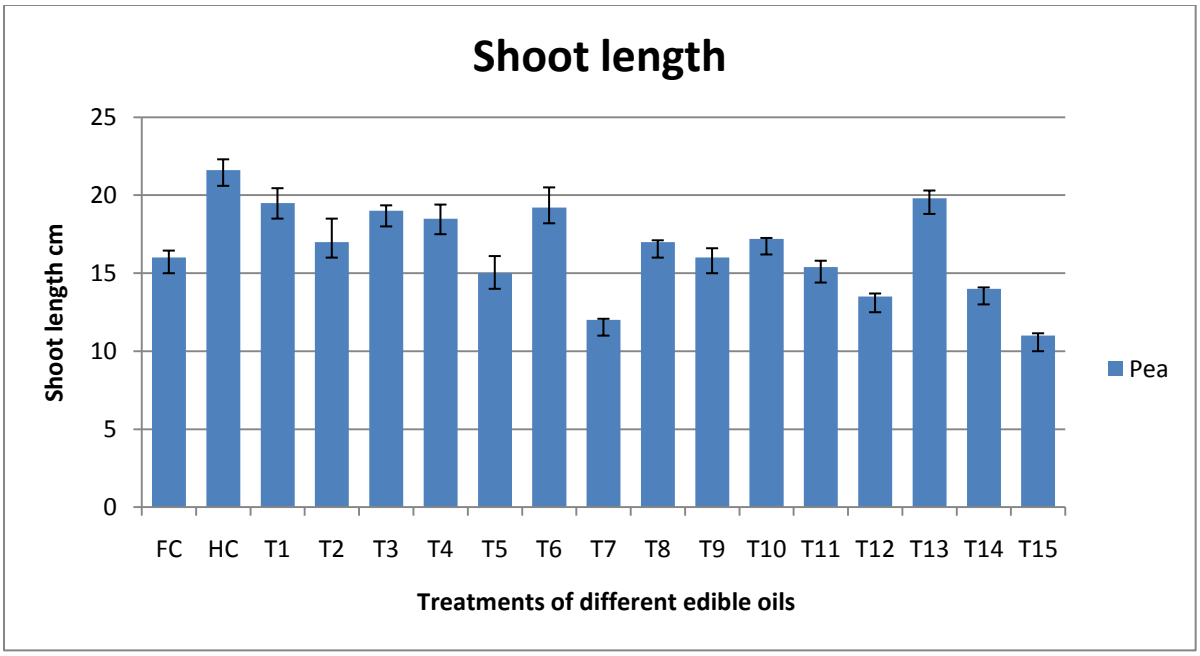


Fig 3.22: Shoot length of Pea after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

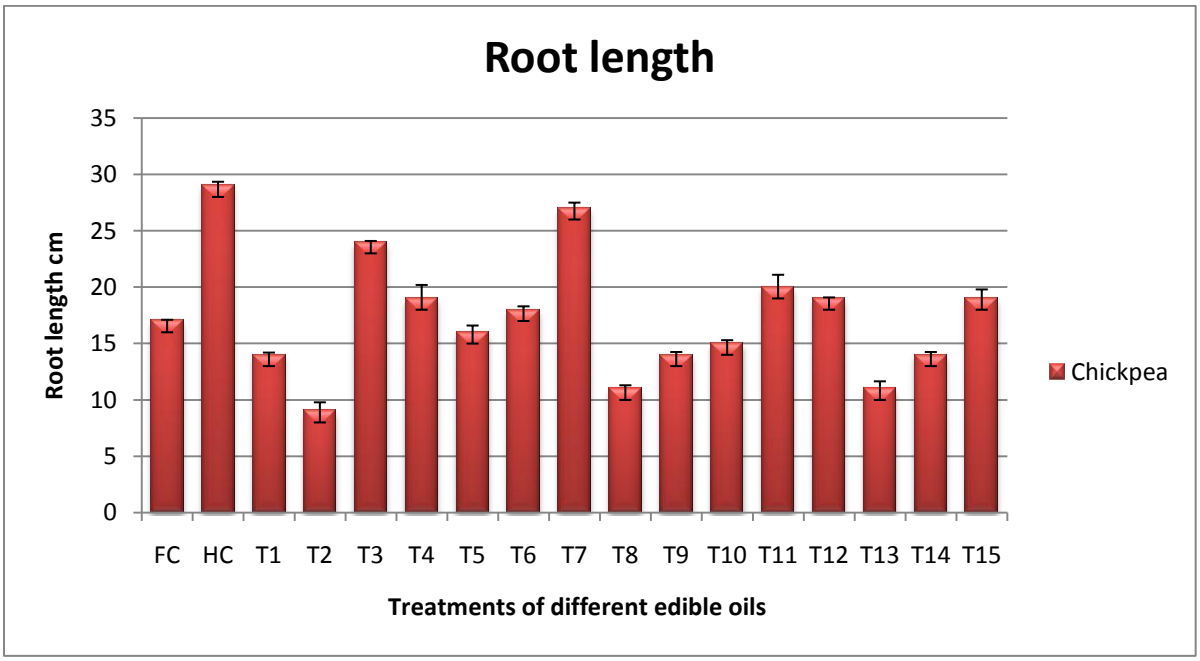


Fig 3.23: Root length of Chickpea after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

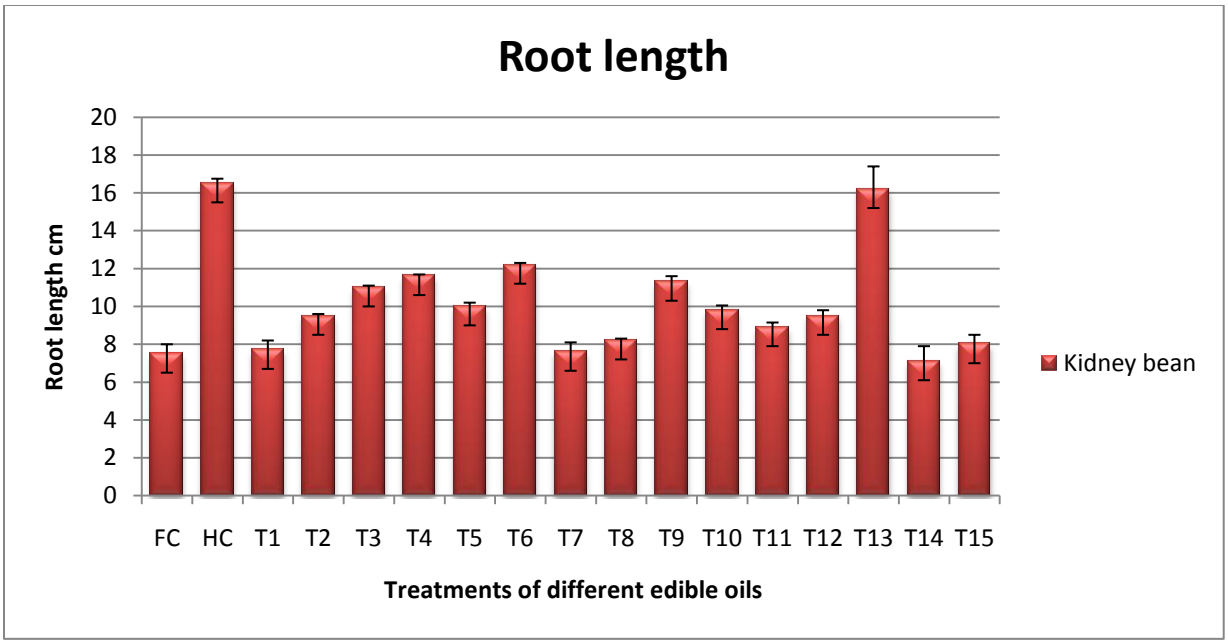


Fig 3.24: Root length of Kidney Bean after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

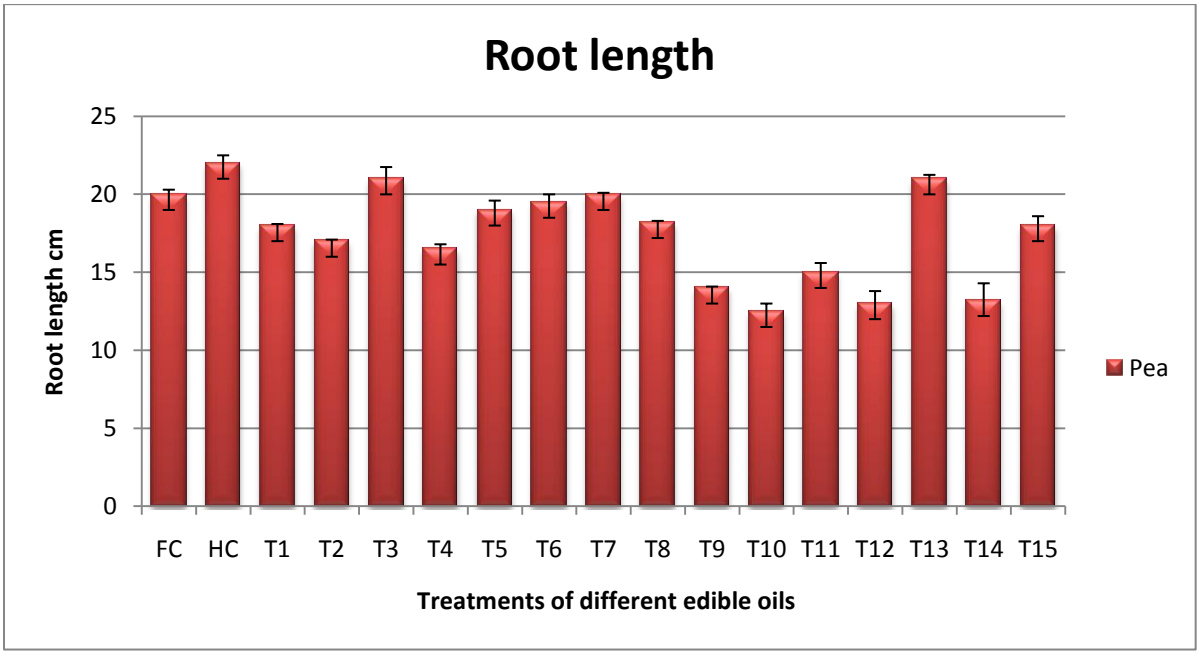


Fig 3.25: Root length of Pea after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

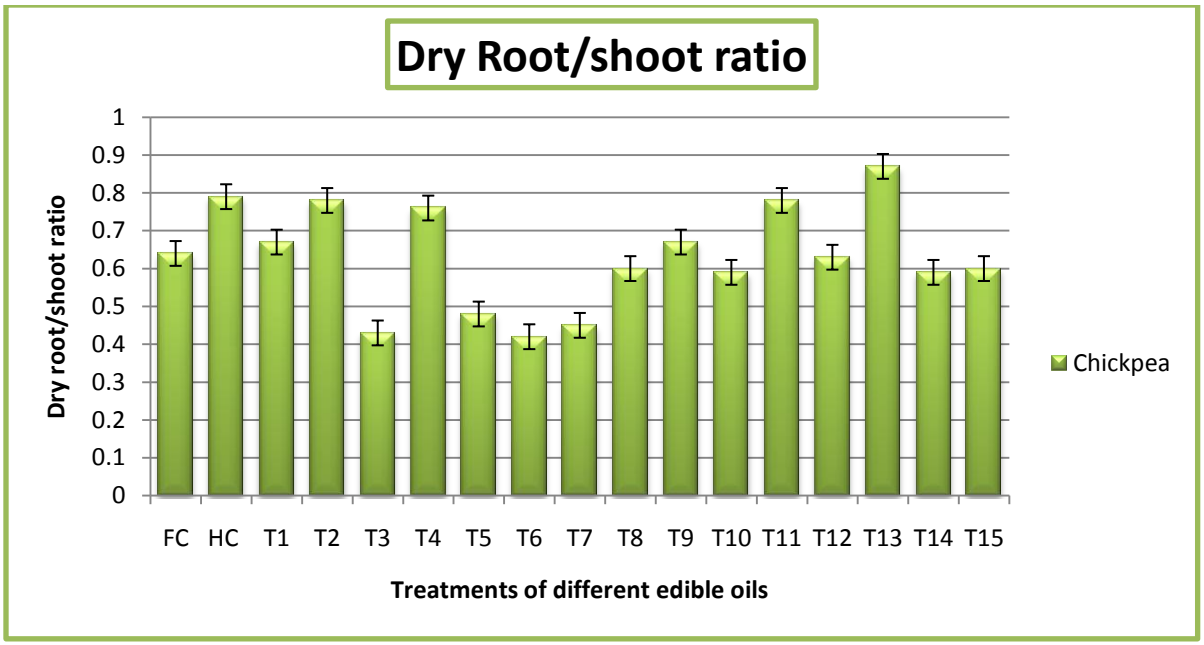


Fig 3.26: Dry root/shoot ratio of Chickpea after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

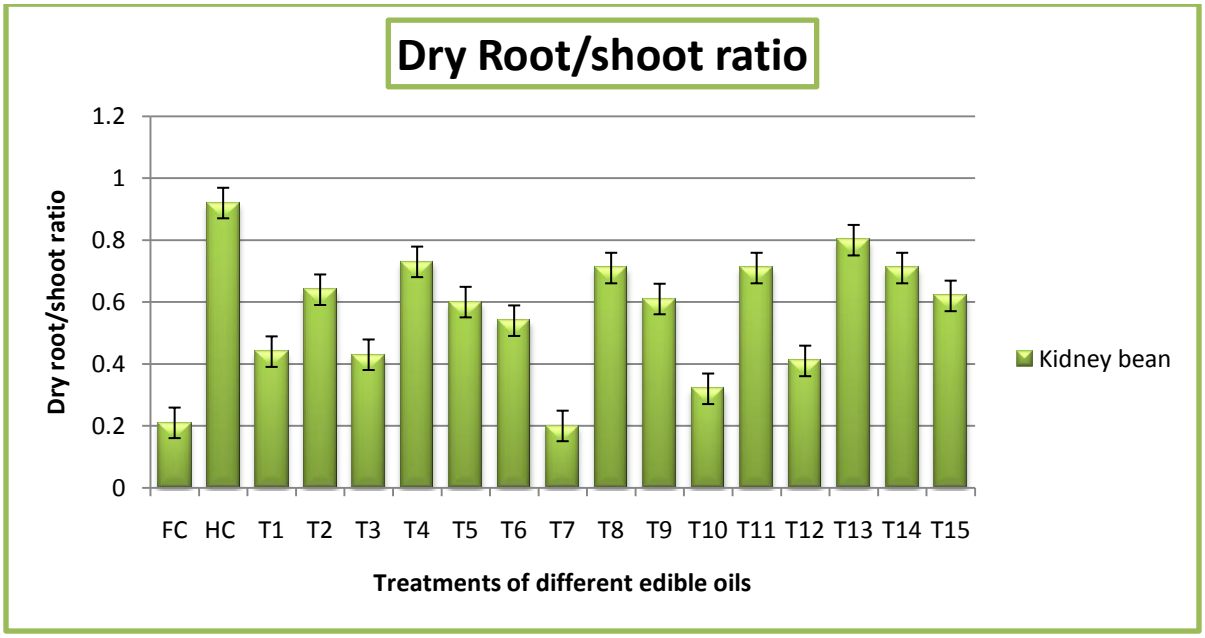


Fig 3.27: Dry root/shoot ratio of Kidney Bean after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

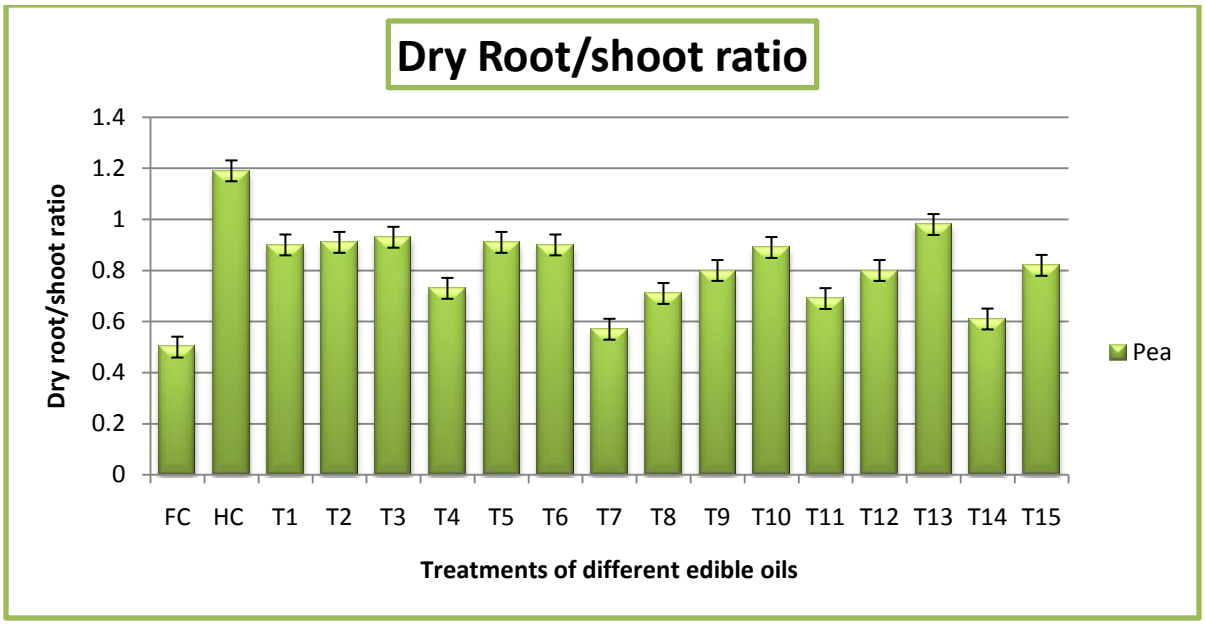


Fig 3.28: Dry root/shoot ratio of Pea after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

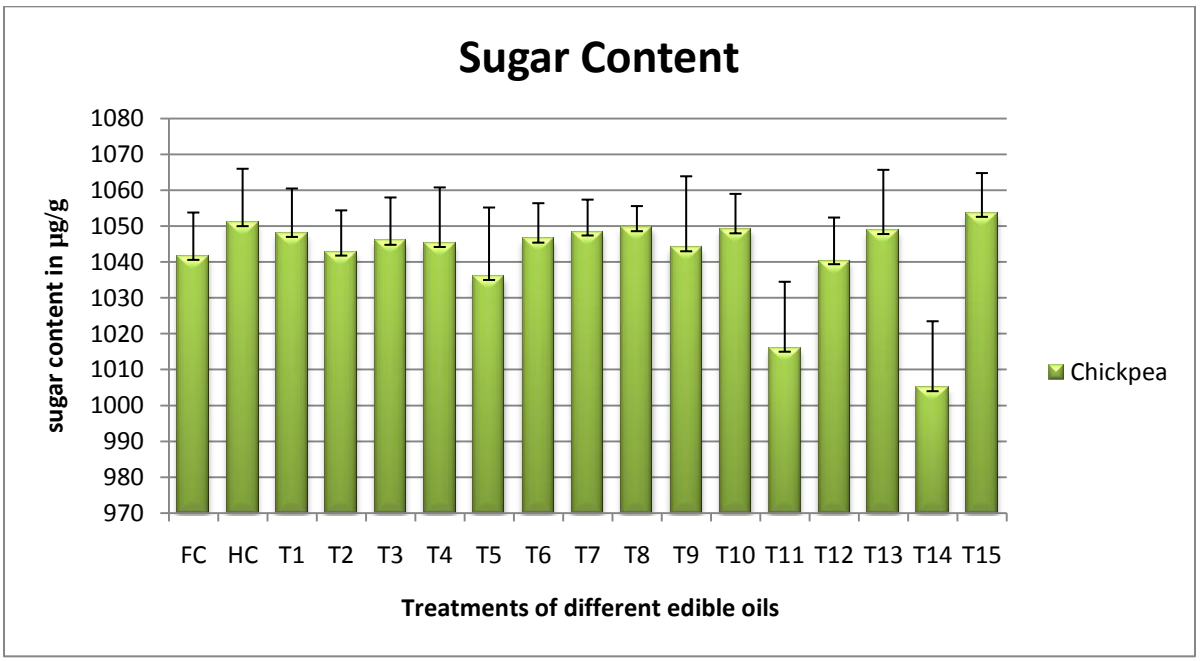


Fig3.29: Sugar content in µg/g of Chickpea after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

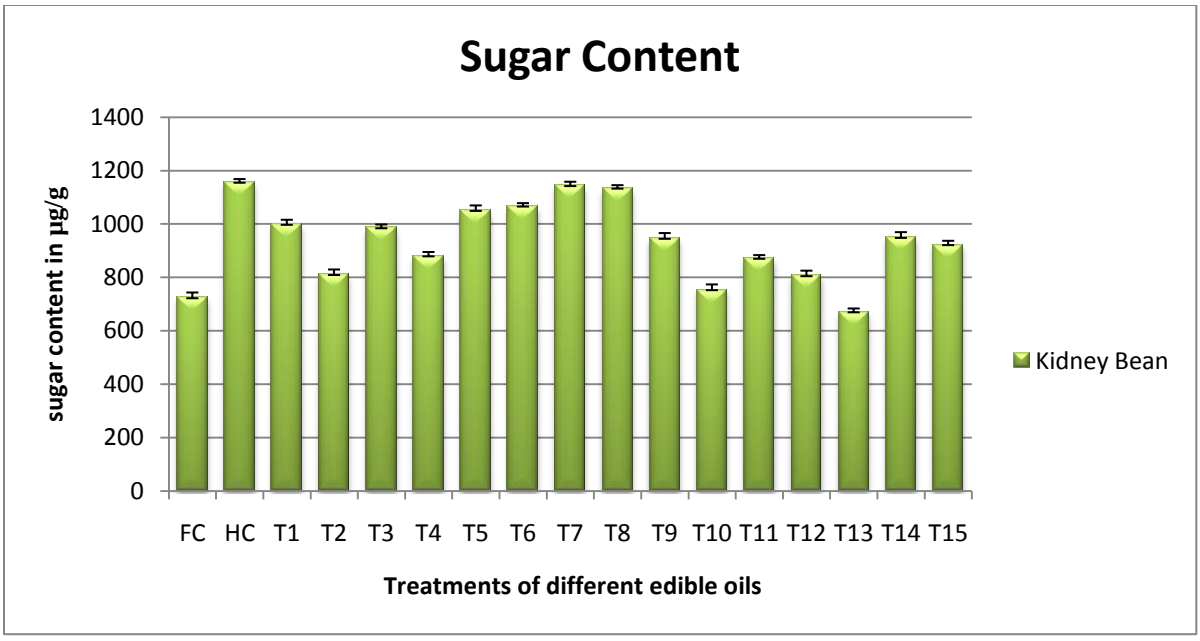


Fig 3.30: Sugar content in µg/g of Kidney Bean after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

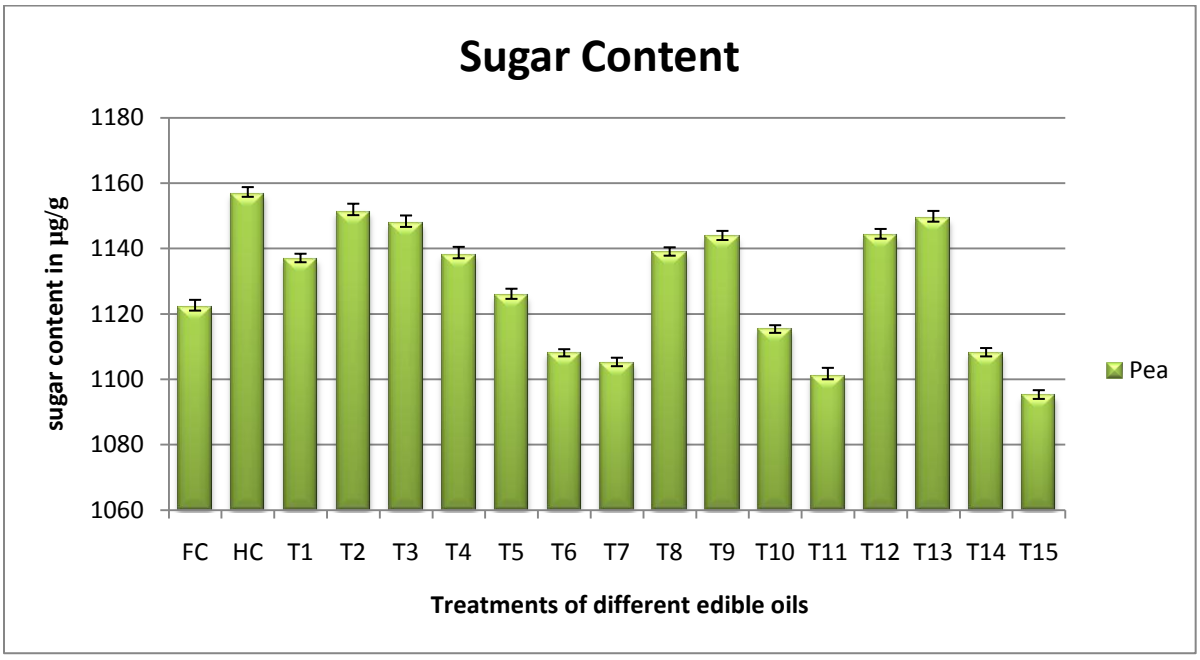


Fig 3.31: Sugar content in µg/g of Pea after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

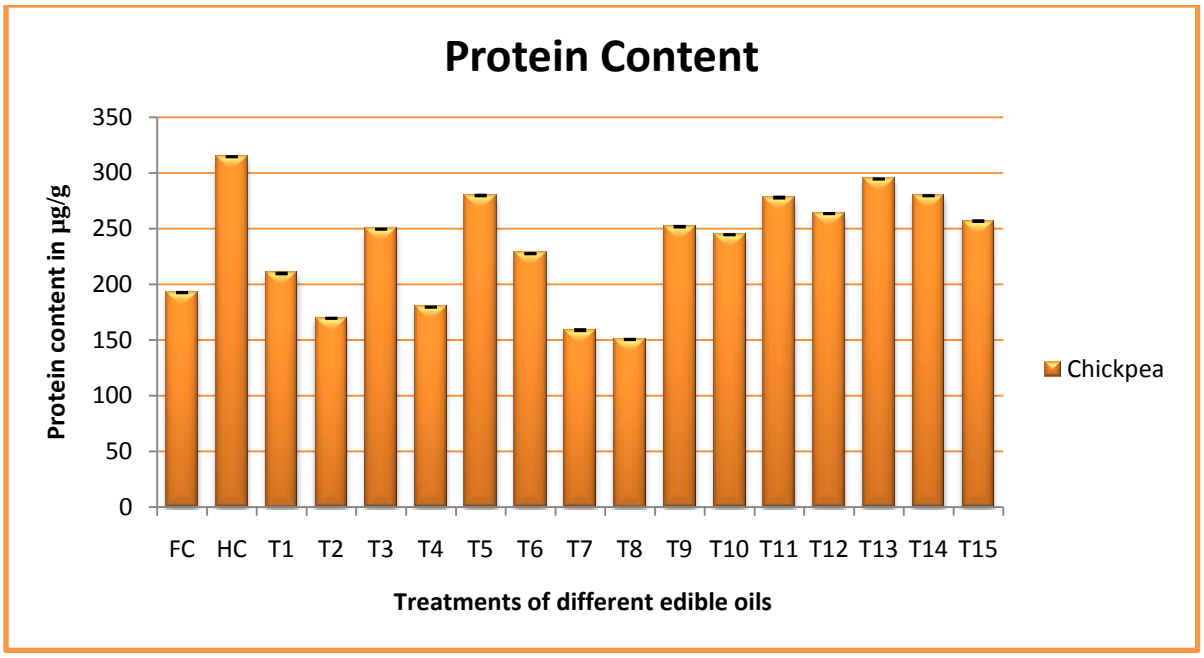


Fig3.32: Protein content in µg/g of Chickpea after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

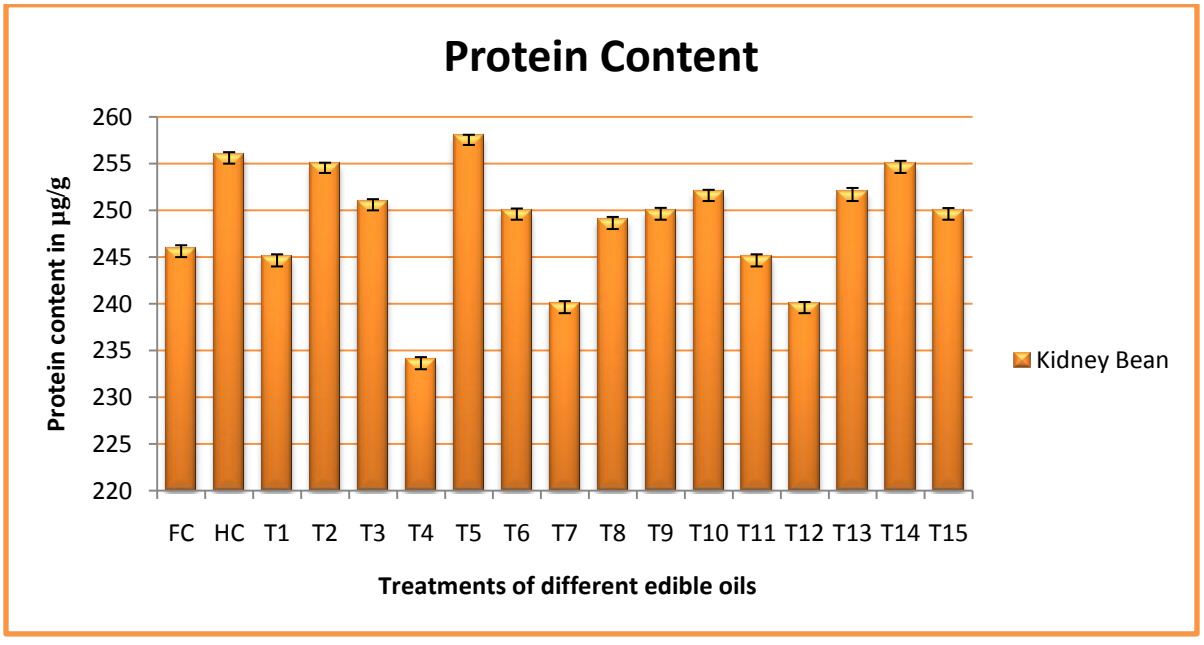


Fig 3.33: Protein content in µg/g of Kidney Bean after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

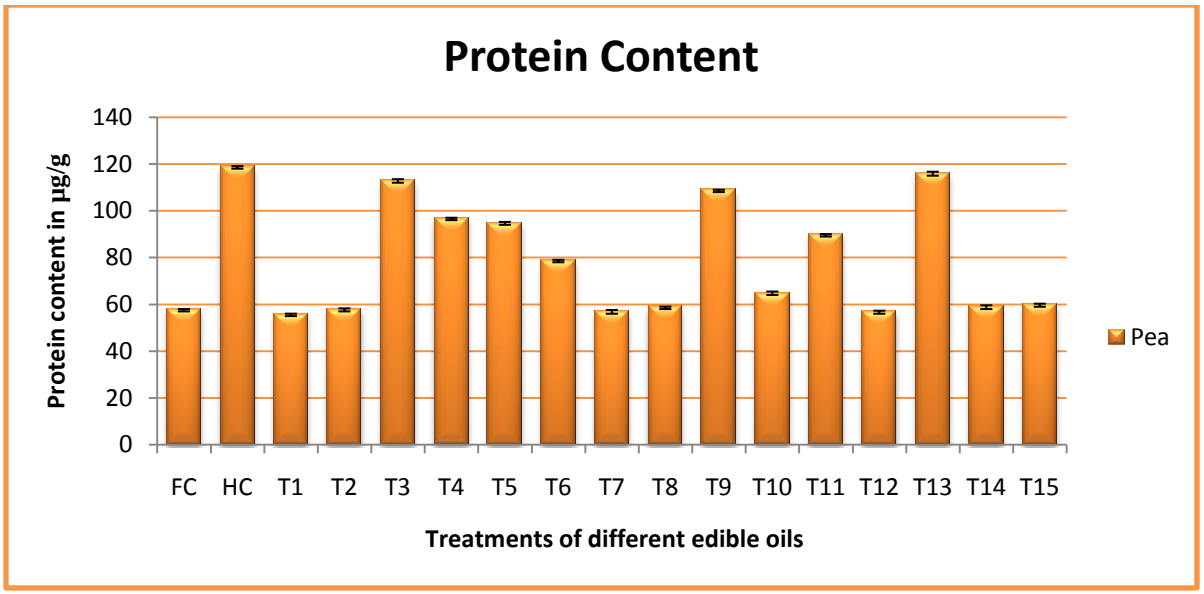


Fig3.34: Protein content in µg/g of Pea after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

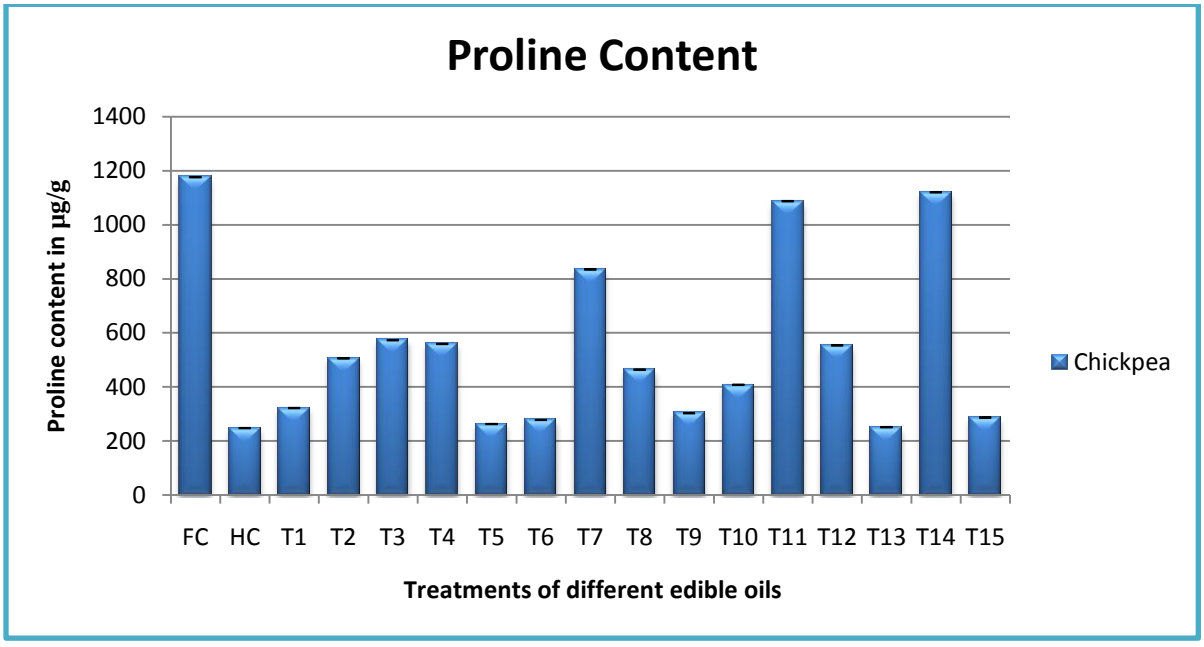


Fig 3.35: Proline content in µg/g of Chickpea after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

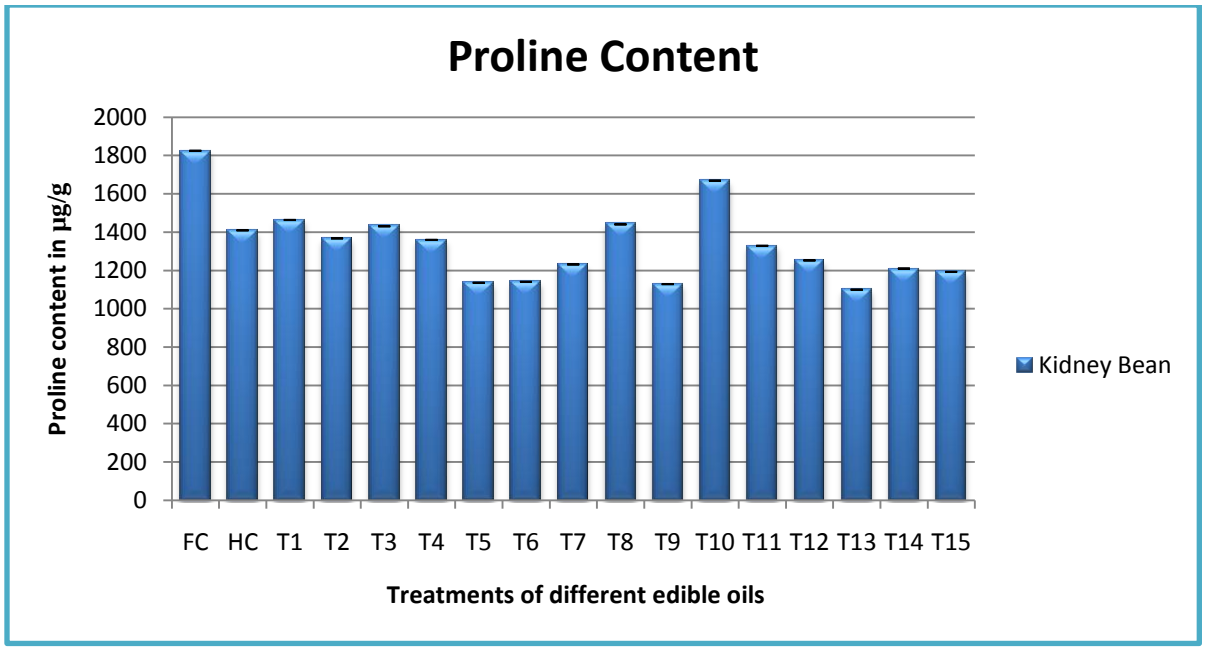


Fig 3.36: Proline content in µg/g of Kidney bean after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

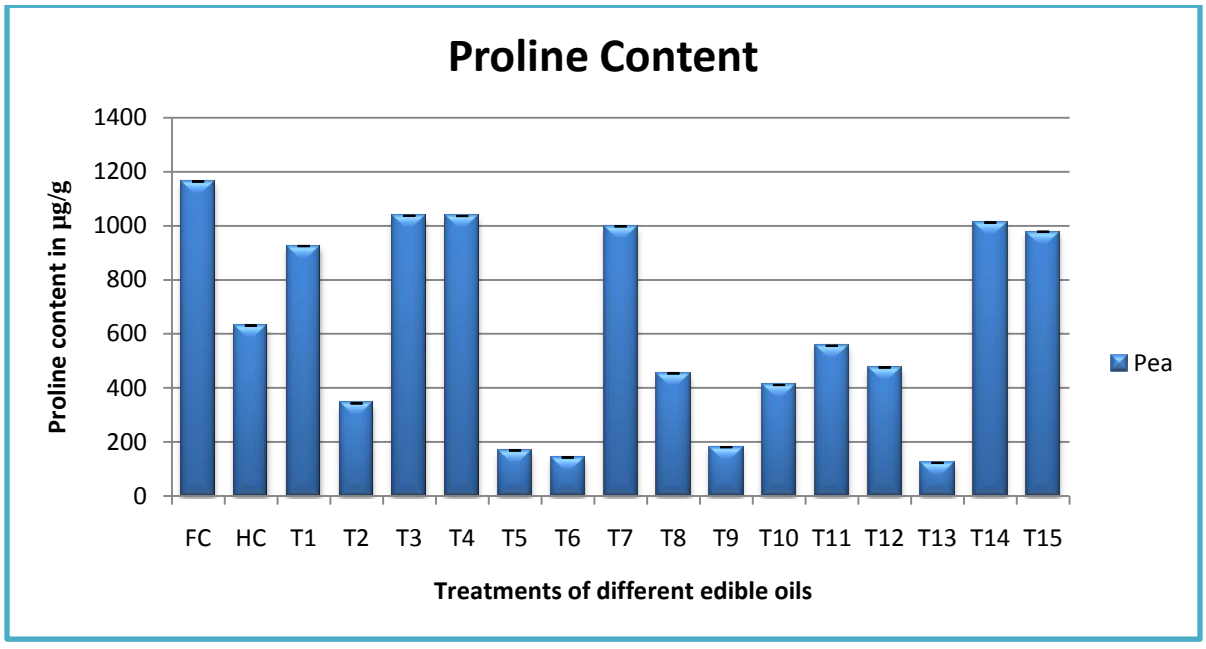


Fig 3.37: Proline content in µg/g of Pea after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

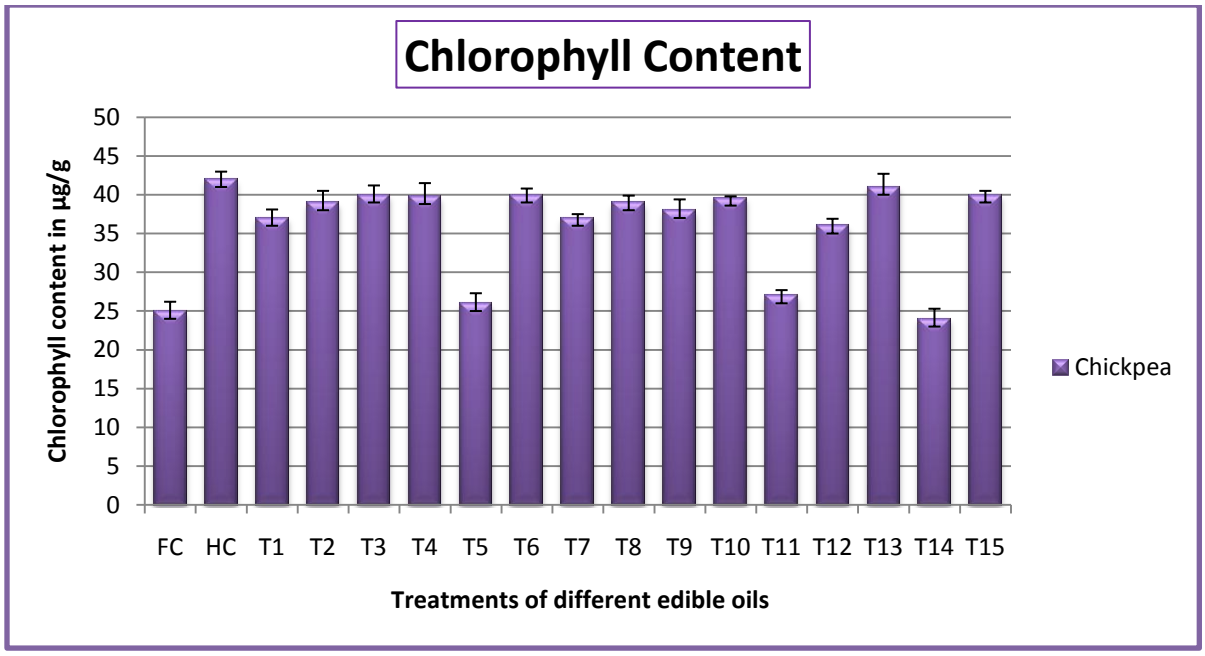


Fig 3.38: Chlorophyll content in µg/g of Chickpea after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

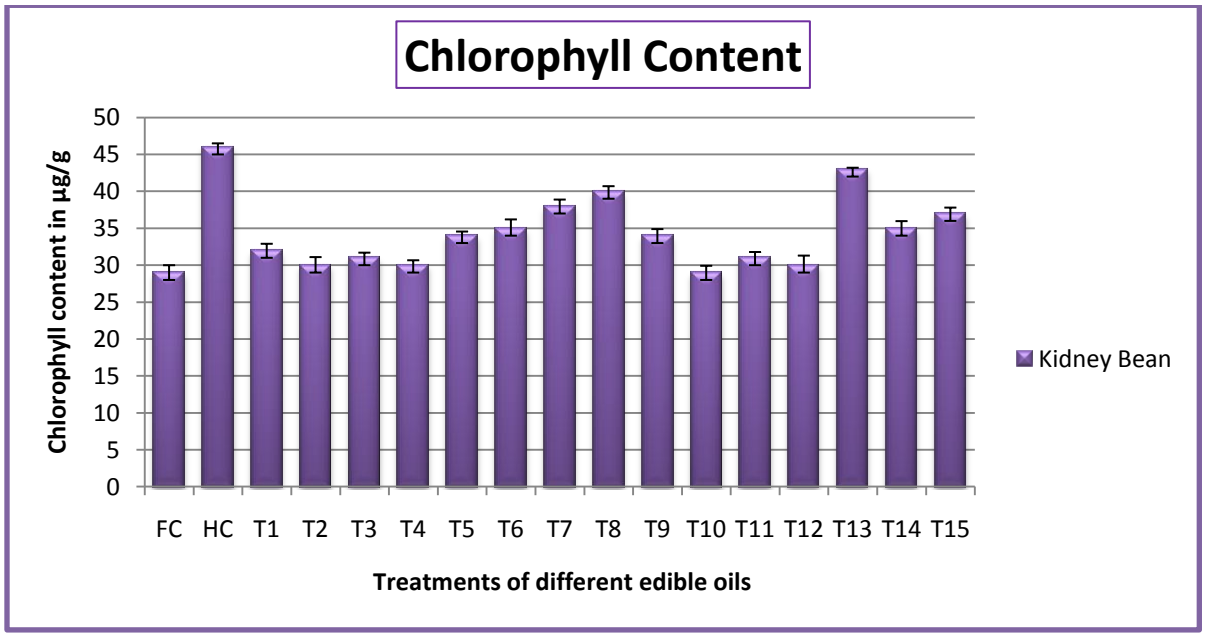


Fig 3.39: Chlorophyll content in µg/g of Kidney Bean after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

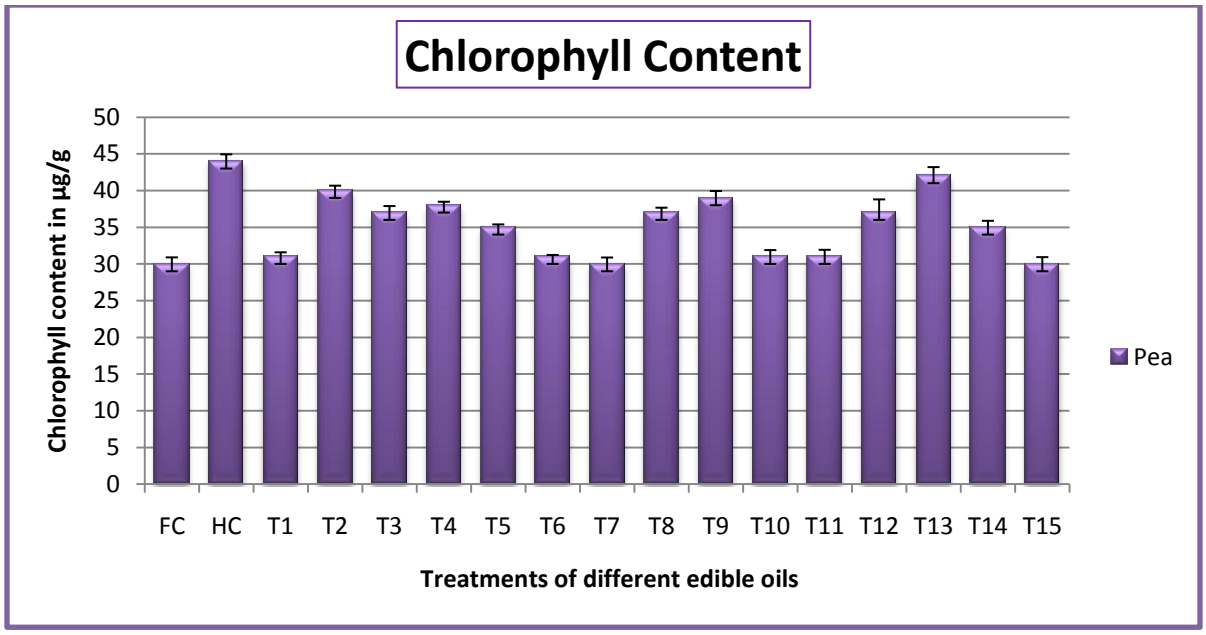


Fig3.40: Chlorophyll content in µg/g of pea after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

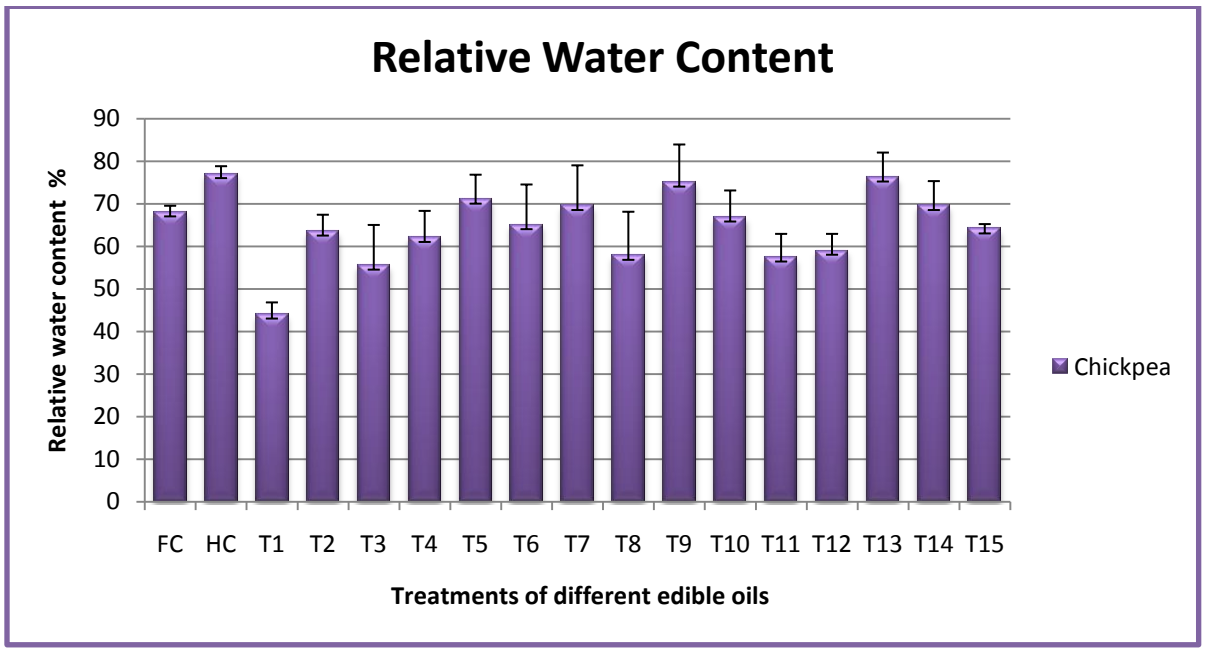


Fig3.41: Relative water content in %age of Chickpea after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

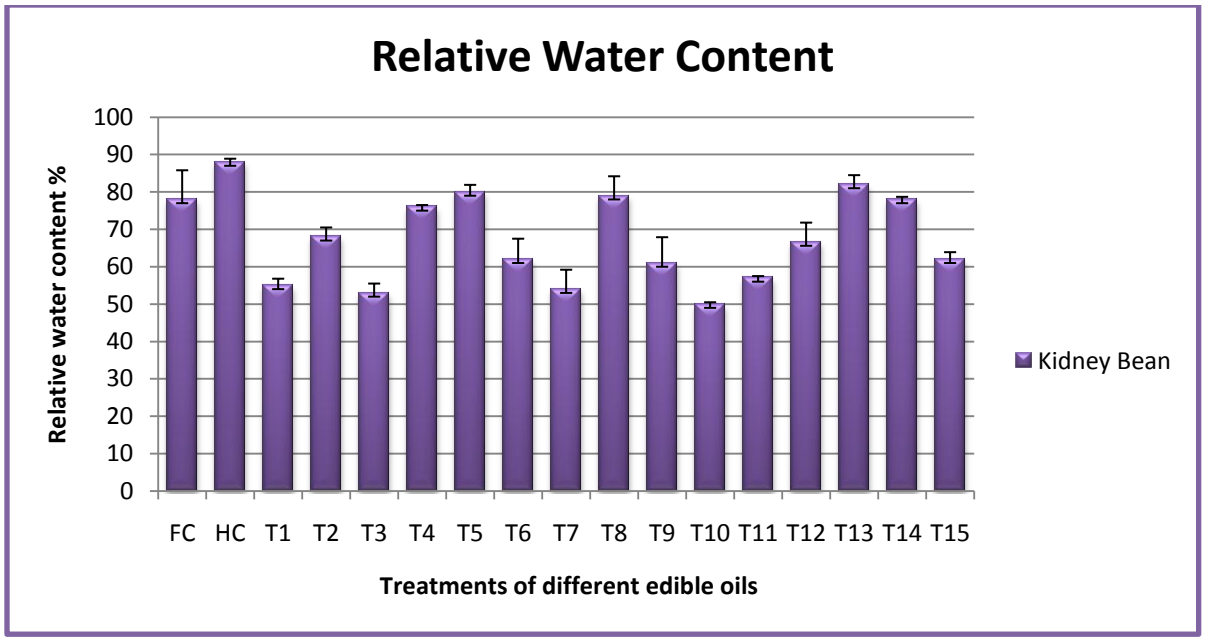


Fig 3.42: Relative water content in %age of Kidney Bean after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

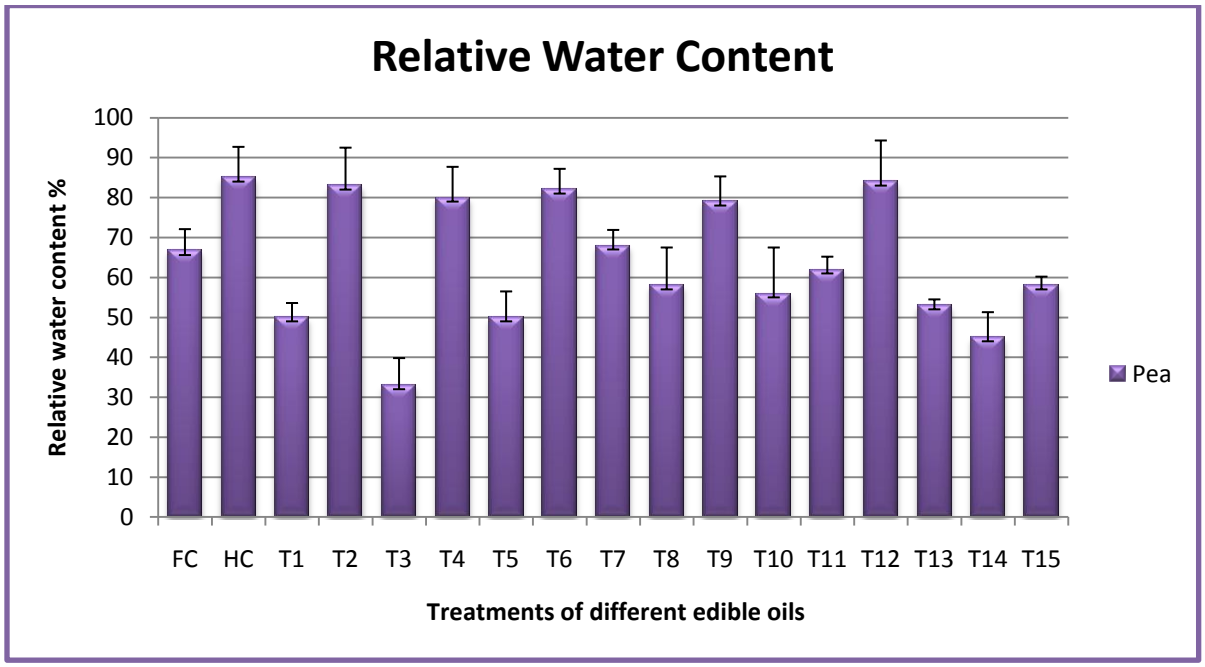


Fig 3.43: Relative water content in %age of Pea after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

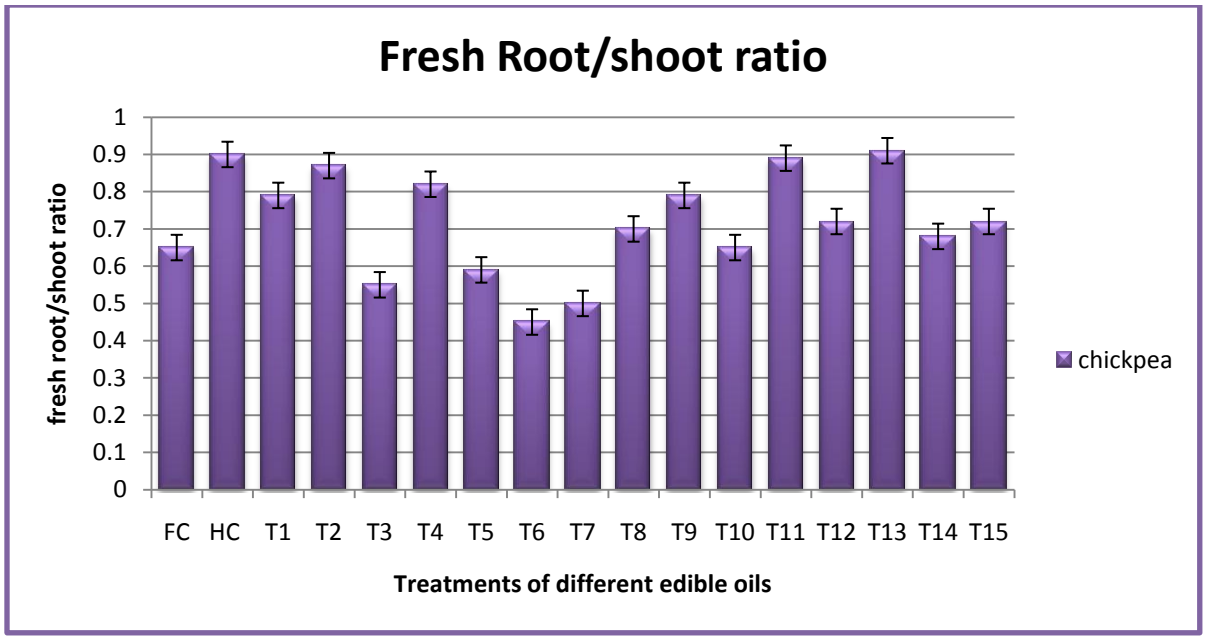


Fig3.44: Fresh root/shoot ratio of Chickpea after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

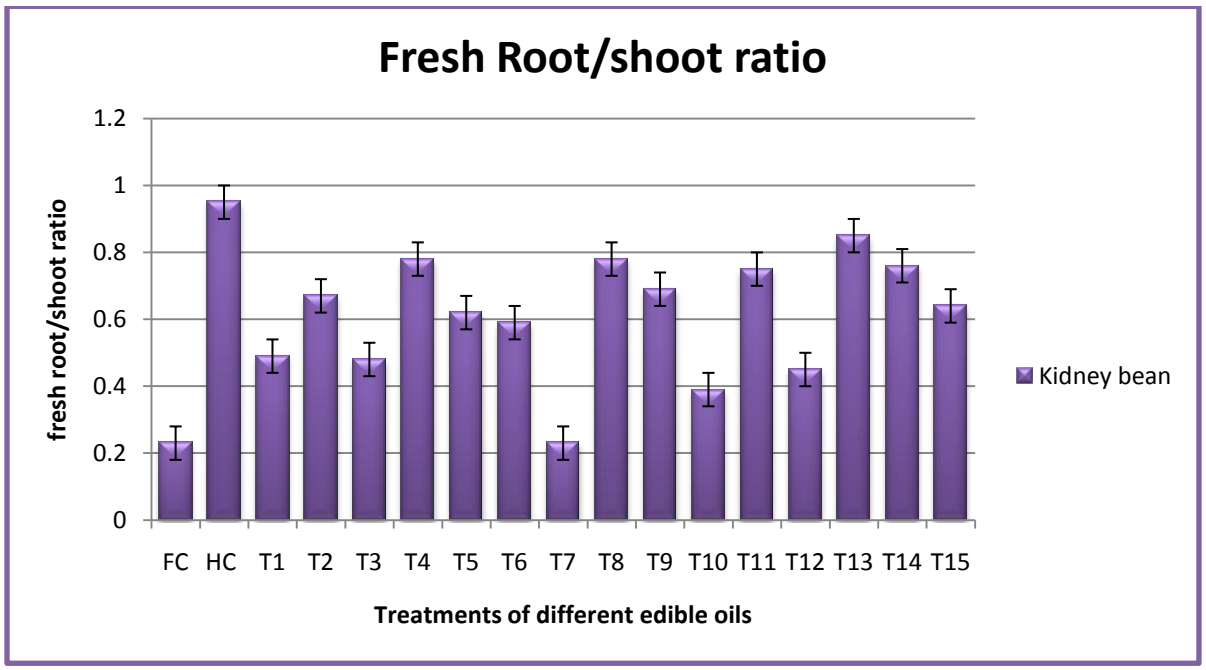


Fig3.45: Fresh root/shoot ratio of Kidney Bean after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

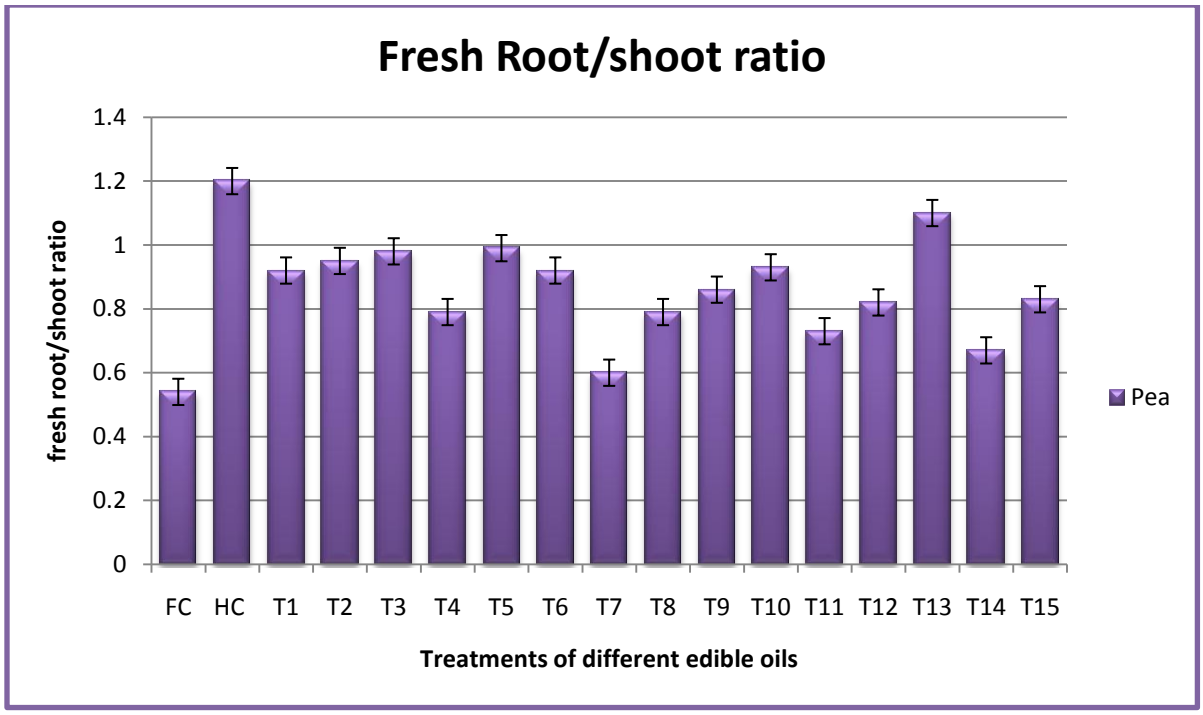


Fig3.46: Fresh root/shoot ratio of Pea after foliar inoculation of fungus and spraying with essential oils (FC = Fungus Control, HC = Healthy Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

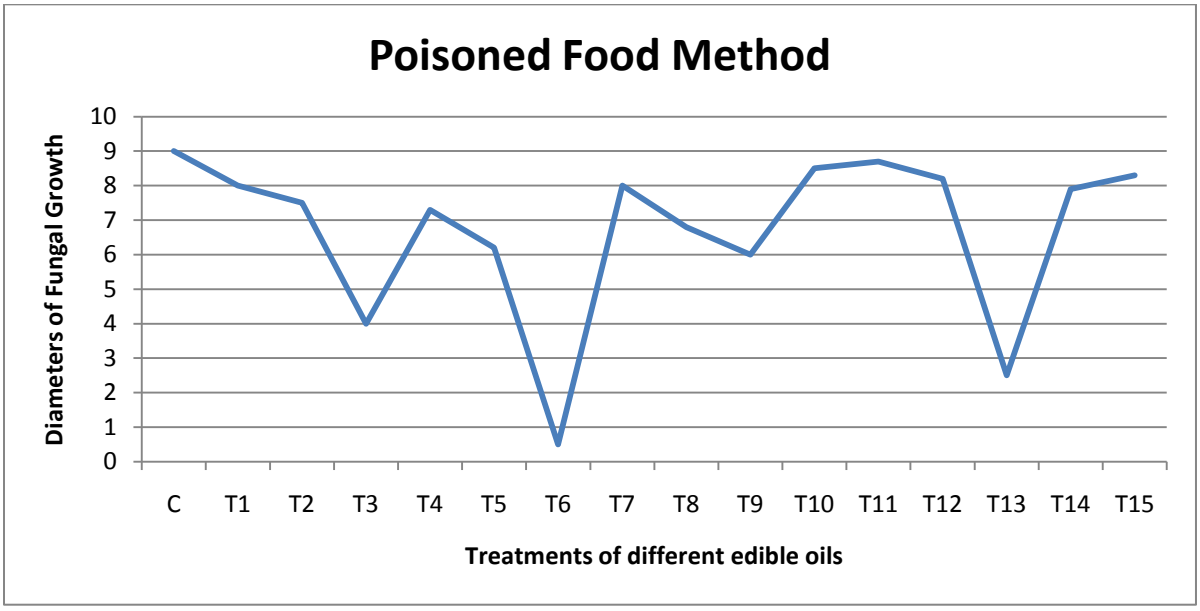


Fig 3.47: Comparative analysis of diameters of growth of fungus on plates treated with essential oils (C= Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).

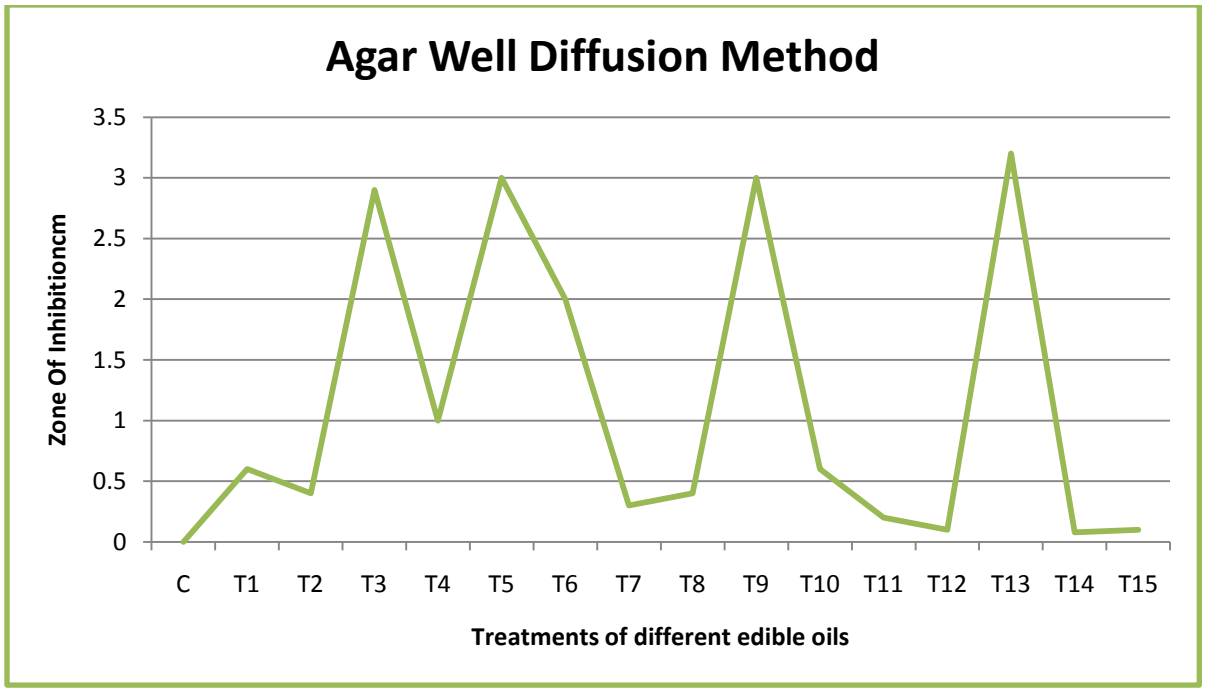
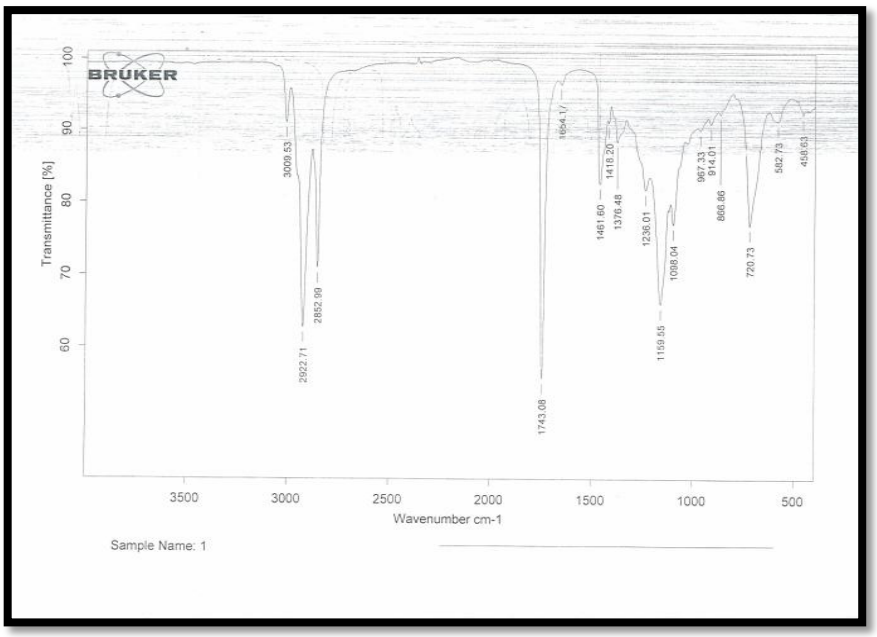


Fig 3.48: Comparative analysis of Zone of Inhibition of fungus on plates treated with essential oils (C= Control, T1-T15 = treatment 1 to 15 as mentioned in table 1.1).



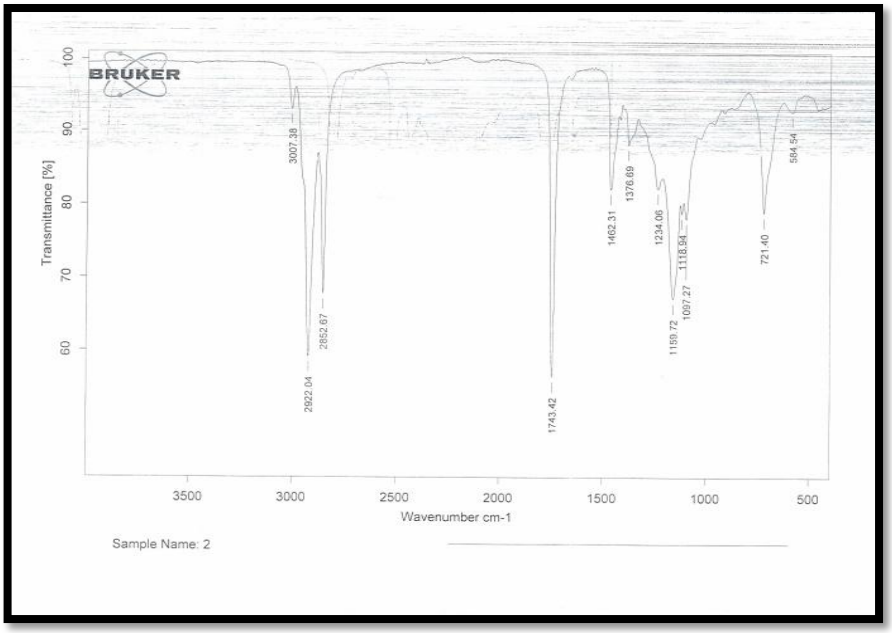
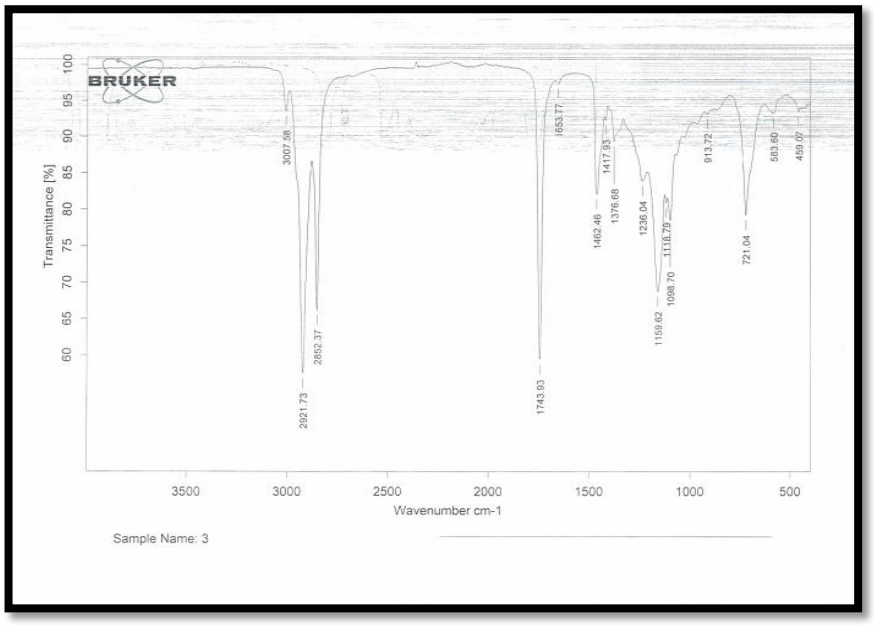


Fig 3.49: FTIR spectra of oils sample 1-2 (as mentioned in table 1.1).



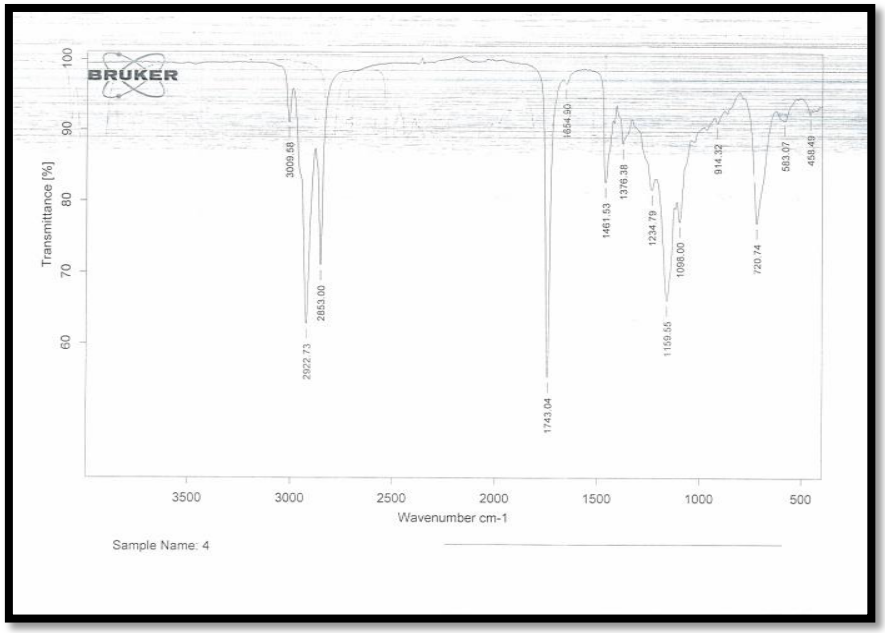
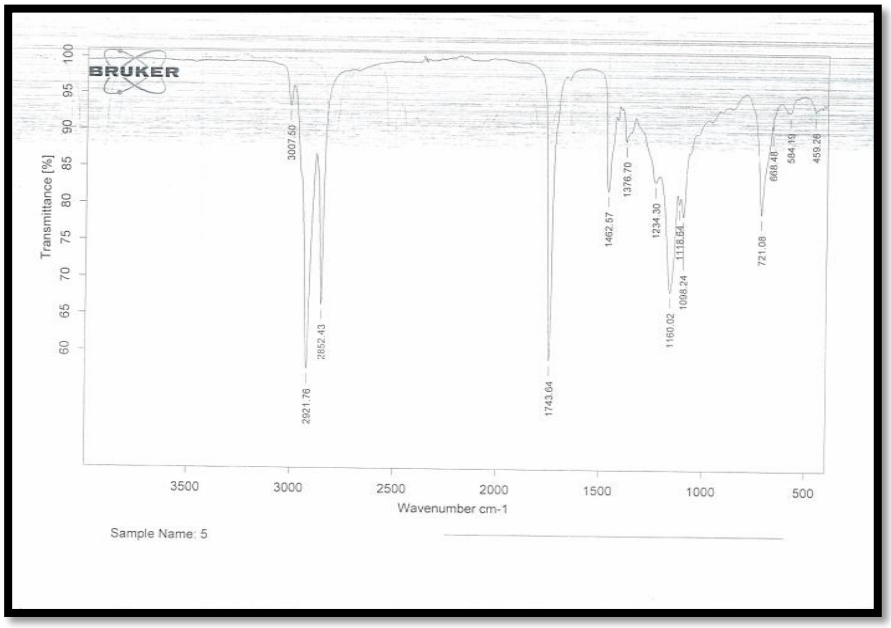


Fig 3.50: FTIR spectra of oils sample 3-4 (as mentioned in table 1.1).



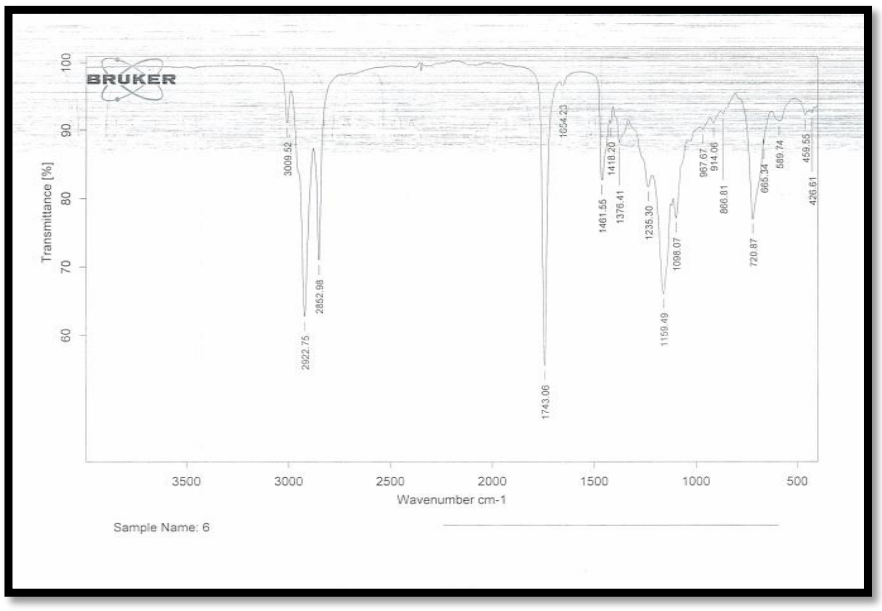
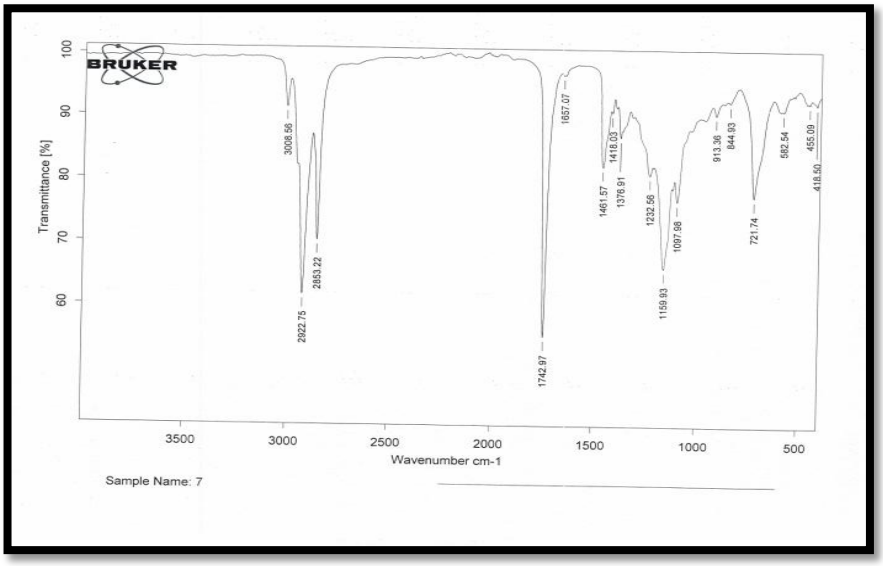


Fig 3.51: FTIR spectra of oils sample 5-6 (as mentioned in table 1.1).



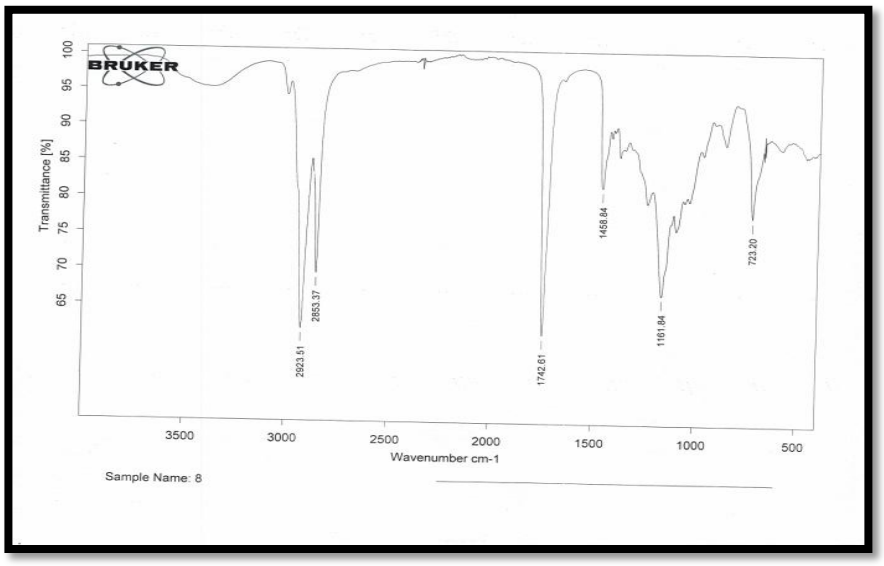
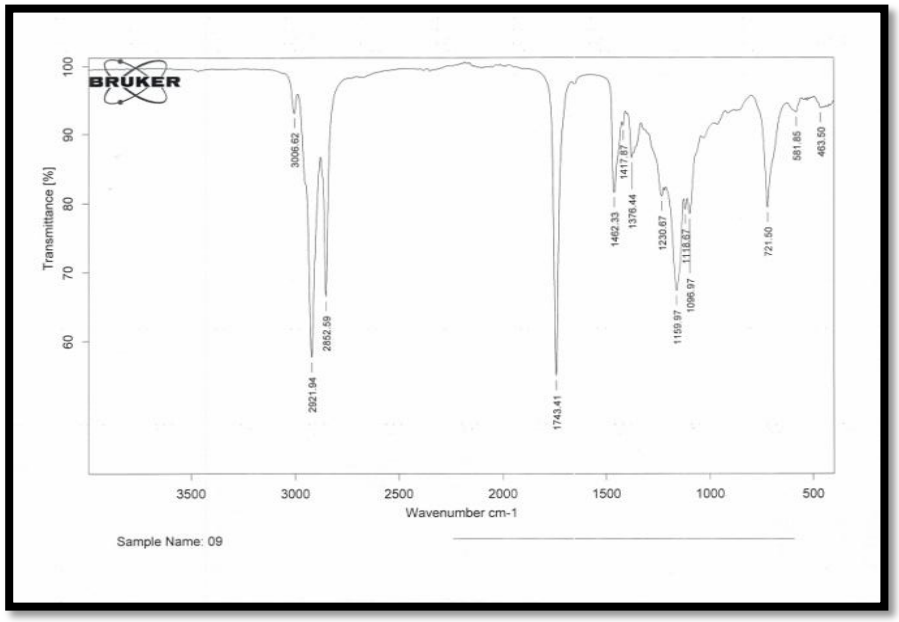


Fig 3.52: FTIR spectra of oils sample 7-8 (as mentioned in table 1.1).



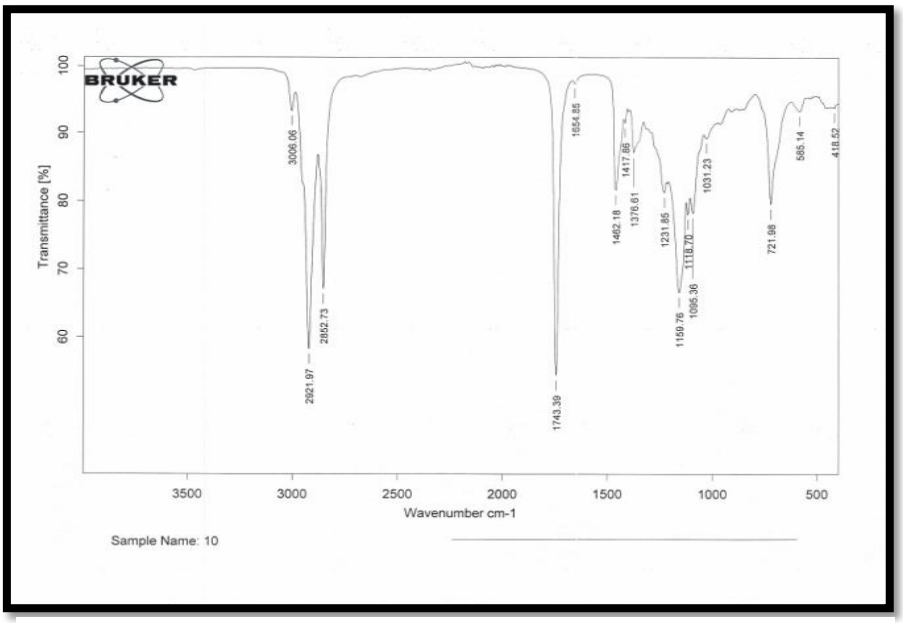
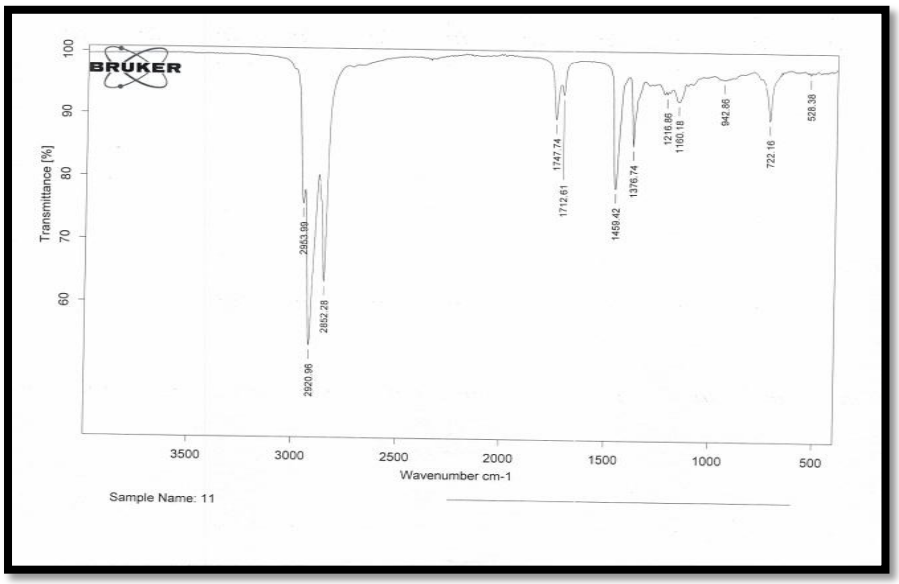


Fig 3.53: FTIR spectra of oils sample 9-10 (as mentioned in table 1.1).



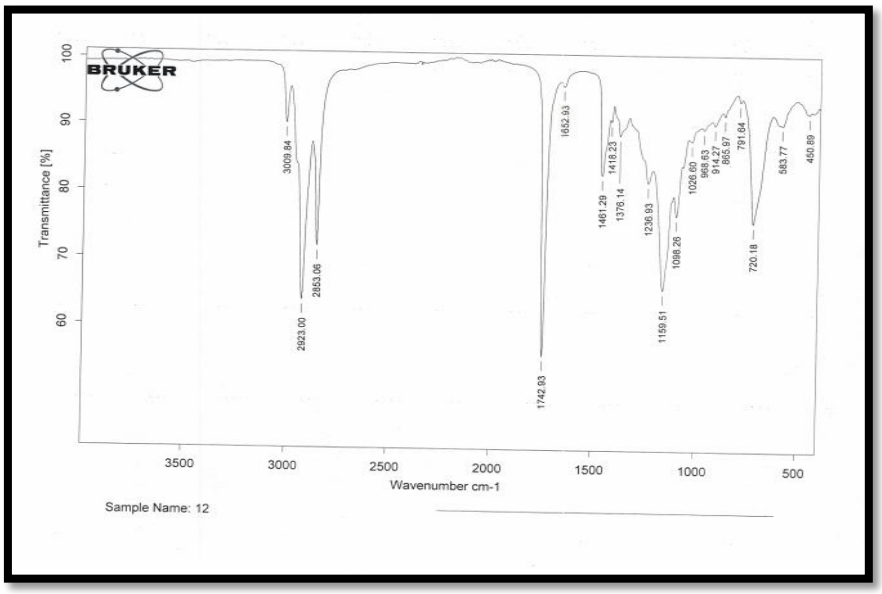
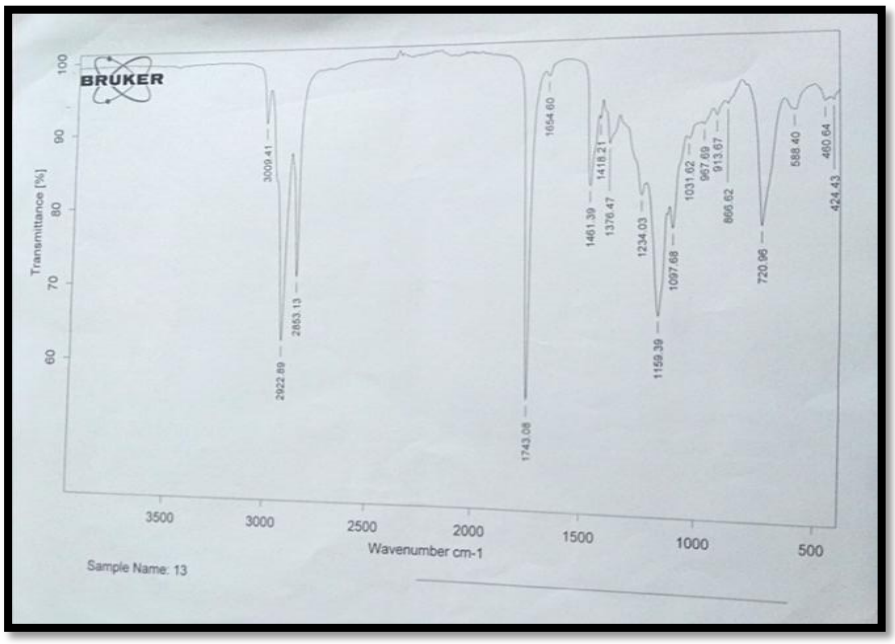


Fig 3.54: FTIR spectra of oils sample 11-12 (as mentioned in table 1.1).



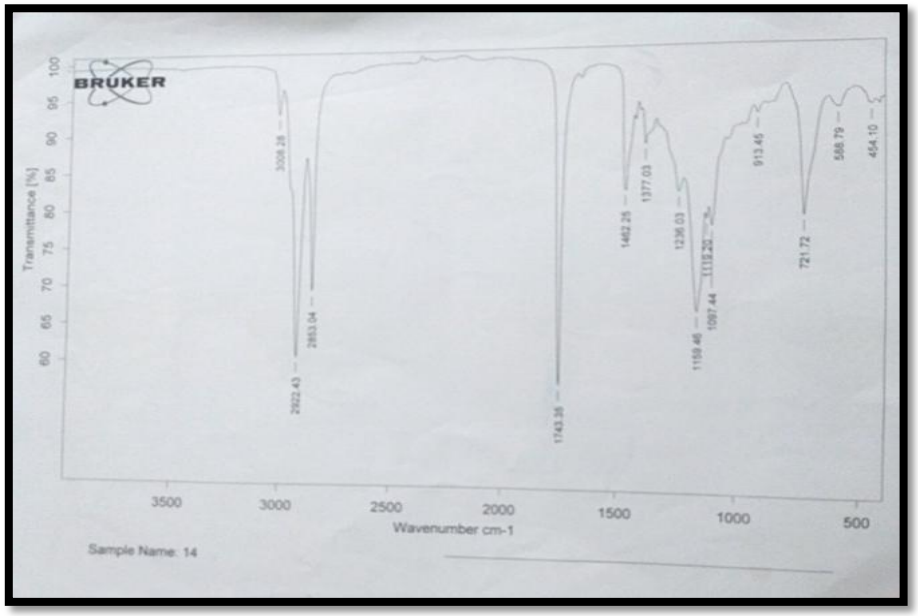


Fig 3.55: FTIR spectra of oils sample 13-14 (as mentioned in table 1.1).

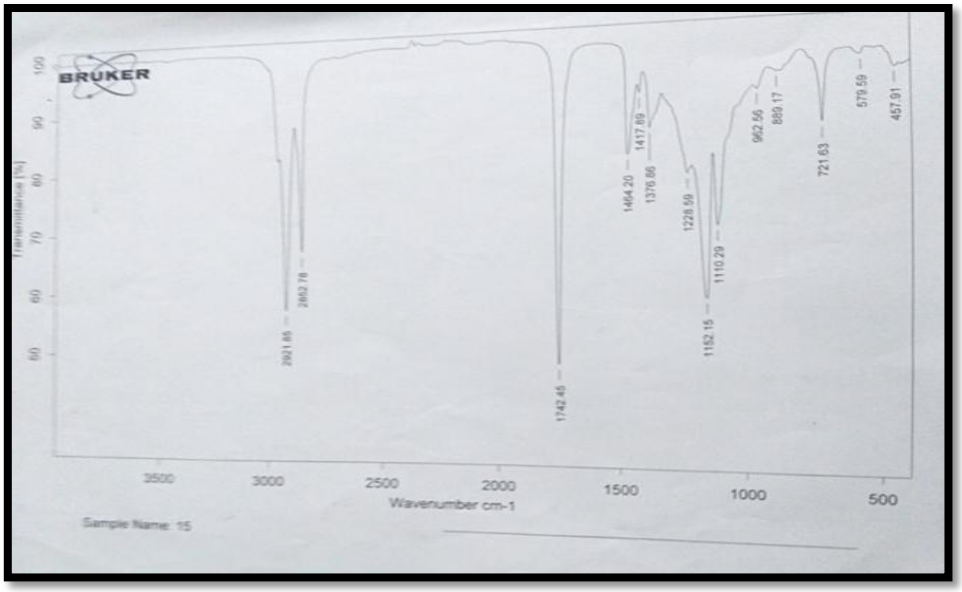


Fig 3.56: FTIR spectra of oils sample 15 (as mentioned in table 1.1).

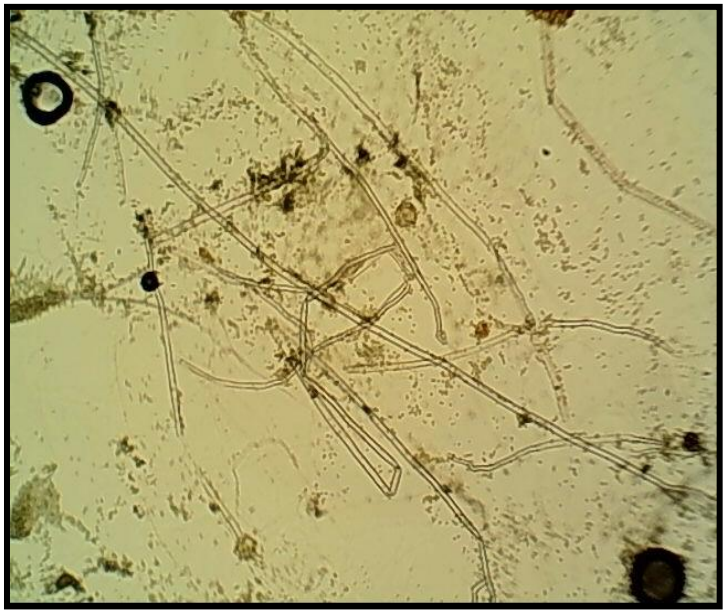


Fig 3.57. Microscopic view of Ascochyta rabiei at 10X

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Fig 3.58 FASTA sequence of *Ascochyta rabiei*

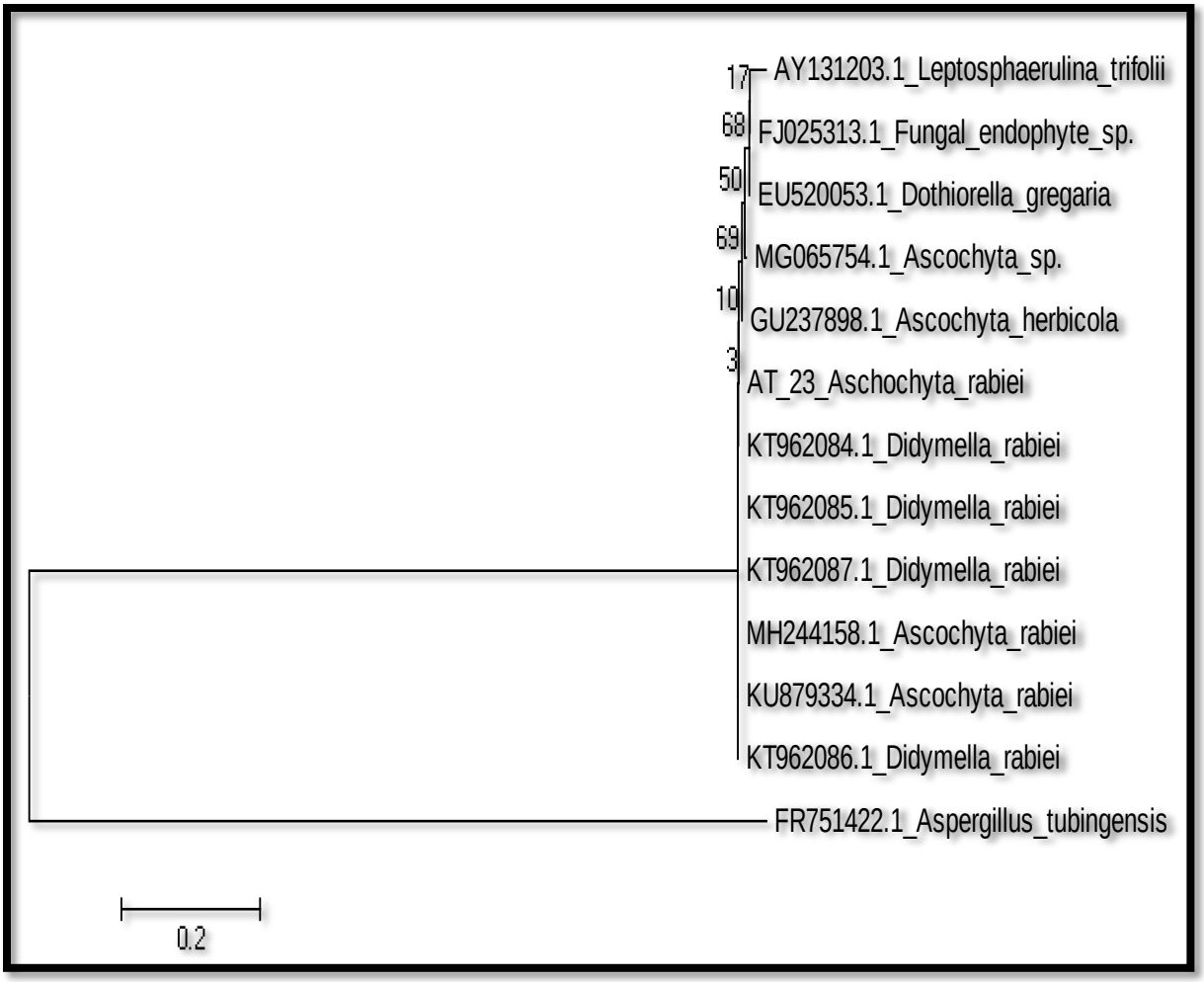

Fig 3.59: Phylogenetic tree showing phylogeny of *Ascochyta rabiei*

Table 3.1: Scientific classification of chickpea

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Fabales
Family	Fabaceae
Genus	*Cicer*
Specie	*Arietinum*.L

Table 3.2: Scientific classification of kidney Bean

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Fabales
Family	Fabaceae
Genus	Phaseolus
Specie	Vulgaris

Table 3.3: Scientific classification of pea

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Fabales
Family	Fabaceae
Genus	Pisum
Specie	Sativum

Table 3.4: Effect of different edible oils on Aschochyta blight disease incidence in chickpea, kidney bean and pea.

	Treatment	Disease incidence %		
		Chickpea	Kidney bean	Pea
1.	FC	54.3±1.73	48.0±1.28	60.5±0.5
2.	T1	51±0.5	42.0±1.66	45.8±1.04
3.	T2	47.5±0.2	43.0±1.66	46.5±0.8
4.	T3	10.3±0.38	15.2±1.22	18.1±1.04
5.	T4	40.2±0.2	34.1±1.72	26.5±0.5
6.	T5	13.2±0.5	17.6±0.9	15.5±0.5
7.	T6	21±1.0	22.1±1.52	19.5±0.5
8.	T7	28.3±0.9	41.8±1.52	36.8±0.76
9.	T8	43.4±0.5	23.8±1.75	25±0.76
10.	T9	18.7±0.2	16.8±0.76	14.1±1.04
11.	T10	43.6±0.3	41.3±1.5	48.6±0.76
12.	T11	53.5±0.4	37.6±1.5	55. ±+0.5
13.	T12	40.3±0.1	31.6±0.76	37.5±0.6
14.	T13	8.2±0.3	10.5±0.5	12.25±0.2
15.	T14	34±0.3	31.3±1.2	44.3±0.6
16.	T15	31.5±0.4	22.5±0.5	38.4±0.5

Values are the average of three replicates

± indicate standard deviation /error.

Table 3.5: Shoot length of chickpea, kidney bean and Pea after foliar inoculation and application of edible oils

	Treatment	Shoot length cm		
		Chickpea	Kidney bean	Pea
1.	FC	7±0.5	22±0.1	16±0.45
2.	HC	24±0.3	40±0.09	21.6±0.7
3.	T1	14±0.4	29±0.25	19.5±0.95
4.	T2	21±0.8	30.2±0.1	17±1.5
5.	T3	9.5±0.01	36±0.3	19±0.35
6.	T4	5.5±0.9	35.5±0.8	18.5±0.9
7.	T5	11±0.6	38±0.15	15±1.1
8.	T6	15±0.8	36±0.2	19.2±1.3
9.	T7	18±0.75	32.7±0.1	12±0.08
10	T8	19±0.36	29±0.07	17±0.11
11	T9	9.5±0.25	34.5±0.12	16±0.6
12	T10	20±0.5	27±0.09	17.2±0.06
13	T11	24±0.35	19.5±0.5	15.4±0.4
14	T12	11±0.27	23±0.1	13.5±0.2
15	T13	22±0.12	38.7±0.25	19.8±0.5
16	T14	6±1.1	24.5±0.3	14±0.09
17	T15	6.5±0.4	28.3±1.2	11±0.15

Values are the average of three replicates

± indicate standard deviation /error.

Table 3.6: Root length of chickpea, kidney bean and Pea after foliar inoculation and application of edible oils

	Treatment	Root length cm		
		Chickpea	Kidney bean	Pea
1.	FC	17±0.11	7.5±0.5	20±0.3
2.	HC	29±0.35	16.5±0.25	22±0.5
3.	T1	14±0.2	7.7±0.5	18±0.1
4.	T2	9±0.79	9.5±0.1	17±0.1
5.	T3	24±0.1	11±0.1	21±0.75
6.	T4	19±1.2	11.6±0.09	16.5±0.3
7.	T5	16±0.6	10±0.2	19±0.6
8.	T6	18±0.3	12.2±0.1	19.5±0.5
9.	T7	27±0.5	7.6±0.05	20±0.1
10	T8	11±0.3	8.2±0.1	18.2±0.1
11	T9	14±0.25	11.3±0.3	14±0.09
12	T10	15±0.3	9.8±0.25	12.5±0.5
13	T11	20±1.1	8.9±0.25	15±0.6
14	T12	19±0.09	9.5±0.3	13±0.8
15	T13	11±0.65	16.2±1.2	21±0.25
16	T14	14±0.25	7.1±0.8	13.2±1.1
17	T15	19±0.8	8±0.5	18±0.6

Values are the average of three replicates

± indicate standard deviation /error.

Table 3.7: Dry root/shoot ratio of chickpea, pea and kidney bean after foliar inoculation and application of edible oils

	Treatment	Root/shoot ratio		
		Chickpea	Kidney bean	Pea
1.	FC	0.64±0.02	0.21±0.01	0.5±0.07
2.	HC	0.79±0.09	0.92±0.05	1.19±0.04
3.	T1	0.67±0.04	0.44±0.05	0.90±0.05
4.	T2	0.78±0.02	0.64±0.05	0.91±0.03
5.	T3	0.43±0.02	0.43±0.05	0.93±0.03
6.	T4	0.76±0.01	0.73±0.01	0.73±0.03
7.	T5	0.48±0.05	0.6±0.02	0.91±0.07
8.	T6	0.42±0.023	0.54±0.02	0.9±0.09
9.	T7	0.45±0.05	0.2±0.07	0.57±0.09
10.	T8	0.6±0.04	0.71±0.01	0.71±0.09
11.	T9	0.67±0.08	0.61±0.01	0.8±0.04
12.	T10	0.59±0.09	0.32±0.05	0.89±0.04
13.	T11	0.78±0.09	0.71±0.02	0.69±0.04
14.	T12	0.63±0.002	0.41±0.02	0.8±0.01
15.	T13	0.87±0.07	0.80±0.02	0.98±0.06
16.	T14	0.59±0.05	0.71±0.05	0.61±0.09
17.	T15	0.6±0.05	0.62±0.02	0.82±0.01

Values are the average of three replicates

± indicate standard deviation /error.

Table 3.8: Sugar content of leaves of chickpea, kidney bean and Pea after foliar inoculation and application of edible oils

	Treatment	Sugar content		
		Chickpea	Kidney bean	Pea
1.	FC	1041.6±18.5	723±0.09	1122±0.05
2.	HC	1051±20.2	1155.6±0.05	1156.8±0.02
3.	T1	1048.4±12.5	998±0.04	1136.8±0.05
4.	T2	1042.8±11.6	810±0.03	1151.2±0.02
5.	T3	1045.8±12.2	985±0.05	1147.6±0.03
6.	T4	1045.2±15.6	880±0.05	1138±0.05
7.	T5	1036±19.2	1050±0.02	1125.6±0.03
8.	T6	1046.4±11.3	1066.4±0.07	1108±0.05
9.	T7	1048.4±15.8	1143±0.04	1105±0.04
10.	T8	1049.6±10.9	1133.6±0.15	1138.8±0.05
11.	T9	1044±19.9	945.6±0.09	1143.6±0.09
12.	T10	1049.6±17.4	753.2±0.03	1115.2±0.02
13.	T11	1016±18.5	870.8±0.07	1101±0.05
14.	T12	1040.4±20.5	805.6±0.05	1144±0.09
15.	T13	1048.8±16.9	670.4±0.07	1149.2±0.07
16.	T14	821.2±18.5	949±0.02	1108±0.08
17.	T15	1053.6±11.2	922±0.05	1095±0.05

Values are the average of three replicates

± indicate standard deviation /error.

Table 3.9: Protein content of chickpea, kidney bean and pea after foliar inoculation and application of edible oils

	Treatment	Protein content		
		Chickpea	Kidney bean	Pea
1.	FC	193±0.2	246±20.6	58±2.3
2.	HC	315±0.05	256±12.8	119±1.98
3.	T1	210±0.5	245±17.9	56±1.6
4.	T2	170±0.09	255±19.7	58±2.5
5.	T3	250±0.01	251±13.3	113±2.5
6.	T4	180±0.06	234±15.3	97±2.5
7.	T5	280±0.4	258±19.6	95±2.1
8.	T6	228±0.07	250±11.7	79±1.2
9.	T7	159±0.8	240±15.3	57±1.6
10.	T8	151±0.14	249±11.9	59±1.56
11.	T9	252±0.45	250±20.6	109±1.79
12.	T10	245±0.08	252±20.6	65±1.34
13.	T11	278±0.7	245±12.8	90±2.5
14.	T12	264±0.02	240±19.7	57±1.98
15.	T13	295±0.01	252±13.3	116±2.3
16.	T14	280±0.07	255±20.6	59±1.56
17.	T15	257±0.5	250±15.2	60±1.67

Values are the average of three replicates

± indicate standard deviation /error.

Table 3.10: Proline content of leaves of chickpea, kidney bean and pea after foliar inoculation and application of edible oils.

	Treatment	Proline content		
		Chickpea	Kidney bean	Pea
1.	FC	1176±0.01	1825±0.4	1164±0.03
2.	HC	248±0.05	1410±0.01	631±0.04
3.	T1	322±0.3	1464±0.06	925±0.01
4.	T2	506±0.08	1368±0.04	343±0.02
5.	T3	573±0.1	1431±0.04	1037±0.01
6.	T4	559±0.5	1360±0.05	1036±0.02
7.	T5	263±0.03	1136±0.1	169±0.04
8.	T6	278±0.05	1141±0.01	143±0.01
9.	T7	835±0.04	1232±0.03	998±0.1
10.	T8	464±0.09	1441±0.03	454±0.01
11.	T9	303±0.03	1129±0.04	181±0.07
12.	T10	408±0.2	1669±0.2	411±0.04
13.	T11	1087±0.7	1329±0.15	556±0.1
14.	T12	554±0.23	1253±0.12	476±0.02
15.	T13	251±0.7	1100±0.02	123±0.3
16.	T14	1120±0.8	1210±0.04	1012±0.06
17.	T15	287±0.05	1193±0.01	978±0.09

Values are the average of three replicates

± indicate standard deviation /error.

Table 3.11: Chlorophyll content of leaves of chickpea, kidney bean and pea after foliar inoculation and application of edible oils

	Treatment	Chlorophyll content		
		Chickpea	Kidney bean	Pea
1.	FC	25±1.2	29±1.0	30±0.9
2.	HC	42±0.98	46±0.5	44±0.94
3.	T1	37±1.1	32±0.9	31±0.59
4.	T2	39±1.5	30±1.1	40±0.67
5.	T3	40±1.2	31±0.7	37±0.89
6.	T4	39.8±1.7	30±0.67	38±0.48
7.	T5	26±1.3	34±0.56	35±0.39
8.	T6	40±0.8	35±1.2	31±0.023
9.	T7	37±0.5	38±0.89	30±0.88
10.	T8	39±0.89	40±0.7	37±0.67
11.	T9	38±1.4	34±0.89	39±0.94
12.	T10	39.6±0.2	29±0.92	31±0.89
13.	T11	27±0.7	31±0.79	31±0.93
14.	T12	36±0.9	30±1.3	37±1.8
15.	T13	41±1.7	43±0.2	42±1.2
16.	T14	24±1.3	35±0.97	35±0.88
17.	T15	40±0.5	37±0.8	30±0.93

Values are the average of three replicates

± indicate standard deviation /error.

Table 3.12: Relative water content of leaves of chickpea, kidney bean and pea after foliar inoculation and application of edible oils

	Treatment	Relative water content %		
		Chickpea	Kidney bean	Pea
1.	FC	68±11.5	78±7.8	66.6±5.5
2.	HC	77±1.8	88±0.9	85±7.7
3.	T1	44±2.8	55±1.8	50±3.6
4.	T2	63.5±3.9	68±2.5	83±9.5
5.	T3	55.5±9.5	53±2.5	33±6.8
6.	T4	62±6.3	76±0.5	80±7.7
7.	T5	71±5.8	80±1.9	50±6.5
8.	T6	65±9.5	62±5.5	82±5.2
9.	T7	69.5±9.5	54±5.2	68±3.9
10.	T8	57.8±10.3	79±5.2	58±9.5
11.	T9	75±8.9	61±6.9	79±6.3
12.	T10	66.8±6.3	50±0.5	56±11.5
13.	T11	57.4±5.5	57±0.5	62±3.2
14.	T12	59±3.9	66.6±5.2	84±10.3
15.	T13	76.2±5.8	82±2.5	53±
16.	T14	69.5±5.8	78±0.7	45±
17.	T15	64±1.2	62±1.9	58±

Values are the average of three replicates

± indicate standard deviation /error.

Table 3.13: Fresh root/shoot ratio of chickpea, pea and kidney bean after foliar inoculation and application of edible oils

	Treatment	Root/shoot ratio		
		Chickpea	Kidney bean	Pea
1.	FC	0.65±0.02	0.23±0.01	0.54±0.07
2.	HC	0.9±0.09	0.95±0.05	1.2±0.04
3.	T1	0.79±0.04	0.49±0.05	0.92±0.05
4.	T2	0.87±0.02	0.67±0.05	0.95±0.03
5.	T3	0.55±0.02	0.48±0.05	0.98±0.03
6.	T4	0.82±0.01	0.78±0.01	0.79±0.03
7.	T5	0.59±0.05	0.62±0.02	0.99±0.07
8.	T6	0.45±0.023	0.59±0.02	0.92±0.09
9.	T7	0.5±0.05	0.23±0.07	0.6±0.09
10.	T8	0.7±0.04	0.78±0.01	0.79±0.09
11.	T9	0.79±0.08	0.69±0.01	0.86±0.04
12.	T10	0.65±0.09	0.39±0.05	0.93±0.04
13.	T11	0.89±0.09	0.75±0.02	0.73±0.04
14.	T12	0.72±0.002	0.45±0.02	0.82±0.01
15.	T13	0.91±0.07	0.85±0.02	1.1±0.06
16.	T14	0.68±0.05	0.76±0.05	0.67±0.09
17.	T15	0.72±0.05	0.64±0.02	0.83±0.01

Values are the average of three replicates

± indicate standard deviation /error.

Table .3.14: 1-15 tables of spectral peak values

Table 1 FTIR spectral peak values and functional groups obtained for oil sample 1

Peak values	Bond	Functional group
3009.53	OH stretch	Alcohol
2922.71	C H stretch	Alkanes
2852.99	-OH bend	Carboxylic acid
1743.08	C=O stretch	Esters, saturated aliphatic
1654.17	C-H bend	Aromatic compound
1461.60	C H bend	Alkanes
1418.20	-OH bend	Alcohol
1376.48	-OH bend	Phenol
1236.01	-CO stretch	Alkyl/aryl ether
1159.55	-CO stretch	Tertiary alcohol
1098.04	-CO stretch	Secondary alcohol
967.33	-C=C bend	Alkenes
914.01	-C=C bend	Alkenes
866.86	-CH stretch	Trisubstituted benzene derivative
720.73	-C-Cl stretch	Halo compound

Table 2 FTIR spectral peak values and functional groups obtained for oil sample 2

Peak values	Bond	Functional group
3007.38	-OH stretch	Alcohol
2922.04	-OH stretch	Carboxylic acid
2852.67	-NH stretch	Amine salt
1743.42	-C=O stretch	Esters
1462.31	-CH bend	Alkane
1376.69	-CH bend	Alkanes
1234.06	-CO stretch	Alkyl/aryl ether
1159.72	-CO stretch	Tertiary alcohol
1118.94	-CO stretch	Secondary alcohol
1097.27	-CO stretch	Aliphatic ether
721.40	-C-Cl stretch	Halo compound

Table 3 FTIR spectral peak values and functional groups obtained for oil sample 3

Peak values	Bond	Functional group
3007.58	-OH stretch	Intra molecular bonded alcohol
2921.73	-OH stretch	Carboxylic acid
2852.37	-NH stretch	Amine salt
1743.93	-C=O stretch	Esters
1653.77	-C=C stretch	Alkenes
1462.46	-CH bend	Alkanes
1417.93	-OH bend	Alcohol
1376.68	-OH bend	Phenol
1236.04	-CN stretch	Amines
1159.62	-CO stretch	Tertiary alcohol
1118.79	-CO stretch	Secondary alcohol
1098.70	-CO stretch	Aliphatic ether
913.72	-C=C bend	Alkenes
721.04	-C-Cl stretch	Halo compound

Table 4 : FTIR spectral peak values and functional groups obtained for oil sample 4

Peak values	Bond	Functional group
3009.58	-OH stretch	Alcohol
2922.73	-OH stretch	Alcohol
2853.00	-NH stretch	Amine
1743.04	-C=O stretch	Esters
1654.90	C=C stretch	Alkenes
1461.53	CH bend	Alkanes
1376.38	OH bend	Phenol
1234.79	CO stretch	Alkyl/aryl ether
1159.55	-CO stretch	Tertiary alcohol
1098.00	CO stretch	Aliphatic ether
914.32	C=C bend	Alkene
720.74	-C-Cl stretch	Halo compound

Table 5 : FTIR spectral peak values and functional groups obtained for oil sample 5

Peak values	Bond	Functional group
3007.50	OH stretch	Alcohol
2921.76	-OH stretch	Alcohol
2852.43	-NH stretch	Amine
1743.64	-C=O stretch	Esters
1462.57	CH bend	Alkanes
1376.70	OH bend	Phenol
1234.30	CO stretch	Alkyl/aryl ether
1160.02	-CO stretch	Tertiary alcohol
1118.64	-CO stretch	Secondary alcohol
1098.24	CO stretch	Aliphatic ether
721.08	-C-Cl stretch	Halo compound

Table 6 : FTIR spectral peak values and functional groups obtained for oil sample 6

Peak values	Bond	Functional group
3009.52	OH stretch	Alcohol
2922.75	-OH stretch	Alcohol
2852.98	-NH stretch	Amine
1743.06	-C=O stretch	Esters
1654.23	C=C stretch	Alkenes
1461.55	CH bend	Alkanes
1418.20	-OH bend	Alcohol
1376.41	OH bend	Phenol
1235.30	CO stretch	Alkyl/aryl ether
1159.49	-CO stretch	Tertiary alcohol
1098.07	CO stretch	Aliphatic ether
967.67	C=C bend	Alkenes
914.06	C=C bend	Alkenes
866.81	CH bend	Tri substituted benzene derivative
720.87	-C-Cl stretch	Halo compound

Table 7: FTIR spectral peak values and functional groups obtained for oil sample 7

Peak values	Bond	Functional group
3008.56	OH stretch	Alcohol
2922.75	-OH stretch	Alcohol
2853.22	-NH stretch	Amine
1742.97	-C=O stretch	Esters
1657.07	C=C stretch	Alkenes
1461.57	CH bend	Alkanes
1418.03	-OH bend	Alcohol
1376.91	OH bend	Phenol
1232.56	CO stretch	Alkyl/aryl ether
1159.93	-CO stretch	Tertiary alcohol
1097.98	CO stretch	Aliphatic ether
913.36	C=C bend	Alkenes
844.93	C-Cl stretch	Halo compound
721.74	-C-Cl stretch	Halo compound

Table 8 FTIR spectral peak values and functional groups obtained for oil sample 8

Peak values	Bond	Functional group
2923.51	-OH stretch	Alcohol
2853.37	NH stretch	Amine
1742.61	-C=O stretch	Esters
1458.84	CH bend	Alkanes
1161.84	-CO stretch	Tertiary alcohol
723.20	-C-Cl stretch	Halo compound

Table 9 FTIR spectral peak values and functional groups obtained for oil sample 9

Peak values	Bond	Functional group
3006.62	OH stretch	Alcohol
2921.94	-OH stretch	Alcohol
2852.59	-NH stretch	Amine
1743.41	-C=O stretch	Esters
1462.33	CH bend	Alkanes
1417.87	-OH bend	Alcohol
1376.44	OH bend	Phenol
1230.67	CO stretch	Alkyl/aryl ether
1159.97	-CO stretch	Tertiary alcohol
1118.67	-CO stretch	Secondary alcohol
1096.97	CO stretch	Aliphatic ether
721.50	-C-Cl stretch	Halo compound

Table 10 FTIR spectral peak values and functional groups obtained for oil sample 10

Peak values	Bond	Functional group
3006.06	OH stretch	Alcohol
2921.97	-OH stretch	Alcohol
2852.73	-NH stretch	Amine
1743.39	-C=O stretch	Esters
1654.85	C=C stretch	Alkenes
1462.18	CH bend	Alkanes
1417.86	-OH bend	Alcohol
1376.61	OH bend	Phenol
1231.85	CO stretch	Alkyl/aryl ether
1159.76	-CO stretch	Tertiary alcohol
1118.70	-CO stretch	Secondary alcohol
1095.36	CO stretch	Aliphatic ether
1031.23	CO stretch	Vinyl ether
721.98	-C-Cl stretch	Halo compound

Table 11 FTIR spectral peak values and functional groups obtained for oil sample 11

Peak values	Bond	Functional group
2953.99	-OH stretch	Alcohol
2920.96	-OH stretch	Alcohol
2852.28	-NH stretch	Amine
1747.74	-C=O stretch	Esters
1712.61	-C=O stretch	Aliphatic ketone
1459.42	CH bend	Alkanes
1376.74	OH bend	Phenol
1216.74	CO stretch	Vinyl ether
1160.18	-CO stretch	Tertiary alcohol
942.86	C=C bend	Alkenes
722.16	-C-Cl stretch	Halo compound

Table 12. FTIR spectral peak values and functional groups obtained for oil sample 12

Peak values	Bond	Functional group
3009.84	OH stretch	Alcohol
2923.00	-OH stretch	Alcohol
2853.06	-NH stretch	Amine
1742.93	-C=O stretch	Esters
1652.93	C=C stretch	Alkene
1461.29	CH bend	Alkanes
1418.23	-OH bend	Alcohol
1376.14	OH bend	Phenol
1236.93	CO stretch	Alkyl/aryl ether
1159.51	-CO stretch	Tertiary alcohol
1098.26	CO stretch	Aliphatic ether
968.63	C=C bend	Alkenes
914.27	C=C bend	Alkenes
791.64	C=C bend	Alkenes
720.18	C-Cl stretch	Halo compound

Table 13 FTIR spectral peak values and functional groups obtained for oil sample 13

Peak values	Bond	Functional group
3009.41	OH stretch	Alcohol
2922.89	-OH stretch	Alcohol
2853.13	-NH stretch	Amine
1743.08	-C=O stretch	Esters
1654.60	C=C stretch	Alkene
1461.39	CH bend	Alkanes
1418.21	-OH bend	Alcohol
1376.47	OH bend	Phenol
1234.03	CO stretch	Alkyl/aryl ether
1159.39	-CO stretch	Tertiary alcohol
1097.68	CO stretch	Aliphatic ether
1031.62	CN stretch	Amine
967.69	C=C bend	Alkenes
913.67	C=C bend	Alkenes
866.62	CH bend	Alkenes
720.96	C-Cl	Halo compound

Table 14 FTIR spectral peak values and functional groups obtained for oil sample 14

Peak values	Bond	Functional group
3008.28	OH stretch	Alcohol
2922.43	-OH stretch	Alcohol
2853.04	-NH stretch	Amine
1743.35	-C=O stretch	Esters
1462.25	CH bend	Alkanes
1377.03	OH bend	Phenol
1236.03	CO stretch	Alkyl/aryl ether
1159.46	-CO stretch	Tertiary alcohol
1119.20	-CO stretch	Secondary alcohol
1097.44	CO stretch	Aliphatic ether
913.45	C=C bend	Alkenes
721.42	C-Cl stretch	Halo compound

Table 15 FTIR spectral peak values and functional groups obtained for oil sample 15

Peak values	Bond	Functional group
2921.85	-OH stretch	Alcohol
2852.78	-NH stretch	Amine
1742.45	-C=O stretch	Esters
1464.20	CH bend	Alkanes
1417.89	-OH bend	Alcohol
1376.86	OH bend	Phenol
1228.59	CO stretch	Alkyl/aryl ether
1152.15	-CO stretch	Tertiary alcohol
1110.29	-CO stretch	Secondary alcohol
962.56	C=C bend	Alkenes
889.17	CH bend	Alkenes
721.63	C-Cl	Halo compound

Table 3.15: Agar well Diffusion method and Zone of inhibition of Aschochyta by different edible oils, *in vitro*.

	Treatment	Zone of Inhibition(cm)
1.	T1	0.6±0.02
2.	T2	0.4±0.02
3.	T3	2.9±0.05
4.	T4	1.0±0.01
5.	T5	3.0±0.06
6.	T6	2±0.03
7.	T7	0.3±0.02
8.	T8	0.4±0.02
9.	T9	3±0.03
10.	T10	0.6±0.02
11.	T11	0.2±0.02
12.	T12	0.1±0.06
13.	T13	3.2±0.01
14.	T14	0.08±0.03
15.	T15	0.1±0.02

Values are the average of three replicates

± indicate standard deviation /error**.**

Table 3.16: Poisoned food method to check growth diameter of Aschochyta by different edible oils *in vitro* analysis.

	Treatment	Rate of growth		
		D_C	D_s	%age
1.	T1	9±0.02	8±0.5	88.8
2.	T2	9±0.02	7.5±0.9	83
3.	T3	9±0.02	4±0.8	44
4.	T4	9±0.02	7.3±1.02	81
5.	T5	9±0.02	6.2±0.5	66.6
6.	T6	9±0.02	0.5±0.5	0.05
7.	T7	9±0.02	8±0.5	88.8
8.	T8	9±0.02	6.8±0.76	75.5
9.	T9	9±0.02	6±0.76	66.6
10.	T10	9±0.02	8.5±1.04	94.4
11.	T11	9±0.02	8.7±0.76	88.8
12.	T12	9±0.02	8.2±0.5	88.8
13.	T13	9±0.02	2.5±0.6	27.7
14.	T14	9±0.02	7.9±0.2	87.7
15.	T15	9±0.02	8.3±0.6	92

Values are the average of three replicates

± indicate standard deviation /error

DISCUSSION

The experiment was conducted to examine the effect of different edible oils on biochemical and physiological parameter of plants and control of Ascochyta blight disease. Among the bio fungicides, edible oils are of immense importance because of minimum health risks. In the present study, fungal disease control by 15 different edible oils was assessed by detached leaf assay. To study disease control and preliminary testing of antifungal agent, detached leaf assay is a rapid and best way (HC Sharma *et al.,* 2005). Various essential oils e.g. Thyme carvacol etc. have been reported to have antimicrobial properties against many pathogens. (Bakkali *et al.,* 2008).

In the present study all edible oils were not able to cut down the severity of blight disease of Chickpea, Pea and Kidney Bean caused by *Aschochyta rabiei*. Some edible oils showed the best results while some, for example Fish oil, proved not so good and caused wilting. Remaining oils showed varied degree of disease control, as evident from disease incidence after the application. Blight disease was considerably controlled by *Riccinus communis, Pappaver somniferum, Sesamum indicum* and *Triticum aestivum* oils indicating that these oils can also produce some inhibitory effects on host parasite interaction and lessen the disease rate.

In present investigation higher Proline content was observed in all the treatments of edible oils in Chickpea, Kidney Bean and Pea except in the plants treated with *Riccinus communis, Pappaver somniferum, Sesamum indicum* and *Triticum aestivum* oils. They showed low Proline accumulation. Osmoregulation (being aided by osmoregulators which may be organic molecules like Proline) functions in reducing the cell osmotic potential to a level to provide high turgor potential for maintaining growth (de lacerda et al., 2005). In treatment with different edible oils showing high level of proline content in the plants, when in stress level, increased turgor pressure and resulted in better resistance but in case of *Riccinus communis, Pappaver somniferum, Sesamum indicum* and *Triticum aestivum* oils, the proline content was low. This might be due to stress level applied did not reach a threshold level. These oils might be containing some compounds that act as an antimicrobial agent at very early phases and prevent the plant to go to that stress level which trigger proline accumulation. According to Claussen (2005), accumulation of proline requires certain stress level called threshold level. Different developmental processes such as flower transition and various stress levels e.g. high salinity ,

drought and the biotic stresses cause proline to accumulate in high concentration (Mattioli et al., 2009).

Sugar content was also high in all healthy plants, used as control (Table 9). it is might be due to the reason that leaves synthesized more soluble sugars and plants without stress stored more soluble sugars contents in their leaves than stressed plants. A very close value of sugar content was found in the plants treated by *Riccinus communis* oil. The reason might be this oil promotes healthy growth by interacting with fungal spores and in this way stress level of the plant is reduced and it produced more sugars. Sugar content was slightly decreased in response to fungal stress in all plants but found minimum in fungus control plants in all the three crops (Table 9). This might be due to maximum stress level and disease severity. Pathogenic infections resulted in the increased production of Phenolic compounds and these phenolic compounds may alter the rate of photosynthesis and sugar formation and storage (A Mahadevan, 1966).

In all the three crops the protein content was found maximum in healthy control and least in fungus control (Table 10). Healthy plant is capable of producing more proteins. Plants encountered by stress used to produce more proteins depending upon stress level and type (Dubey, 1999). In case of pathogens, pathogen related proteins are produced and these pathogen related proteins activate defence mechanism of plant making it resistant and reducing further infection, but if the pathogen is strong enough it will interfere with the plant protein manufacturing machinery and the protein formation process may be reduced and plant may be more stressed and die. More stressed plants may have less protein content because of interference like in fungus control in all the three crops. Secondly if there is overall increase in protein content and decrease in sugar contents, this might be due to fungal inoculution which is a sign of stimulating ostmotic material synthesis in stress conditions by these fungi (farkas and kiraly,1962; klement and goodman 1967).

Chlorophyll content also showed variation and this variation might be because of the different factors. Chlorophyll content was found higher in healthy control and least in fungus control and this pattern was observed in the values of all the three crops. Maximum photosynthesis was shown by healthy plants and sugar contents also increased in them. In abiotic stress, plant produces other proteins and this production shift may affect the proteins which form the basic structure of chlorophyll and different oils which upon spraying on plants show the enhanced

chlorophyll content might be due to reduction in stress levels so that plant can function normally. Secondly under stress conditions if they are prolonged the chlorophyll degradation may occur (Khan *et al.,* 2009).

In present study, higher relative water contents were observed due to the various treatments. High or low water content depends upon the fresh weight of root and shoots. Healthier they are, more they are capable to maintain turgor pressure. High relative water content was observed in healthy control in all the three crops. But relative water content is also affected by two factors namely hormones such as Saliycylic acid Absiccic Acid and Proline content. Salicylic acid accumulates because of system acquired response in plants (Gaffney *et al.,* 1993). Under stress conditions, salicylic and *Abscisic* acid accumulate and increase the fresh weight of roots and shoots (Khodary, 2004). More the fresh weight, more the water content. Proline is an osmolyte and it helps to maintain turgor pressure that is why it might be responsible for high water content.

To study the differences in chemical composition of oils FTIR has immense value. The bands or peaks shift themselves according to change in fatty acid dimensions (functional groups) (Vlachos et al., 2006). Fish oil showed certain negative effects along with positive effects on plants. This may be described in regard to functional group transition. Excess of carboxylic acids, aliphatic amine, alkenes, alcohol, and ether and ester functional group containing compounds in fish oil might be the cause of wilting in leaves when fish oil is sprayed on it. Oils act as efficient bio fungicides because microorganisms cannot make resistance the reason might be their structure having variety of functional groups (Gomez-Castillo et al., 2013; F Patrignani *et al.,* 2015). Antifungal activity of oils depends upon these functional groups (Burt, 2004). Reason behind antifungal activity of castor, wheat, opium and sesame oil is not clear. Organic nitrogen containing compounds called alkaloids are having a strong evidence for antimicrobial potential. Morphine was the first medicinally important alkaloid isolated from poppy plant (*Papaver somniferum*) in 1805 (Fessenden and Fessenden, 1982). Alkaloids have antimicrobial properties (Omulokoli et al., 1997). Antifungal activity is also concerned with Amides but they are not more active in this regard (Goodman and Gilman, 1958).

Other studies along with our results represent essential oils as a natural and healthy fungicide and an effective way to control the diseases caused by pathogens. More research and studies will

transfer the synthetic means of controlling the disease to biological means. But to evaluate the affect in detail of these suggested oils, further studies on mechanism of disease control and efficacy are required.

CONCLUSION

CONCLUSION

The study confirms that

- All edible oils have not a positive effect on chickpea, kidney bean and pea plant morphology and physiology.
- Some edible oils like fish oils are detrimental for chickpea, kidney bean and pea plants and cause loss of the plant vigor and result in wilting of leaves.
- *R. communis (castor), P. somniferum, S. indicum and T. aestivum* oils proved best in blight disease resistance in chickpea, kidney bean and pea plants and these can be used successfully in lab and field conditions.

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