Botanical pesticides in the management of *Xanthomonas campestris* pv. *campestris* and *Alternaria brassicae* of cabbage

THESIS

Submitted in partial fulfillment of the requirements for the award of

DOCTOR OF PHILOSOPHY

in

BOTANY

by

Ms. NEELAM

DEPARTMENT OF BIOLOGICAL SCIENCES

Sam Higginbottom University of Agriculture,

Technology and Sciences

Allahabad-211007

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Place: Allahabad

Date: 13.02.2017

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CERTIFICATE OF RECOMMENDATION BY STUDENT ADVISORY COMMITTEE

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ABSTRACT

The experiments were conducted in the field and research laboratory of Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad (Deemed to-be University) during two *rabi* seasons of 2012-2014. The field experiment was planned in Randomized Block Design (RBD) with three replications for each treatment and Complete Randomized Design (CRD) in laboratory with four replications for each treatment. The site selected for experimentation was uniform, cultivable with typical sandy loam soil having good drainage. Observations were recorded at screening of 75 plant leaf extracts and their antimicrobial activity against *Alternaria brassicae* and *X. campestris* pv. *campestris*, assayed of secondary metabolites and active compound from the selected 12 botanicals. Results summarized that Bordeaux mixture @ 0.2% was recorded minimum mycelial radial growth (8.67, 12.07, 20.65 and 22.85 mm) and maximum % inhibition as compared with all the botanicals leaf extract. The least mycelium growth of *Alternaria* was found in the neem leaf extract @ 50 % concentration (7.70 and 10.45mm) at 48 and 72 hrs after incubation. Neem oil was observed maximum population reduction of *X. campestris* and inhibition % over control at the rate of 2, 4 and 6% concentrations on 48 hrs (6, 5.7 and 2.7, respectively) and 72 hrs (15, 9.7 and 8.7, respectively) . Per cent disease intensity of black leaf spot (*Alternaria brassicae*) was reduced maximum in the treated plots with Bordeaux mixture (15.23, 16.33, 19.93 and 21.94), While in the botanical extracts, neem oil was found maximum reduction of disease intensity at different D.A.S. Maximum cabbage head diameter (cm) was observed in the sprayed plots with *Azadirachta indica* oil (14.10, 25.20) at 90 and 120 days after sowing. The edible head weight (g) at harvesting time, it was recorded that maximum in the Bordeaux mixture (1782.06 g) and *Azadirachta indica* (1740.46) followed by all other essential oils.

Key words: *Alternaria brassicae*, botanical extracts, essential oil, *Xanthomonas campestris* pv. *campestris*

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Key words: *Alternaria brassicae*, botanical extracts, essential oil, *Xanthomonas campestris* pv. *campestris*

CHAPTER – I

INTRODUCTION

Cabbage (*Brassica oleracea* var. *capitata*) is leafy green vegetable crop, a popular cultivar of the species *oleraceae* under the family Brassicaceae. It is an herbaceous, biennial, dicotyledonous flowering plant distinguished by a short stem upon which is crowded a mass of leaves, usually green **(Helen, 2006).** The only part of the plant is normally eaten is the leafy head; more precisely, the spherical cluster of immature leaves, excluding the partially unfolded outer leaves **(Mathewson, 1998).** Cabbage heads generally range from 0.5 to 4 kilograms (1 to 9 lb), can be green, purple and white. It is a member of the brassicaceae family and closely related to other cole crop such as broccoli, cauliflower and Brussels sprouts.

It has excellent source of vitamin K, C, B_1, B_2, B_5, B_6 and also a very good source of manganese, dietary fiber, potassium, foliate, magnesium, calcium, selenium, iron, copper, chlorine, phosphorus, sodium and protein. On a full array of nutrients, including carbohydrates, sugar, soluble and insoluble fiber, fatty acids, amino acids and more use of cabbage prevents constipation, increases digestion and appetite.

Nutritional value per 100g of cabbage is reported by USDA Nutrient Data 2015 base as in carbohydrates (5.8g), sugar (3.2g), dietary fiber (2.5g), fat (0.1g), protein (1.28g), fluoride (1 µg), thiamine (B_1 5%), riboflavin (B_2 3%), niacin (B_3 2%), pantothenic acid (B_5 4%), vitamin B_6 (10%), folate (B_9 11%), vitamin C (44%), vitamin K (72%), calcium (4%), iron (4%), magnesium (3%), manganese (8%), phosphorus (4%), potassium (4%), sodium (1%) and zinc (2%) **(USDA Nutrient Database, 2015)**.

Cabbage has anti-bacterial and anti-viral powers. It also contains numerous anti-cancer and antioxidant compounds, this is possible due to the glucosinolates. These compounds are concentrated in the highest quantities in the seeds; lesser quantities are found in young vegetative tissue, and they decrease as the tissue ages **(Katz and Weaver, 2003).** Cooked cabbage is often criticized for its pungent, unpleasant odor and taste due to overcooked and hydrogen sulfide gas is produced **(Corriher, 2001).** Research suggests that boiling of vegetables reduces their anti-carcinogenic properties **(Warwick Medical School, 2007).** Hence, eating raw cabbage is best; light cooking is recommendable**.** It is used for the various production *i.e.* pickled, fermented such

as sauerkraut, steamed, stewed, sautéed and braised. Pickling is one of the most popular ways of preserving cabbage, creating dishes such as sauerkraut and kimchee. In India, it is often included in spicy salads and braises **(Gil, 2008).**

The nations with the largest production of cabbage are China (46%) followed by India (12%). China and India used a surface area of 958,746 hectares and 369,000 hectares, respectively **(FAOSTAT, 2015).**

The cultivation of cabbage was undertaken in almost all states in India. The highest cultivated (M ha) state was West Bengal (77.80) followed by Odisha (40.98), Bihar (39.73), Assam (31.57), Jharkhand (31.13), Gujarat (30.92), Madhya Pradesh (20.94), Maharashtra (20.00), Chhattisgarh (16.55) and Haryana (14.88). Whereas, the highest production (MT) was West Bengal (2179.20) followed by Odisha (1148.21), Assam (652.67), Jharkhand (480.22), Gujarat (663.53), Madhya Pradesh (413.91), Maharashtra (421.00), Chhattisgarh (295.52) and Haryana (270.31) **(Anonymous, 2015)**.

Cabbage consumption varies widely around the world: Russia has the highest annual per capita consumption at 20 kilograms followed by Belgium at 4.7 kilograms the Netherlands at 4.0 kilograms and Spain at 1.9 kilograms. Americans consume 3.9 kilograms annually per capita **(Boriss and Kreith, 2006; Louis Bonduelle Foundation, 2012).**

Cabbage (*B. oleracea* var. *capitata*) is severely affected by *A. brassicae* (Berk.) Sacc., and *A. brassicicola* (Schw.) Wiltsh **(Maude and Humpherson-Jones, 1980; Humpherson-Jones 1985)**. These are most common pathogens, usually cause black spot disease, spotting of cabbage leaves, blackleg of heads (head cabbage and Chinese) and spotting/browning of cabbage. Among them, black spot is the most common disease in the crucifer plants in tropical and sub-tropical regions.

Black leaf spot of cabbage crop has been reported from many countries, *viz*., India **(Kadian and Saharan, 1983),** Italy **(Tosi and Zazzerini, 1985),** USA, UK and several other European countries **(Gladders, 1987),** Canada **(Berkenkamp and Kirkham, 1989; Conn and Tewari, 1990)** and Iran **(Nourani *et al.*, 2008).**

Black leaf spot disease in cabbage is caused by *Alternaria brassicicola* (Schw.) Wiltshire and *Alternaria brassicae* (Berk) Sacc. Although this is a common disease of cruciferous vegetables worldwide, the disease caused by *A. brassicicola* is more common in Taiwan. The pathogen is

easily spread through the contamination of seeds **(Maude and Humpherson-jones, 1980).** Leaf spot incited by *A. brassicicola* appear as small, dark coloured areas which expand rapidly to form circular lesions up to 1 cm in diameter. In humid weather the fungus may appear as a bluish growth in the center of the spots. Concentric rings may also form in the lesions. The spots caused by *A. brassicae* are similar but tend to remain smaller and lighter in color.

This species have the ability to survive in seeds for several months at different temperatures and relative humidity **(Kumar and Gupta, 1994; Abul-Fazal *et al.*, 1994).** Losses up to 30% and 47% were caused by *Alternaria brassicae* in cauliflower (*Brassica oleracea* var *botrytis*) **(Tamayo *et al.*, 2001)** and Indian mustard (*Brassica juncea*) **(Chattopadhyay, 2008)** respectively.

The genus *Alternaria* was first recognized by **Nees and Esenbeck** in **(1817). Berkeley (1836**) identified the causal fungus on plants belonging to family Brassicaceae as *Macrosporium brassicae* Berk., which was later renamed as *Alternaria brassicae* (Berk.) Sacc. There after, **Elliot (1917)** studied the taxonomy of *Alternaria* in detail. The morphological variations of *Alternaria* species were described by **Joly (1959).** In India, the first report of *Alternaria* was made from Pusa (Bihar) on a herbarium material of Sarson (*Brassica* sp.) **(Mason, 1928).** In U.P. the appearance of *Alternaria* spp. was noticed by **Dey (1948).** A comprehensive account of distinguishing characters of the Indian species of *Alternaria* has been described by **Subramanian (1971).** The characteristic features of a number of *Alternaria* species are described in "Dematiaceious Hyphomycetes" **(Ellis, 1971).** *Alternaria brassicae* also produces a host-selective pathotoxin 'dextruxin B' **(Pedras and Smith, 1997).**

Second most important disease, black rot of cabbage caused by bacterium *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson, is the most common and destructive disease of the cabbage family worldwide. It was first reported from Iowa, North America, on rutabaga by **Pammel (1895).** The disease was reported on cabbage from Wisconsin by **Russel (1898).** Since then, there has been a growing recognition of seriousness of black rot on cruciferous crops throughout the world. On cauliflower, the disease was first recorded in Norway **(Jorstad, 1922).**

In India, the disease was recorded for the first time on cauliflower by **Patel *et al.* (1949)** and subsequently it was reported from Katrain (Kullu district) area of Himachal Pradesh **(Rao and Srivastava, 1964).** Since then, it has also been reported from other parts of the country by various workers **(Patel *et al.*, 1970; Shyam *et al.*, 1994; Gupta and Chaudhary, 1995).**

The disease is very destructive and causes considerable yield losses by premature defoliation and reducing the quality of curd and heads in cauliflower and cabbage, respectively, during transit and renders them unmarketable. A crop loss up to 50 per cent in cabbage and 50 to 70 per cent in cauliflower has been recorded **(Jorgensen and Walter, 1955).** Substantial crop losses have been reported from the rapid spread of the bacteria under favourable conditions, especially during seedling production by **Berg *et al.* (2005).**

The pathogen moves systemically through the vascular bundles turning them black and produces main symptoms on leaves as described by various workers. The pathogen produces two types of symptoms. Initially, the marginal chlorotic spots appear on the leaves followed by darkening or blackening of mid rib and veins **(Cook *et al.*, 1952).** However, **Sutton and Williams (1970)** reported that vein blackening was the first visible symptom which was due to the accumulation of melanin among xylem cells. The pathogen colonizes the vascular system after its entry into the plant and produces plentiful extracellular polysaccharide called xanthan **(Jeanes, 1973)** which along with the bacterial cells plug the xylem vessels, restricting the water flow and resulting in the characteristic V-shaped chlorotic lesions originating from the margins of the leaves. As the bacterium moves throughout the plant, the vascular tissues darken, resulting in blackening of the veins **(Agrios, 1997).**

X. campestirs pv. *campestris* is a small, straight rod shaped, 0.5-0.75, 1.5-2.0 mm. mobile, aerobic, gram negative, non-spore forming and obligate plant parasite bacterium. The bacterium has a single polar flagellum and it is catalase positive, hydrogen sulphide positive, oxidase negative and does not produce nitrate or indole. It produces a yellowish extracellular polysaccharide (EPS) called xanthan on media containing glucose. The survival temperature range is very wide (5- 38^{o}C), with the optimum temperature 30^{o} C and lethal at about 50^{o} C **(Sidhu *et al.*, 2008; Dezordi *et al.*, 2009).** The bacterium can survive in the soil for 14-42 days and in old cabbage stem for 244 days. The initial infections may enter from debris of crucifer plants (mustard, cabbage and cauliflower etc.), weeds and irrigation water **(Roberts *et al.*, 1999)**.

Biological screening of plant extracts was carried out throughout the world for their antifungal activity. Botanical pesticides have received attention of the growers because these are considered as less toxic and environmentally safe. The antimicrobial property of some plant extracts under *in-vitro* and *in-vivo* have been reported by **Mehta and Mehta, 2005; Kumar *et al.*, 2006).** Natural plants derived compounds contribute a lot in fight against pathogens **(Vyvyan, 2002).** Due to their inappropriate properties such as inducing pesticide resistance, enhanced pest

resurgence, bioaccumulation in the food chain and toxicological implications in human health **(Barnard *et al.*, 1997).** Considering the injurious effects of synthetic pesticides on life supporting system, there is a very strong need for alternative agents for the management of pathogenic microorganisms **(Mahajan and Das, 2003; Britto *et al.*, 2011).** Green plants can be used as a source of a reservoir and can be effective chemotherapeutants which can provide valuable sources of natural pesticides because of environmental and economic considerations, plant scientists are involved to find the cheaper and more environmental friendly bio-compounds for the control of plant diseases using different forms of botanicals **(Jayalakshmi *et al.*, 2011; Nidhi *et al.*, 2013; Biplab *et al.*, 2013).**

Bio-active compounds that effectively control various pests and pathogens are known from approximately 2400 plant species **(Saleem, 1988).** Extracts from leaves have been reported to have antifungal **(Kumar *et al.*, 2006; Srivastava and Singh, 2011; Mostafa *et al.*, 2011)**, anti-proliferative **(Hussain *et al.*, 2011)**, antibacterial **(Mary, 2011; Mani *et al.*, 2010; Barreto *et al.*, 2010)**, nematicidal **(Verma *et al.*, 2006)**, termicidal **(Bequm *et al.*, 2008)**, anti-helmintic **(Mohapatra *et al.*, 2011)** and anticancer activities **(Tripathi *et al.*, 2002).** Beside this, the essential oil of the plants also possesses antifungal **(Sonibare and Effiong, 2008)** and antibacterial activities **(Pattnaik and Pattnaik, 2010; Tesch *et al.*, 2011).**

Several leaf extracts like, *Eucalyptus citriodora* **(Ramezani, 2006),** *Lantana camara* **(Deena and Thoppil, 2000)**, *Ocimum basilium* **(Boyraz and Ozcan, 2005)** and *Azadirachta indica* **(Bhonde *et al.*, 1999)** can be efficient in controlling agents against *Alternaria brassicae*. The plants like *Solanum nigrum* **(Muto *et al.*, 2006)** and *Azardirachta indica* **(Bhonde *et al.*, 1999)** are proved to contain secondary metabolites at high concentration. Glycoalkaloids and saponins **(Zhou *et al.*, 2006)** are reported to be present in very high concentration in *Solanum* species. The alkaloids in these plants have established to have antifungal properties **(Kusano *et al.*, 1987; Quetin-Leclercq *et al.*, 1995).**

Lantana camara is a noxious weed belonging to family Verbenaceae. Three varieties of *L. camara* have been reported from India in which *L. camara* var. *aculeata* is the most common **(Anonymous, 1972; Morton, 1994; Ross, 1999)**.

Azadiracta indica is belonging to family meliaceae. It's a medicinal plant, which has been found useful in the protection of a number of diseases. Neem extract has been reported to have antidiabetic, antibacterial and antiviral activity **(Kirtikar and Basu, 1987).** The extract from bark,

leaves, fruits and root have been used to control leprosy, intestinal helminthiasis and respiratory disorders in children **(Chattopadhyay *et al.*, 1993)**. Flavonoids, flavonoglycosides, dihydrochalocones, tannins and others are also important constituents of bark, leaves, fruits and flowers of neem **(Venugopal and Venugopal, 1994)**. The neem leaves contain nimbin, nimbinene, 6-desacetylnimbinene, nimbandiol, nimbolide and quercetin. The fruits containing nimbiol and azadirachtin. Azadirachtin and limonoids are considered as most bioactive in gradients having insecticidal and microbial property **(Prajapati *et al.*, 2003)**.

Twenty plants screened namely *Acacia nilotica, Ageratum conyzoides, Boerhaavia diffusa*, *Cynodon dactylon*, *Cleome viscosa*, *Datura stramonium, Euphorbia hirta, Ficus benghalensis*, *Hyptis suaveolens, Hibiscus rosa-sinensis, Jatropha gossypifolia*, *Phyllanthus niruri*, *Prosopis juliflora, Polyalthia longifolia, Sida cordifolia, Tephrosia purpurea, Tridax procumbens, Zizyphus jujube, Solanum nigrum* for their antibacterial activity against *Xanthomonas campestris* pv. *campestris* **(Siddiqui, 2014).** Among all the tested species, nine plant species *viz. Acacia nilotica, Ageratum conyzoied*, *Boerhaavia diffusa*, *Cleome viscose*, *Datura stramonium, Euphorbia hirta, Hyptis suaveolens, Hibiscus rosa sinensis, Prosopis juliflora* and *Tridex procumbens* showed medium to light antibacterial activity against the selected pathogens. Significant antibacterial activity was observed in aqueous extracts of *Prosopsis juliflora*, *Hyptis suaveolens, Euphorbia hirta* and *Acacia nilotica*.

Eucalyptus leaf extract has shown significant reduction in radial growth, sporulation and spore germination of *Alternaria* sp. **(Patni and Kolte, 2006)**

Garlic extract has been found as best biocide in controlling the early blight of Indian mustard (*Alternaria brassicae*) **(Singh, 1994).**

Abo-El-Seoud *et al.* (2005) tested essential oils of fennel, peppermint, caraway, eucalyptus, geranium and lemongrass have displayed antimicrobial activities against *Fusarium oxysporum, Alternaria alternata, Penicillium italicum* and *Botrytis cinerea.*

Plant extracts are least expensive and cause less health hazards. Several higher plants and their constituents have shown success in plant disease control and are proved to be harmless and non-phytotoxic unlike chemical fungicides **(Singh *et al.*, 1983; Alam *et al.*, 2002b)**. Evidence suggests that plant extracts can be used against microbes causing diseases in plants. Thus remarkable antifungal effects of plant extracts on the germination of fungal spores **(Dubey, 1991)**, and extracts of plant parts for controlling the diseases are reported earlier **(Singh and Dwivedi,**

1990). Plants are a rich source of thousands of secondary metabolites (SM). These consist of low molecular weight compounds that are regarded as not essential for sustaining life, but as crucial for the survival of the producing organism **(Hadacek, 2002).** In these botanicals active compound are found Papaya (caffeic acid, p-coumaric acid, protocatechuic acid, Kaempferol, quercetin and 5, 7-dimethoxycoumarin.), Lantana (calarane and 3-hydroxy-10,19-enurs-28-oic acid), Basil (ursolic acid, apigenin and luteolin), Eucalyptus (1,8cineole (eucalyptol), limonene, α pinene, Pcymene and terpineol), Rauvolfia (reserpine), Ginger (zingiberene, β- bisabolene, α-farnesne, β-sesquiphellandrene, α-curcumene and phenolic, gingerol and shogaol) Mentha (menthone, isome nthone, cyclodecadiene, dodecatriene, n –decanoic acid, 3,7,11,1 5 - tetramethy l-2-hexadecenol, phytol, octadecanol and 1,2-benzenedicarboxylic acid), Thorn apple (hyocymine scopolamine), Neem (azadiractin) and Garlic (allicine). These compounds are frequently accumulated by plants in smaller quantities than the primary metabolites **(Croteau *et al.*, 2000, Dewick, 2002).** Plants secondary metabolites are synthetized in specific pathway and sites of production can vary between kinds of compounds as well as between plant species. Moreover, some molecules can be synthesized by all plant tissues, whereas others are produced in a specific tissue or even cell specific fashion **(Yazdani *et al.*, 2011).** The site of synthesis for SM is not certainly the site of accumulation. Therefore, there is a great demand for novel natural fungicides. Higher plants are rich source of bioactive secondary metabolites of wide variety such as tannins, terpenoids, saponins, alkaloids, flavonoids and other compounds reported to have *in vitro* antifungal properties. Thus, secondary metabolites with antifungal activity represent an alternative for achieving a sustainable control of phytopathogenic fungi and to reduce the heavy reliance of synthetic pesticides used to control them. Plant antifungal metabolites may be preformed inhibitors that are present constitutively in healthy plants (phytoanticipins), or they may be synthesized *de novo* in response to pathogen attack or another stress conditions (phytoalexins). These molecules may be used directly or considered as a precursor for developing better fungicidal molecules. This review presents a selection of antifungal agents induced in plants during fungal attack that can be potentially used for phytopathogenic fungal and antibacterial. This, hydrophilic compounds are mainly stored in the vacuole while the lipophilic SM are commonly sequestered in resin ducts, laticifers, oil cells, trichomes or in the cuticle **(Engelmeier and Hadacek, 2006).**

Plant extracts are screened to detect secondary metabolites with biological activities, including antimicrobial activities. Keeping this in mind the present study entitled **"Botanical pesticides in the management of *Xanthomonas campestris* pv. *campestris* and *Alternaria brassicae* of cabbage"** was proposed with the following objectives –

1. To isolate and identify *Xanthomonas campestris* pv. *campestris* and *Alternaria brassicae* from infected leaves of cabbage.
2. To screen available botanicals for their antimicrobial activities against *Xanthomonas campestris* pv. *campestris* and *Alternaria brassicae*.
3. To detect the secondary metabolities from the selected botanicals.
4. To evaluate the efficacy of selected botanical extracts and essential oils against *Xanthomonas campestris* pv. *campestris* and *Alternaria brassicae* of cabbage.
5. To study the effect of selected botanicals on edible head diameter (cm) and weight (g) of cabbage boll.

CHAPTER - II

REVIEW OF LITERATURE

The present study entitled **"Botanical pesticides in the management of *Xanthomonas campestris* pv. *campestris* and *Alternaria brassicae* of cabbage"** has been made to study and examine all the available finding of previous research work and these have been summarized and presented in this chapter.

2.1 Screening of different botanical leaf extracts against the pathogens :

Pawar (2014) screened the *Azadirachta indica* leaf extract of various plants against 25 strains of *Xanthomonas campestris* pv. *mangifera indica* (Xcmi). Maximum activity against Xcmi 0.09 (Mean activity zone– 22.86 mm) followed by Xcmi 0.07 (Mean activity zone- 22.55 mm) and minimum against Xcmi 0.23 (Mean activity zone – 18.30 mm) strain under investigation.

Siddiqui (2014) screened the twenty plants namely *Acacia nilotica, Ageratum conyzoides*, *Boerhaavia diffusa, Cynodon dactylon*, *Cleome viscosa*, *Datura stramonium, Euphorbia hirta, Ficus benghalensis, Hyptis suaveolens, Hibiscus rosa-sinensis*, *Jatropha gossypifolia, Phyllanthus niruri, Prosopis juliflora, Polyalthia longifolia, Sida cordifolia, Tephrosia purpurea, Tridax procumbens, Zizyphus jujube* and *Solanum nigrum* against *Xanthomonas campestris* pv. *campestris*. He showed among nine plant species *viz., Acacia nilotica, Ageratum conyzoied, Boerhaavia diffusa, Cleome viscose, Datura stramonium, Euphorbia hirta, Hyptis suaveolens, Hibiscus rosa sinensis, Prosopis juliflora* and *Tridex procumbens* were medium to light inhibition per cent of selected pathogen while, significant antibacterial activity was observed in aqueous extracts of *Prosopsis juliflora, Hyptis suaveolens, Euphorbia hirta* and *Acacia nilotica*.

Zaker *et al.* (2013) screened the methanol and pure methanol:water (50:50 v/v) extracts of peppermint, lavandula, eucalyptus, datura and nettle against *Alternaria sesame* at 5, 10 and 15% concentrations. Mancozeb 80wp (0.2%) was used for better comparison. They indicated the results that methanolic extracts of peppermint (15 and 10%), lavandula (15%) and eucalyptus

(15%) were more effective than methanol: water extracts and completely inhibited the growth of the pathogen. Further they showed that the methanolic extracts of peppermint (15%) and eucalyptus (15%) were best in preventing the spore germination of the pathogen.

Bhardwaj *et al.* (2011) screened the aqueous petal-extracts of 20 plants against *Xanthomonas campestris* pv. *campestris*. They observed that the *Tagetes erecta* and *Chrysanthemum coronarium* were maximum inhibition of *X. campestris* pv. *campestris.* Whereas, *Acacia fernesiana, Anthocephalus cadamba, Bombax malabaricum, Lathyrus odoratus, Rosa damascena* and *Thevetia nerifolia* also showed the inhibitory effect against the test bacteria.

Seniya *et al.* (2011) screened the leaves extract of *Calotropis gigantea* against *Bacillus cereus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Salmonella typhi* and *Micrococcus luteus*. They found that among solvents, Ethyl acetate leaves extract was to be most effective with MIC value ranging from 0.25 to 2.0 mg/ml. A small portion of the dry extract was used for the phytochemical tests for compounds which include alkaloids, cardic glycoside, anthraquinone, tannins, saponins, flavnoid, steriods, terpeniods, reducing sugars and resins in accordance with the methods.

Bhardwaj and Laura (2009) screened the twenty plants extract against *Xanthomonas campestris* pv. *campestris*. They reported that the leaf extract of *Camellia sinensis* was found maximum inhibition of *Xanthomonas campestris* pv. *campestris* growth, followed by *Acacia arabicae, Aegle marmelos, Acacia catechu, Achyranthus asper, Asparagus racemosus, Azadirachta indica, Callistemon lanceolatus* and *Acacia fernesiana.*

Flavia *et al.* (2008) screened the antimicrobial effect of essential oils and methanol, hexane, ethyl acetate extracts from guava leaves against diarrhea causing bacteria: *Staphylococcus aureus, Salmonella* spp. and *Escherichia coli*. They showed maximum inhibitory activity against *S. aureus* and *Salmonella* spp in the essential oil extract.

Tripathi *et al.* (2008) screened twenty six essential oils against *Botrytis cinerea*. They found the essential oils of *Chenopodium ambrosioides, Eucalyptus citriodora, Eupatorium cannabinum, Lawsonia inermis, Ocimum canum, O. gratissimum, O. sanctum, Prunus persica, Zingiber cassumunar* and *Z. officinale* were maximum inhibition of mycelia growth (100% growth

inhibition). Whereas, the oil of *O. sanctum, P. persica* and *Z. officinale* were found to be mic values at 200, 100 and 100 ppm (mg/l) respectively.

2.2 Phytochemical compounds from botanical leaf extracts :

Konathala *et al.* (2014) evaluated the extract of *Calotropis procera* stem samples from the air-dried stem powder with different solvents such as hexane, chloroform, methanol and sterile water to establish its macro and microscopical standards, physicochemical parameters and preliminary phytochemical to the characters of the plant. The preliminary phytochemical analysis was done long with measurement of the leaf constants, fluorescence characteristics and extractive values. Quantitative estimation of total ash value, acid insoluble ash and water soluble ash may useful for identification of the powdered drug.

Enyiukwu *et al.* (2014) demonstrated a wide range of activity of plant extracts against plant pathogenic organisms. These plants extracts have been found to contain broad spectra of phytochemicals (secondary metabolites) such as alkaloids, flavonoids, tannins, saponins, phenols, glycosides, terpenoids, phlobatannins, polyphenols and steroids. Complexes of these secondary metabolites occur in crude extracts.

Singh *et al.* (2014) selected methanolic leaves extracts of *Parthenium hysterophorus*, *Vernonia amygdalina*, *Eucalyptus camaldulensis*, *Nerium oleander*, *Lantana camara* and *Ocimum sanctum* against *A. alternata* at 5, 10 and 20% concentrations. Highest reduction in mycelial growth was achieved by *Oleander* followed by *Parthenium*, *Ocimum*, *Lantana*, *Vernonia* and *Eucalyptus*, respectively. Thin layer chromatography (TLC) was carried out by using solvent system of toluene: ethyl acetate: formic acid (3:1:2) to separate the compounds responsible for antifungal activity. They reveled also the phytochemical analysis of leaf extracts were the presence of alkaloids, terpenoids, phenols, saponins and tannins at various concentrations.

Padalia *et al.* (2013) hydrodistilled the essential oils of 16 cultivars of *Mentha*, *viz*. *M. arvensis*, *M. spicata* and *M. citrata* analysed and compared by gas chromatography and gas chromatography-mass spectrometry. Fifty-seven constituents representing 92.8-99.8% of the total essential oil composition were identified. Monoterpenoids (88.1-98.6%) are the major constituents of the essential oils. The major constituents of the oils in 9 cultivars of *M. arvensis*

are menthol (73.7- 85.8%), menthone (2.5-12.0%), menthyl acetate (0.5-5.3%), isomenthone (2.1-3.9%), limonene (2.2-3.3%) and neomenthol (2.9-2.5%). Carvone (52.3-65.1%), limonene (15.1-25.2%), α-pinene (2.3-3.2%) and 1,8-cineole (≤0.1-3.6%) are the major constituents in 5 cultivars of *M. spicata*, while in one cultivar (ganga) of *M. spicata* the major constituents are piperitenone oxide (76.7%), α-terpineol (4.9%) and limonene (4.7%). Linalool (59.7%), linalyl acetate (18.4%), nerol (2.0%), *trans*-p-menth-1-en-2-ol (2.8%), α-terpineol (2.5%) and limonene (2.1%) are the major constituents of *M. citrata*.

Rai *et al.* (2013) revealed that the saponin, saponin glycosides, steroid, tannins, volatile oils, phenols and balsam (gum) were present in methanolic extract of *Eucalyptus globules.* They also observed that the methanolic extract of the plant was inhibited by the growth of *Staphylococcus aureus* and *Candida albicans* but had no inhibitory effects on *Escherichia coli*. While, minimum inhibitory concentration (mic) of the extract was ranged from 2.25g/ml to 5g/ml.

Manzoor *et al.* (2013) evaluated the extraction solvents, 100% methanol, 80% methanol, 100% ethanol and 80% ethanol, antioxidant and antimicrobial components from *Lantana camara* flowers. The extracts produced contained considerable amounts of total phenolics (8.28-52.34 mg GAE/100 g DW) and total flavonoids (2.24-7.88 mg CE/100 g DW). They concluded that both extraction solvent and techniques employed affected the antioxidant and antimicrobial attributes of the extracts from *L. camara* flowers.

Ribera and Zuniga (2012) reported the bioactive secondary metabolites of wide variety such as tannins, terpenoids, saponins, alkaloids, flavonoids and other compounds on phytopathogenic fungi in *in-vitro.* Plant antifungal metabolites may be preformed inhibitors that are present constitutively in healthy plants (phytoanticipins). They reviewed a selection of antifungal agents induced in plants during fungal attack that can be potentially used for phytopathogenic fungi control in crops.

Raphael (2012) obtained the yield of *A. vera* and neem plants 8.6% and 14.3% respectively through soxhlet (chloroform) extraction, while the aqueous extracts of the two plants were 5.4% and 6.2%, respectively. He observed that the screening of phytochemical in presence of tannins, flavonoids, terpenoids, carbohydrates and alkaloids against *A. vera* plant with saponins, glycosides phlobatannins, antiquinones carbohydrates and steroids been absent, while the neem

plant contains flavonoids, steroids, carbohydrates, glycosides, antiquinone, terpenoides and alkaloids.

Tripathi *et al.* (2012) studied the extraction of *Psidium guajava, Syzygium cumini, Eucalypts* with methanol soluble phytochemicals. They analyzed soluble fraction of phenols using HPLC coupled to UV-vis detector at 254 nm. Which gives different peaks at this region, it may be phenolic compounds.

Hasan *et al.* (2012) evaluated that the seven component of ginger extracts in different solvents by HPLC. They results showed that the two extracts of *Zingiber officinale* had inhibitory effects on the growth of *Candeda albicans* fungi.

Britto *et al.* (2011) investigated that the leaves, stem, flowers and fruits of *Azadiracta indica* against *Xanthomonas campestris.* Phytochemical analysis gave positive results for steroids, triterpinoids, reducing sugars, sugars, alkaloids, phenolic compounds, flavonoids and tannins. They showed that the methanol extract of the leaves and fruits showed significant inhibitory effect when compared with positive controls, neomycin and kanamycin, respectively. While, stem and flowers extracts were also found antibacterial activity.

Britto and Gracelin (2011) investigated the leaves, stem, flowers and fruits of *Azadiracta indica* against *Xanthomonas campestris*. They analyzed phytochemicals and gave positive results for steroids, triterpinoids, reducing sugars, sugars, alkaloids, phenolic compounds, flavonoids and tannins. The methanol extract of the leaves and fruits showed significant inhibitory effect when compared with positive controls, neomycin and kanamycin respectively. The stem and flowers extracts show marked antibacterial activity.

Murgan and Mohan (2011) extracted the phytochemicals from the leaves and stem bark of *Bauhinia purpurea* and *Hiptage benghalensis* using different solvents of various polarities such as petroleum ether, chloroform, acetone, methanol and water. They carried out the antibacterial activity against *Staphylococcus aureus, Klebsiella pneumoniae, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi.* They reported that the methanol extract was most effective against the tested microorganisms and phytochemical analysis revealed the presence of alkaloids, coumarin, flavonoids, phenols, tannins and terpenoids also.

Aprotosoaie *et al.* (2010) characterized the chemical profile constituents of the essential oils of fennel (*Foeniculum vulgare*) using gas chromatography and mass spectroscopy analysis (gc-ms). The main compounds in all fennel volatile oils were: t-anethole, estragole, fenchone and limonene.

Jain *et al.* (2009) assessed the anti-inflammatory activity of methanolic and petroleum ether extracts of *Ipomoea fistulosa* leaves. Anti-inflammatory activity was screened by formalin (0.1%) induced rat paw edema method in albino mice. The petroleum ether extracts caused significant decrease in paw edema in 24-hour observation. Anti-inflammatory activity may be due to the presence of b-sitosterol. Dose dependent activity may be attributed to high concentration of phyto b-sitosterol.

2.3 Botanical leaf extracts against the pathogens :

Atar *et al.* (2015) studied ten botanicals and two bioagents against *Xanthomonas axonopodis* pv. *punicae*. Neem oil, was found best effective for control of *X. axonopodis* pv. *punicae* at all concentration by forming 14.47 per cent inhibition followed by Garlic leaf extract, Tulasi leaf extract, Ginger extract, Guava leaf extract and *Aloe vera* extract with 12.86%, 12.28%, 12.70%, 12.13%, 9.36% and 7.34 % average inhibition, respectively.

Taware *et al.* (2015) evaluated all the eleven plant extract/botanicals against *Alternaria carthami* in *in vitro*. They recorded that mean inhibition percentage of mycelial growth was ranged from 19.26 per cent in the treatment of *A. sativum*. However, *A. sativum* was found most fungistatic which recorded significantly highest mean mycelial growth. The second and third best botanicals found were *D. metal* (49.87%) and *C. longa* (46.91%).

Ali *et al.* (2014) evaluated the aqueous decoction and aqueous infusion extract of *Cuscuta pedicellata* L. against different pathovar of *Xanthomonas campestris*. The results obtained, both extracts found inhibited the bacterial growth of pathogen with inhibition zone diameter ranging from 2.0 to 5.0 cm. They revealed that the aqueous decoction was strongest antibacterial activity on *X. campestris* pv. *mangiferae indicae* and *X. campestris* pv. *punicae* whereas aqueous infusion extract showed strongest antibacterial activity on *X. campestris* pv. *citri* and *X. campestris* pv. *pinicae*.

Kaushik *et al.* (2014) prepared the leaf extracts of *Solanum torvum*, *Adhatoda vasica*, *Terminalia chebula* and *Asparagus racemosus* in organic solvents namely methanol, ethanol and methanol-ethanol (1:1) and tested against *Fusarium oxysporum* and *Aspergillus parasiticus.* Irrespective of the extraction solvent used all the plants extracts showed certain degree of antimicrobial activity against *Aspergillus parasiticus*. They obtained the maximum zone of inhibition was recorded in the methanolic and ethanolic extracts of *Solanum torvum* and *Adhatoda vasica.* Similarly *Asparagus racemosus* and *Adhatoda vasica* were most effective against *Aspergillus parasiticus*. Rest of the plant extracts exhibited moderate to minimal antifungal activity.

Ibrahim and Al-Ebady (2014) tested ethanolic extracts of tarragon (*Artemisia dracunculus*), rosemary (*Rosemarinus officinalis*) and thyme (*Thymus vulgaris*) and the essential oil of oregano (*Origanum vulgare*) against *A. niger*, *A. flavus*, *Penicillium* spp., *Rhizopus* spp. and *Fusarium* spp at concentrations of 100, 500, 1000, 1500, 2000 ppm. The results showed that oregano essential oil was very strong antifungal activity against *Fuazarium* spp. (mic: 0.8 mg/ml), *A. niger* (mic: <1 mg/ml) and *Penicillium* spp. (mic: 4.5 mg/ml) whereas, rosemary was most effective on *Rhizopus* spp.

Rodino *et al.* (2014) tested ethanolic and aqueous extracts of absinth (*Artemisia absinthium*), rosemary (*Rosmarinus officinalis*), jimson weed (*Datura stramonium*) and cocklebur (*Xanthium strumarium*) against *Alternaria alternata*. They obtained the results that all extracts were presented antifungal potential and demonstrated by the inhibition of the mycelial growth. Generally, the ethanolic extracts showed a higher antifungal activity than the aqueous extracts, for all the tested variants.

Mokhtar *et al.* (2014) evaluated the extracts powder of chilli pods (*Capsicum annuum*), leaves of cabbage (*Brassica oleracea*) and eucalyptus (*Eucalyptus obliqua*) against *Fusarium solani* and *Rhizoctonia solani.* They observed that different concentrations of botanicals were superior inhibitory effect on radial fungal growth when compared with powdered ones. Further they also reported that the botanical plant powders were reduced root rot incidence comparing with untreated control in field conditions. Higher significant reduction in disease incidence was observed for combined treatment than that of individuals, and it was interesting to note that

botanical plants powder gave a similar effect to the fungicide rhizolex-t in reducing root rot incidence either at pre- or post-emergence stages of bean growth.

Sheema and Durai (2014) tested ten plant extracts against *Alternaria brassicae* causing black leaf spot in the common cabbage (*Brassica oleracea* Linn.). The majority of the extracts, *Solanum nigrum* was more effective and the activity is almost found equal to chemical fungicides. They discussed the results in relation to the literature and steroidal saponins were concluded to be the active principle in controlling the pathogen.

Didwania *et al.* (2013) investigated the leaves extracts of twenty medicinal plants against *Xanthomonas campestris* pv. *campestris* causing black rot disease on cauliflower at 12.5%, 25% and 50% concentrations. It was found that twenty extracts of all the plant samples showed significant activity. They showed that the plant extracts of *Allium cepa, Azadirachta in-dica, Tamarix aphylla, Vernonia anthelmentica, Plumbago zelanicum,* and *Tagetes erecta* were significantly good antibacterial activity in 50% concentration against *X. c.* pv. *campestris in vitro* and resulted in better seed germination and plant vigour than streptomycin.

Rani *et al.* (2013) evaluated the piperine (piperamides which are the pungent bioactive alkaloids) against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa, Escherichia coli, Alternaria alternata, Aspergillus niger, Aspergillus flavus* and *Fusarium oxysporum.* Piperine showed antimicrobial activity against all tested bacteria with zone of inhibition ranged from 8-18mm. Maximum zone of inhibition was against gram positive bacteria *Staphylococcus aureus* (18mm) and minimum against gram negative bacteria *Escherichia coli* (8mm). Further, they reported that the Piperine was maximum antifungal activity towards *Fusarium oxysporum* (14 mm) and very least effect against *Aspergillus niger* (38mm).

Singh *et al.* (2013) prepared extracts of *C. procera, W. somnifera, A. indica* leaves, *A.indica* seed kernel, *A. sativum* bulb and *S. suratteuse* green berries in distilled water, acetone or methanol were significantly inhibited mycelial growth as well as spore germination of *A. brassicae*. *Azadirachta* leaves prepared in distilled water, acetone and methanol were inhibitory to mycelial growth of *A. brassicae* in PDA and also germination of spores of the fungus. The inhibition of growth and spore germination of the fungus increased with the increase in concentration of the test extracts prepared in all the three solvents. The inhibition was most spectacular when the

extracts were used at 15 per cent concentration. *Azadirachta* seed kernel and *Calotropis procera* leaf extracts were also highly inhibitory to mycelial growth and spore germination. While, *A. sativum, W. somnifera* and *S. suratteuse* were relatively less toxic to it. Further they revealed that the two foliar sprays of aqueous extracts (10%) of *A. indica* seed kernel, *C. procera* or *A. indica* leaves were found suppressed *Alternaria* blight and enhanced seed yield in mustard under field conditions.

Kumari *et al.* (2013) tested the aqueous extracts of neem cake, karanj cake and vermicompost against *Helminthosporium pennisetti*, *Curvularia lunata* and *Colletotrichum gloeosporioides* f. sp. *mangiferae.* They observed that aqueous extract of karanj cake was found most effective against *C. gloeosporioides* f. sp. *mangiferae* where GI_{50} was found to be 0.41% drug concentration followed by neem cake (0.46%) and vermicompost (0.86%). While, neem cake extract was most effective with GI_{50} value of 0.27% and 0.11% against *C. lunata* and *H. pennisetti,* respectively. The GI_{50} values noted for *C. lunata* with extracts of karanj cake (0.70%), vermicompost (0.88%) and for *H. pennisetti* were (0.20% and 0.22%), respectively.

Mahapatra and Das (2013) evaluated the aqueous extract of neem (*Azadirachta indica*), bulbs of garlic (*Allium sativum*) and rhizome of ginger (*Zingiber officinale*) against alternaria leaf blight of mustard at 5, 10, 15, 20% concentrations. They revealed that spraying of neem leaf extract @15% was more effective against this disease as well as increased the seed yield of mustard. Seed yield was significantly highest ($p < 0.05$) on application of neem leaf extract @ 15% over other treatments (1403.83 kg/ha) where as in check had only 820.33kg/ha. Garlic bulb extract @10% also gave better yield (1366.17kg/ha) which is similar to that of 10% of neem leaf extract (1383.00kg/ha). Cost benefit ratio was also highest (1:2.5) at 15% neem leaf extract which was similar to that of 10% of the same botanicals (1:2.0) and 10% garlic bulb extract (1:2.9).

Khan and Nasreen (2013) tested the thirty-nine extracts from 10 medicinal plants against *Helminthosporium sativum* and *Aspergillus niger* for their fungitoxicity *in vitro.* They reported that the methanol leaves extracts of *Lawsonia inermis, Withania somnifera, Datura metel, Datura stramonium* and stem bark extract of *Bauhinia racemosa* were significantly inhibited

mycelia growth of both target fungi. While, some extracts were exhibited greater fungitoxicity than that of synthetic fungicide dithane M-45.

Kebede *et al.* (2013) evaluated the potency of mustard and ginger rhizome extracts, lemon juice, Atella (residue of traditional Ethiopian beer) and cow urine in controlling tomato seed borne pathogen, *Xanthomonas campestris* pv. *vesicatoria* (Xcv). Streptomycin (0.5%), sodium hypochlorides (1%) and control treatments were similarly applied. Atella at all treatment durations completely inactivated (0 cfu/ml) Xcv from inoculated tomato seeds and induced 92 to 98% seed germination. Ginger and mustard extracts showed 0 cfu/ml on Xcv with 24 h soaking, 97 to 81% germination and 2303 to 2270 vigor indexes of tomato seed.

Mamgain *et al.* (2013) evaluated the new chemicals along with various biological control agents including bacteria, *Actinomycetes* and fungi. They are found some plants and plant products to be useful in controlling on *Alternaria* infection.

Sajid *et al.* (2013) evaluated the three chemicals (plant protector, agrimycine and copper oxy chloride) and plant extracts (*Citrullus colocynthis*, *Nicotiana tobaccum* and *Curcuma lunga*) against colony growth of *Xanthomonas axonopodis* pv. *malvacearum*. They found that the plant protector was most effective at 600ppm after 72 hours of treatment while, chemicals inhibition zone area increased with increasing dose and time. They showed, among plant extracts were no relation in colony growth inhibition of bacteria with respect to time and concentrations but, tobacco's extract was most significant at 10% concentration.

Swami and Alane (2013) tested the aqueous extracts of *Annona squamosa, Azadirachta indica, Adhatoda vasica, Ocimum sanctum, Polyalthia longifolia, Tridax procumbens,* rhizome extracts of *Curcuma longa* and *Zingiber officinale,* bulb extract of *Allium cepa* and clove extract of *Allium sativum* against the growth of the fungi isolated from the seeds of green gram. They reported that all the plant extracts were significantly inhibited the mycelia growth of *Alternaria alternata, Curvularia lunata, Phytophthora, Fusarium oxysporum, Asperillus niger* and *Rhizoctonia solani.*

Al-Hazmi (2013) evaluated the aqueous and ethanolic extracts of the leaves and seeds of *Azadirachta indica* on *Pythium aphanidermatum, Alternaria alternata, Bipolaris sorokiniana,*

Fusarium oxysporium, Helminthosporium sp. and *Thilaeviobsis* sp at 1:1, 1:10, 1:100, 1:1000 (v:v) concentrations. They showed that the growth of *P. aphanidermatum, A. alternata* and *Helminthosporium* sp. was mostly inhibited by the ethanolic extract of the neem seeds compared to the other extracts, while no inhibitory effect was noticed for the tree seed water extract on the growth of *P. aphanidermatum, Alternaria alternata* and *Helminthosporium* sp. The least affected fungus by these seeds and leaves extract was *Thialeviopsis* sp. The seeds and leaves extracts were mostly affective in growth retardation of the fungi when applied at the highest concentration (1:1 v:v), *P. aphanedermatum* was retarded by 22.74%, *A. alternata* by 33.2%, *B. sorokinianae* by 40.27%, *F. oxysporum* by 57.26%, *Helminthosporium* sp. by 38.56%, *T.* sp. by 23.40%.

Gupta *et al.* (2013) stated that black rot disease of cabbage caused by *Xanthomonas campestris* pv. *campestris* can be managed through cultural practices, resistant hosts, seed treatment with hot water and antibiotics, fungicides, biological control as well as chemical control methods.

Singh *et al.* (2013) evaluated the plant extracts of *C. procera, W. somnifera, A. indica* leaves, *A.indica* seed kernel, *A. sativum* bulband *S. suratteuse* against *A. brassicae.* They reported that the *Azadirachta* seed kernel and *Calotropis procera* leaf extracts were inhibitory to mycelial growth and spore germination at 15 per cent concentration. While, *A. sativum, W. somnifera* and *S. suratteuse* were relatively less toxic to it. Further, they revealed that two foliar sprays of aqueous extracts (10%) of *A. indica* seed kernel, *C. procera* were suppressed *Alternaria* blight and enhanced seed yield in mustard under field conditions.

Rizvi *et al.* (2013) evaluated the extract of *Lawsonia inermis, Lantana camara* and *Swertia angustifolia* against *Staphylococcus aureus* and *Klebsiella pneumoniae*. They reported that the *L. camara* was not much active against any bacterial strain. While, inhibition zone was range about 9- 12.3 mm against all bacterial strains tested. In case of fungal growth inhibition *L. inermis* showed good percent inhibitions (78.8, 65.3 and 72.1) of 3 fungal strains, *Fusarium solani, Alternaria* and *Mucor,* respectively.

Prakash and Karmegam (2012) tested of *Aegle marmelos, Aristolochia indica, Ocimum canum* and *Plumbago zeylanica Xanthomonas campestris* at different concentrations. They reported that the all solvent extracts were found significant activity against five plant pathogenic bacteria at

concentrations of 1000, 2000, 3000, 4000 and 5000 ppm. They showed also the largest inhibition zone in the extract of *Aegle marmelos* and *Ocimum canum* at 5000 ppm.

Thirumalesh *et al.* (2012) evaluated the crude extracts, by using solvents like petroleum ether, chloroform, methanol and water against *X. campestris* pv *mangiferae indica*. They reported that the crude fruit extract of *Sapindus laurifolia* (15.0 mm) and leaf extracts of *Asclepias curassavica* (7.50 mm), *Helicteres isora* (7.0 mm), *Piper betel* (7.0 mm), *Tamarindus indica* (5.0 mm), *Tridax procumbens* (5.0 mm) and *Azadirachta indica* (7.0 mm) were greater antibacterial activities with MIC ranging from 3.0% to 12.0%.

Manimegalai and Ambikapathy (2012) investigated the extract of *Bergia capensis, Marselia quadrifolia, Parthenium hysterophorus, Lippia nodiflora, Azadirachta indica, Eclipta prostrata, Datura metal, Cyperus compressus, Ocimum santcum* and *Adhatoda vasica* against *Bipolaris oryzae*. They recorded that the methanolic extract of *Parthenium hysterophorus* and *Azadirachta indica* were exhibited maximum zone of inhibition as 28mm and 25mm.

Jalander and Gachande (2012) tested the aqueous leaf extract of *D. stramonium, D. innoxia, D. metal* and *D. ferox* against *Alternaria solani* and *Fusarium oxysporum* f.sp. *udum* at 5, 10, 15 and 20% concentrations. They found that the leaf extract of *D. stramonium* and *D. innoxia* was found more inhibitory activity against *F. oxysporum udum*, while the extract of *D. stramonium* at 20% concentration was inhibitorier against *A. solani*.

Paradza *et al.* (2012) tested the neem (*Azadirachta indica*), leaf garlic (*Allium sativum*) and cloves against *Pectobacterium carotovorum* subspecies *carotovorum* (Pcc), *Pectobacterium atrosepticum* (Pa) and *Dickeya dadantii* (Dd). Neem and garlic extracts were significantly inhibited the growth of Pa and Dd but ineffective against Pcc.

Sheikh *et al.* (2012) observed the aqueous leaf extracts against *Xanthomonas campestris, Agrobacterium rhizogenes* and *Aspergillus fumigates.* They showed that the *Prosopis juliflora* was maximum and significant inhibitory effect on the growth of all the three pathogens. Whereas, strength of *Xanthomonas campestris* in the leaf extracts was almost equal to the streptomycin 10 mcg. Aqueous extracts of some plants formed low zone of inhibition. While some plant extracts were comparatively more effective against all the three pathogens.

Dwivedi and Dwivedi (2012) tested *Eugenia caryophyllata* (clove), *Moringa oleifera* (sehjan), *Trachyspermum captivum* (ajwain) and *Zingiber officinale* (zinger) against *Fusarium solani* at 10%, 25% and 50% concentrations. They reported in among all four plant extracts, *Eugenia caryophyllata* was found to be most effective against *Fusarium solani* on 3rd, 5th and 7th day at 50% concentration and conquered the colony growth by 100% compared to control followed by *Trachyspermum captivum* and *Moringa oleifera*, 3rd day (92.70% and 67.42%), 5th day (92.74% and 72.35%) and 7th day (94.35% and 78.04%) respectively. Whereas, *Zingiber officinale* has shown least efficiency to suppress the colony growth of *Fusarium solani* on 3rd day (60.65%), 5th day (63.55%) and 7th day (73.50%) after inoculation at 10%, 25% and 50% concentration over control.

Bajpai and Kang (2012) reported the *Magnolia liliflora* leaf extracts such as hexane, chloroform, ethyl acetate and methanol (1,500 μg $disc^{-1}$) against *Botrytis cinerea* kacc 40573, *Colletotrichum capsici* kacc 40978, *Fusarium oxysporum* kacc 41083, *Fusarium solani* kacc 41092, *Phytophthora capsici* kacc 40157, *Rhizoctonia solani* kacc 40111 and *Sclerotinia sclerotiorum* kacc 41065 as radial growth inhibition percentages of 38 to 65.6% and 7.6 to 57.3%, respectively along with their respective MIC and MFC values ranging from (125 to 500 and 125 to 100 μg ml-1) and (500 to 4,000 and 500 to 8,000 μg ml-1).

Kunasakdakul and Suwitchayanon (2012) evaluated the chili and black pepper extracts on *Xanthomonas campestris* and *Alternaria brassicicola* causing black rot and leaf spot disease of chinese cole crop at concentrations of 3.0%, 2.5% and 0.75% (w/v). They reported that the black pepper extract completely inhibited the bacteria after soaking in a 1:1 mixture (v/v) of bacterial suspension (1x104 cfu/ml) and diluted extracts. While, chilli extract concentrations varied in their inhibition ability, from highest (3.0%) to moderate (2.5%) to none (0.75%).

Uma *et al.* (2012) tested eight fruit seed extract against *Ralstonia solanacearum*, *Xanthomonas campestris*, *Phytophthora capsici* and *Pythium aphanidermatum.* They showed the methanolic extract of *Eugenia jambolana* had maximum inhibitory effect against *X. campestris* with the inhibitory zone of 34 mm, whereas the aqueous extract recorded an inhibition zone of 22 mm.

Gracelin *et al.* (2012) evaluated the antimicrobial potency of five solvents (petroleum ether, benzene, chloroform, methanol and water) extracts of five medicinal ferns *Pteris biaurita*,

Lygodium flexiuosam, Hemionitis arifolia, Actinopteris radiate and *Adiantum latifolium* against *Xanthomonas campestris*. They concluded that the *P. biaurita* was maximum MIC (8µg/ml) and rpi (199.36%) values of *X. campestris.*

Sheikh *et al.* (2012) studied the eleven aqueous leaf extracts on *Xanthomonas campestris*, *Agrobacterium rhizogenes* and *Aspergillus fumigates.* They showed the leaf extract of *Prosopis juliflora* was maximum and significant inhibitory effect on the growth of all the three pathogens. While, in case of *Xanthomonas campestris* the effect of this plant extract was almost equal to the strength of Streptomycin 10 mcg.

Neycee *et al.* (2012) evaluated the antibacterial effects of methanol extract of seed-fruits, leaves and flowers of chinaberry (*M. azedarach*) via growth inhibitory zone against strains of *Pseudomonas syringae* pv. *syringae, Xanthomonas campestris* pv. *campestris*, *Rathayibacter tritici* and *Escherichia coli*. They reported the positive effect and size of growth inhibition zone in each of the factors A, B, C (organ, concentration and bacteria) found significant difference, while there was also a significant difference between types of organ and bacteria in forming growth inhibitory zone.

Thirumalesh *et al.* (2012) reported the antibacterial activity of crude extracts of eight plants including *A. indica*, by using solvents like petroleum ether, chloroform, methanol and water against *X. campestris* pv. *mangiferaeindicae.*

Upadhyay *et al.* (2012) evaluated the plant bark extracts of *Chloroxylon switenia* against *E. coli, P. aeruginosa* and *S. aureus*. They reported that the *Chloroxylon switenia* was exhibited good results for antimicrobial activity against *S.aureus* and *P. aeruginosa*.

Britto *et al.* (2011) investigated the methanol and aqueous extracts of *Acalypha indica, Aerva lanata, Phyllanthus amarus, Phyllanthus emblica, Cassia auriculata* and *Caesalpinia pulcherrima* against *Xanthomonas campestris* and *Aeromonas hydrophila*. They reported that the methanol extracts of *Acalypha indica, Aerva lanata* and *Phyllanthus amarus* exhibited clear zone of inhibition against the tested micro organisms.

Lalitha (2011) evaluated the *Allium sativum, Capsicum annum Cassia fistula, Coriandrum sativum, Cuminum cyminum* and *Curcuma longa* against *Pyricularia oryzae, Bipolaris oryzae,*

Alternaria alternata, Tricoconis padwickii, Drechslera tetramera, Drechslera halodes, Curvularia lunata, Fusarium moniliforme, F. oxysprorum and *F. solani* and five human pathogenic bacteria *viz., Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholera* and *Streptococcus pneumonia* at 500, 1000, 1500 and 2000 ppm concentration. They recorded that the essential oil of *A. sativum* was complete inhibition against eight fungi followed by *C. sativum, C. longa* and *C. cyminum* including with control. Whereas, assayed in antibacterial, *A. sativum* was found maximum inhibition of all test bacteria in the range of 10.9 to36.9 mm followed by *C. longa* (5.6 -25.6 mm), *C. cyminum* (10.9-30.2 mm).

Lin *et al.* (2011) discovered the ethanol (EtOH) extract of *Solanum nigrum* was inhibited spore germination of *Alternaria brassicicola* at 500 mg/L. Furthermore, an n-butanol fraction of the EtOH extract exhibited strong antifungal activity; at a concentration of 25 mg/L, a derived subfraction (Bu-11-13) showed complete inhibition of spore germination. A white powder was collected from fraction Bu-11-13, and its minimum inhibitory concentration was determined to be 8 mg/L. Using NMR and LC-MS/MS analysis, this white powder compound was identified as degalactotigonin.

Dellavalle *et al.* (2011) investigated the 10 plant extracts against *Alternaria* spp. They used three solvents on different tissues of the plants and among the 29 extracts, 31% of the extracts inhibited growth, similar to the effects of a chemical fungicide. Acid extracts of the plants were more effective than the aqueous or buffer extracts against *Alternaria* spp. They determined the MIC values of the extracts were ranging between 2.25 and 25 μg $ml^{-2.}$ Whereas, the MFC values of the extracts ranged between 2.25 μg ml^{-1} (*Rosmarinus officinalis* L.) and 10 μg ml^{-1} (*Cynara scolymus* L.). MICs and MFCs values obtained from leaves (*Salvia officinalis* L. and *R. officinalis*) and seeds extracts (*Salvia sclarea* L.) were quite comparable to values obtained with the conventional fungicide captan (2.5 μg ml^{-1}).

Kumar *et al.* (2011) evaluated the fungicides, botanicals and bio-agent against *Alternaria* leaf blight of chrysanthemum. They recorded that the Hexaconazole (0.1%) was found effectively controlled the disease incidence which recorded very less per cent disease index (4.49) followed by Chlorothalonil (0.2%) and Mancozeb (0.2%).

Mathur *et al.* (2011) investigated the *Mentha piperita* (lamiaceae) extracted essential oil against *Staphylococcus aureus, Streptococcus mutans, Streptococcus faecalis, Streptococcus pyogenes, Lactobacillus acidophilus, Pseudomonas aeruginosa, E. coli, Bacillus subtilis, Salmonella typhimurium* and the fungal strains *Candida albicans, Aspergillus niger, Penicillium notatum* and *Saccharomyces cerevisae* at different concentrations (1:1, 1:5, 1:10, and 1:20). They observed that menthol was highest inhibitory effect against all tested bacteria. Furthermore, menthol achieved considerable antifungal activity against all the fungal strains except *A. niger*. The isolation of an antimicrobial compound from *M. piperita* leaves validates the use of this plant in the treatment of minor sore throat and minor mouth or throat irritation as well as diseases such as typhoid.

Chavan *et al.* (2011) investigated the ethanolic and aqueous extracts from the leaves of *Cassia tora* against *Pseudomonas aeruginosa, Lactobacillus, Salmonala typhi, P. vulgaris, Bacillus subtilis, Staphylococcus aureus, Streptococcus pneumoniae, E. coli* and *Enterobacter*. They showed that the ethanolic extract (0.15mg) and aqueous extract (0.31mg) of *Cassia tora* antibacterial activity against all tested bacteria but maximum activity aqueous extract against *Staphylococcus aureus* and *Lactobacillus*.

Hasan *et al.* (2011) investigated the solvent of ethyl alcohol, ethyl acetate, chloroform, acetone, n-hexane and dichloromethane extracts from the leaf, stem and root of akanda (*Calotropis gigantea*) against *Xanthomonas oryzae* pv. *oryzae, Ralstonia solanacearum* and a symbiotic bacterium *Rhizobium* sp. They observed that the maximum inhibition zone of *X. oryzae* pv. *oryzae* in the leaf extracts of ethyl acetate followed by the stem and the root extracts of acetone, respectively. The maximum zone of inhibition of *R. solanacearum* and *Rhizobium* sp. was found with the ethyl alcohol leaf extract followed by the stem and the root extracts.

Kuri *et al.* (2011) evaluated the aqueous leaf extracts of *Azadirachta indica, Calotropic procera, Clerodendron* spp*., Croton spasriflorous, Luffa cylidrica, Salvadora persica, Senna alata, Trema orientales* and *Trichosanthes dioica;* leaf and seed of *Lantana camara, Moringa oleifera, Putranjiva roxburghii* against *Phomopsis vexans, Fusarium oxysporum, Aspergillus flavus, Aspergillus nigar, Curvularia lunata* and *Penicillium* spp. They observed that the leaf extract of *M. oleifera* was increased 92% seed germination as compared with control treatment. Whereas,

leaf extracts of *A. indica* and *P. roxburghii* gave the best potential against all tested pathogen with 4% seed infection, followed by leaf extract of *S. persica* and *C. procera* were shown activities of 5.33% seed infection. *L. camara* and *Clerodendron* spp were significantly 6.67 % inhibited as seed infection. While, the leaf extracts of *S. alata, T. orientales* and *L. cylidrica* showed seed infection of 8 % and leaf extracts of *M. oleifera, C. spasriflorous, P. roxburghii, T. dioicahae* were also shown 9.33 % seed.

Mesta *et al.* (2011) reported that garlic clove extract was found effective in inhibiting the mycelial growth of *A. solani* and *A. helianthi*, respectively.

Yanar *et al.* (2011) investigated 27 plant extracts against *Alternaria solani*. They revealed the results of *Circium arvense, Humulus lupulus, Lauris nobilis* and *Salvia officinalis* showed significant antifungal activities. The treatment of *L. nobilis* was most effective in inhibiting the mycelial growth of *A. solani* (79.35%) at 4% concentration, followed by *S. officinalis, H. lupulus*, and *C. arvense* with 76.50, 62.50 and 55.83% inhibition, respectively. While, the lowest antifungal activity was observed on *Hypericum perforatum* extract.

Begum *et al.* (2010) tested the 15 plant extracts against *Fusarium oxysporium* f. sp. *capsici, Rhizopus artocurpi* and *Alternaria tenuis.* They showed that the leaf and seed extracts of *Azadirachta indica* was maximum inhibitory mycelial growth than tested with other plant extracts. Whereas, extract of *Moringa oleifera* showed least inhibitory effect against all the fungi tested. While, *Datura metel, Plygonum oriantale, Tagestes patula* and *Micania scandens* also showed promising inhibition on spore germination and mycelial growth of all the pathogens tested.

Neeraj and Verma (2010) evaluated *Canna indica, Convolvulus arvensis, Ipomoea palmata, Cenchrus catharticus, Mentha piperita, Prosopsis spicigera (Mant), Allium cepa, A. sativum, Lawsonia inermis, Argemone mexicana, Datura stramonium* and *Clerodendron inerme* against *A. brassicae.* They reported that the some plants and plant products were also found to be useful in controlling *Alternaria* infection.

Nunboon *et al.* (2010) tested the crude extracts from *Curcuma longa, Cymbopogon narus* and *Stemona tuberose*, three edible mushrooms (*Pleurotus osteatus, Lentinus squarrosulus* and

Lentinus polychrous) with five solvents (water, acetone, methanol, ethanol and hexane) *Alternaria brassicicola*. They revealed the results of crude acetone extract from *C. longa* inhibited vegetative growth of *A. brassicicola* by 10,000 ppm at 77.50%, and the ed50 value was 50 ppm. While, showed crude acetone from *C. longa* gave good result to control leaf spot disease, with no side effects on growth and seed germination at 10,000 ppm.

Belabid *et al.* (2010) evaluated *Anacyclus valentinus, Artemisia herba alba, Eucalyptus* sp., *Inula viscosa, Laurus nobilis, Mentha pepirita, Rosmarinus officinalis, Salvia officinalis, Tetraclinis articulata* and *Thymus vulgaris* on *Fusarium oxysporum* f. sp. *lentis.* They obtained the results showed that the treatments 10 and 5% with the powders of *I. viscosa* and *M. pepirita* and the essential oils formulation in all treatments have significantly reduced the soil population densities of *Fol* and the disease incidence on lentil.

Sharma *et al.* (2010) investigated the ethanolic and aqueous extracts of *Cassia tora* against various bacteria. Their concentrations 0.15mg, 0.31mg ethanolic and aqueous extracts respectively against *Staphylococcus aureus, Lactobacillus* and showed moderate activity against *Pseudomonas aeruginosa, P. vulgaris* and *Enterobacter* and show less activity against *Bacillus subtilis* and *Eschieria coli.*

Pundir and Jain (2010) evaluated black pepper and turmeric in three solvents against antibacterial and antifungal activity. They showed that aqueous extract of black pepper and turmeric were good inhibitory activity against *Staphylococcus aureus* with zone of inhibition 25 to 30 mm and 26 to 28 mm, respectively. Whereas, ethanolic extract of black pepper showed antibacterial activity against all test bacteria with zone of inhibition ranged between 15 mm and 22 mm while turmeric showed activity with zone of inhibition ranged between 13 mm and 24 mm. Further they reported that the turmeric ethanolic extract was maximum antifungal activity against *Rhizopus stolonifer* and *Mucor* sp. with percent mycelial growth inhibition ranged between 25% and 30%.

Ahameethunisa and Hoer (2010) tested the six organic solvent extracts of *Artemisia nilagirica* against phytopathogens and clinically important bacterial strains. They showed that the all the extracts were found inhibitory activity for all phytopathogens with low MIC of 32 μg/ml and the methanol extract exhibited a higher inhibition activity against *Escherichia coli, Yersinia*

enterocolitica, Salmonella typhi, Enterobacter aerogenes, Proteus vulgaris, Pseudomonas aeruginosa (32 µg/ml), *Bacillus subtilis* (64 µg/ml) and *Shigella flaxneri* (128 µg/ml). After they screened the phytochemical derivative of alkaloids, amino acids, flavonoids, phenol, quinines, tannins and terpenoids.

Pawar and Papdiwal (2010) screened the 30 plants leaf extracts against 11 strains of *Xanthomonas campestris* pv. *mangiferae indicae* (*Xcmi*). Whereas, out of which, 12 leaf extracts showed antibacterial activity. While the extract of *Terminalia thorelii* and *Azadirachta indica* were maximum activity against the *Xcmi* strains.

Nwinyi *et al*. (2010) found the aqueous seed extract of *C. papaya* was suppressed the growth of fungal mycelia of *Rhizopus* sp., *Mucor* sp. and *Aspergillus* sp. They also reported the effectiveness of *C. papaya* seed extract against *P. aphanidermatum* and *P. capsici.*

Sunder *et al*. (2010) reported the extracts of onion bulbs and garlic cloves were effective against *Drechslera oryzae*.

Jain *et al.* (2010) evaluated the antibacterial activity of methanolic and hexane leaf extracts of *D. Stramonium, Ricinus communis, Calotropis gigantean, Malva verticillata* and *Malvastrum coromandelium* against *E. coli, S. aureus* and *B. subtilis*. They observed that the maximum antimicrobial activity expressed in terms of zone of inhibition by methanolic and hexane leaf extract of *D. stramonium* followed by *Ricinus communis, Calotropis gigantean* to and *Malva verticillata* against the tested organism.

Madhanraj *et al.* (2010) studied that antifungal ability of some plant extracts against *Fusarium solani*. They reported that the leaves of *Adathoda vasica, Azadirachta indica* and *Vitex negundo* extract were more effective at 20% concentration against the pathogen.

Suprapta and Khalimi (2009) evaluated the six formulation of *Eugenia aromatica*, *Piper betle*, *Alpinia galanga* and *Sphaeranthus indicus* against *F. oxysporum* f.sp. *vanillae*. They observed that the 5% solution of F5 formulation was highest inhibitory (6.7 mm) effect against the radial growth of *F. oxysporum* f.sp. *vanilla* as compared to non amended control (87.9 mm). Whereas, stem rot disease was obviously suppressed on vanilla seedlings grown in the soil treated with 5% solution of each plant extract. While, the treatment of F5 was lowest disease incidence, in which

only 7% of the vanilla seedlings were infected and low population density of *F. oxysporum* f.sp. *vanillae* in the soil.

Simone *et al.* (2009) evaluated the *Azadirachta indica* (neem) extracts on mycelial growth, sporulation, morphology and ochratoxin A production by *P. verrucosum* and *P. brevicompactum*. They found that the neem oil extract from seeds and leaf showed significant ($p \leq 0.05$) reduction of growth and sporulation of the fungi at 0.125, 0.25 and 0.5% and 6.25 and 12.5 mg/mL, respectively, in Yeast Extract Sucrose (YES) medium.

Suwitchayanon and Kunasakdakul (2009) tested clove (*Syzygium aromaticum*) and turmeric (*Curcuma longa*) extracts against crucifer pathogens. They revealed that antimicrobial activity of clove and turmeric extracts were inhibited in the ranges of 950-3800 ppm of clove and 3800-15000 ppm of turmeric extracts. Whereas, clove extract had minimum inhibitory concentration (MIC) of *Alternaria brassicicola* and *Fusarium oxyporum* at 1900 ppm and 2300 ppm, respectively. While, the lower MIC at 470 ppm and 230 ppm of clove and turmeric extracts respectively were observed with *Xanthomonas campestris*.

Mesta *et al.* (2009) evaluated new molecules of fungicides and plant extracts against *Alternaria helianthi*. They reported that the neem leaf extract was maximum % inhibition of spore germination (38.49) and per cent inhibition of mycelial growth (43.90) as compared to all other plant extracts.

Manonmani *et al.* (2009) reported *Acalypha indica, Achyranthes aspera, Aloe vera, Azadirachta indica, Datura metel, Hibiscus rosasinensis, Nerium oleander, Ocimum sanctum, Ocimum basilicum, Phyllanthus emblica, Polyalthia longifolia, Piper betle, Punica granatum, Solanum torvum and Solanum trilobatum* against *X. axonopodis*, and all the extracts have antibacterial action of citrus canker disease.

Sharma and Kumar (2009) demonstrated that *Lantana* extract was moderately active against different fungal strains. While *S. angustifolia* extract was inactive against *Mucor* species and did not inhibit its growth.

Yadav (2009) showed the extracts of *Allium sativm, A. cepa* and *Azadirachta indica* were found antifungal activity against white rust and blight of mustard.

Shafique and Shafique (2008) investigated the antimycotic potential of *D. metel* against *Ascochyta rabiei*. They reported that the pathogen was exposed to various- hexane concentrations (1, 2, 3 and 4% w/v) of shoot and root extracts of *D. metel*. All the tested concentrations of both root and shoot extracts inhibited the growth of the target fungal pathogen.

Hassanein *et al.* (2008) investigated neem (*Azadiracta indica*) and chinaberry (*Melia azedarach*) against *Alternaria solani* and *Fusarium oxysporum* at 5%, 10%, 15% and 20 %. They observed that the neem extract was maximum inhibition percentages 17.88%, 23.66, 52.77 % and 70.55% for *Alternaria solani*, while those for *F. oxysporum* were 14.77 %, 23.88%, 32.22 % and 100%, respectively. Whereas, the chinaberry leave extracts were 3.11 %, 5.22%, 5.53 % and 5.77 %, recorded for *Alternaria solani* and 5.44 %, 6.11 %, 6.35 % and 6.55% for *F. oxysporum*. While, both ethanol and ethyl acetate extracts of neem leaves assayed at a concentration of 20%, completely suppressed the growth of *F. oxysporum* and inhibited *Alternaria solani* by ratios between 52.44% and 62.77%, the same extracts but from chinaberry (20%) slightly inhibited the growth of both pathogenic fungi and values of inhibition not exceeded the 7 %. Further, recorded disease incidence under field condition, the highest value (94.29%) was +ve control while the lowest (19.04%) was recorded with seeds treated with pathogen and irrigated with neem aqueous extracts whereas the highest disease control (80.96%) was recorded for seeds treated with pathogen and irrigated with neem extract.

Siva *et al.* (2008) determined the 20 plants species using water, ethanol and acetone as a solvent against *Fusarium oxysporum* f. sp. *melongenae*. They reported that the all plant extracts was found in reducing the mycelial growth of *Fusarium oxysporum* f. sp. *melongenae* at 50% concentration. But among the 20 plant extracts, *Adhatoda vasica, Jatropha curcas, Sapindus emarginatus* and *Vitex negundo* in different solvents, higher inhibition was noticed. Further these plants were selected for different concentrations of 10%, 20% 30% and 40%. Among them *Adhatoda vasica* at 40% alone recorded 100% inhibition and remaining three plants produced almost similar inhibitory effect. At the low concentration of 10% *Vitex negundo* had more inhibitory effect (82%), while *Jatropha curcas* extracts showed very low inhibition (25%).

Ghosh *et al.* (2008) evaluated the antibacterial potentiality of hot aqueous and methanol solvent extracts of mature leaves of *Polyalthia longifolia* against six reference bacteria. They reported

that the highest antibacterial activity against *K. pneumonia* in both the extracts followed by *E.coli* in hot aqueous extract and *B. subtilis* in methanol extract as evident from MIC values.

Satish *et al.* (2007) investigated the fifty-two aqueous extract against *Aspergillus candidus, A. columnaris, A. flavipes, A. flavus, A. fumigatus, A. niger, A. ochraceus* and *A. tamari* which isolated from sorghum, maize and paddy seed samples. They recorded among fifty-two plants, aqueous extract of *Acacia nilotica, Achras zapota, Datura stramonium, Emblica officinalis, Eucalyptus globules, Lawsonia inermis, Mimusops elengi, Peltophorum pterocarpum, Polyalthia longifolia, Prosopis juliflora, Punica granatum* and *Sygigium cumini* have significant antifungal activity against *Aspergillus* species. Further they tested among the solvent extracts, methanol gave more effective than ethanol, chloroform, benzene and petroleum ether, except for *Polyalthia longifolia,* where petroleum ether extract recorded highly significant antifungal activity than other solvent extracts.

Shafique *et al.* (2007) studied the allelopathic tree *Accacia nilotica, Alstonia scholaris, Azadirachta indica, Eucalyptus citriodora, Ficus bengalensis, Mangifera indica, Melia azedarach* and *Syzygium cumini* on germination and seed-borne mycoflora of wheat (*Triticum aestivum*). They reported that the all aqueous extracts were significantly reduced the frequency of *A. alternata* and *F. solani*. Generally there was not any pronounced difference between effectiveness of 10 and 20 minutes treatments.

Mohana and Raveesha (2006) extracted the leaf and pod material of *Caesalpinia coriaria* with water and successively with petroleum ether, benzene, chloroform, methanol and ethanol solvent against *Xanthomonas* pathovars. They, observed that among five solvents extracts, methanol extract of both leaf and pod was most active against all the test bacteria, followed by ethanol extract. Comparison of the inhibitory activity of the extracts with the antibiotics bacterimycin 2000 and streptocycline revealed that methanol and ethanol extract of both leaf and pod and aqueous extract of pod were significantly higher than that of the antibiotics.

Patni and Kolte (2006) showed that eucalyptus leaf extract exhabit significant reduction in radial growth, sporulation and spore germination of *Alternaria brassicae*. They reported that the leaf extracts of eucalyptus, ocimum and anagallis showed maximum reduction (92.5%, 92.6%, 92.4% decrease over check respectively) in radial growth whereas ocimum, eucalyptus and urtica

were minimum sporulation intensity (0.26, 0.28, 0.81x105 respectively). Further, observed that the significantly lowest reduction of spore germination in urtica followed with ocimum and eucalyptus (86.6%, 79.4% and 78.9%, respectively). Whereas, revealed that eucalyptus spray gave significantly lesser number of sports/leaf (2.05), minimum size of sport (2.28 mm), minimum sporulation intensity (2.22x105) and minimum disease index (13.96) followed by calotropis, ocimum and polyanthai extracts spray under glasshouse conditions.

Guleria and Kumar (2006) observed the aqueous leaf extract of neem *(Azadirachta indica)* against *Alternaria sesami*. Treatment with this extract led to the changes in plant metabolism as leaves of the treated plants exhibited significantly high level of enzyme phenylalanine ammonialyase (PAL), peroxidase (PO) and content of phenolic compounds. Further, they reported germination of *A. sesami* spores was not significantly inhibited by neem extract.

Akhter *et al.* (2006) reported that *Vinca rosea*, *Piper betle* and *A. indica* extracts have inhibitory (100%) effect against spore germination of *Bipolaris sorokiniana*.

Basu *et al.* (2005) reported the presence of Emodin and Physcion in *L. camara* extract that showed mild activities against different bacterial strains.

Das and Das (2005) showed the extracts of *Allamanda cathartica, Cassia tora, Bixa orellana, Clerodendron inerme* and *Terminalia chebula* had antifungal activity against *Alternaria brassicola*.

Kagale *et al.* (2004) recorded that leaf extract of *Datura metel* was found significantly reduced the growth of *Rhizoctonia solani* and *Xanthomonas oryzae* pv. *oryzae*. Among the solvent, methanolic extract showed the best control of the pathogens recording 10-35% more toxicity than aqueous extract. Further they observed the foliar application of leaf extracts effectively reduced the incidence of sheath blight and bacterial blight disease of rice under green house.

Prajapati *et al.* (2003) studied the neem leaves contain nimbin, nimbinene, 6-desacetylnimbinene, nimbandiol, nimbolide and quercetin. Whereas, fruits containing nimbiol and azadirachtin. Azadirachtin and limonoids were considered as most bioactive in gradients having insecticidal and microbial property. The *Allium sativum* has germicidal properties and has

inhibitory effects on gram negative bacteria. A number of antibiotic principles like allistatin I, allistatin II and garrticin are isolate from garlic.

Chaudhary *et al.* (2003) tested the plant extracts including *A. indica* against *Alternaria alternata* causing early blight of potato. They reported that the bulb extracts of *Allium sativum* caused 59% inhibition followed by extracts of *A. indica* (54%).

Upasana *et al.* (2002) found that both ether and methanol neem seed extracts gave the best results at 3000 µlitre/100 ml concentrations but methanol was most effective against *A. niger*, *F. oxysporum* and *Trichoderma resii.* Whereas, maximum inhibition zone was seen in ether extract only against *F. oxysporum*.

Paul and Sharma (2002) showed the aqueous leaf extract of *Azadirachta indica* induced resistance in barley against *Drechslera graminea* through biochemical changes in the host plant.

Sharma and Mehta (2001) reported that leaf extracts from *Prosopis juliflora, Allium sativum, Vitis quadranquralis, Carucuma longa, Occimum sanctum* and *Eucalyptis citridosa* contained antimicrobial activity against *X. c.* pv. *campestris.*

Kumudini *et al.* (2001) reported that the leaf extract of *D. metel* exhibited 80% pro-tection against the downy mildew pathogen, *Sclerospora graminicola*, and induced resistance in the highly susceptible HB3 cultivar of pearl millet.

Dwivedi and Shukla (2000) found that the mycelia growth inhibition rate increased with plant extract concentration. Whereas, 100% aqueous neem (*A. indica*) leaf extract caused complete inhibition of spore germination of *Fusarium* spp.

Satish *et al.* (1999) investigated that the 30 plants extracts against *Xanthomonas campestris*. They observed that the aqueous extract of *Prosopis juliflora, Oxalis corniculata* and *Lawsonia inermis* showed significant antibacterial activity. Whereas, the extracts of a few plants were comparable with that of the synthetic antibiotics, bacterimycin and streptocycline.

Satish *et al.* (1999) studied the leaf extracts of *Catharanthus roseus* and was found to have significant antibacterial activity against *Xanthomonas campestris.*

Vijayalakshmi *et al.* (1999) derived the effectiveness of garlic clove extract as a pesticide was due to volatile oil which contains dialyl disulphide, diallyl trisulphide and sulphodoxides by fromallicin.

Lirio *et al.* (1998) reported the antibacterial activity of *Allium cepa, A. porrum, A. sativum, Euphorbia tisucalla* and piper bettle against *Erwinia carotovora* pv. *carotovora, X.c.* pv. *campestris* and *Pseudomonas solanacearum* when used in aqueous extracts.

Lovang and Wildt-Persson (1998) reported that *M. azedarach* aqueous leaf extract was a good inhibitor of *Bipolaris micropus* but it was partially inhibitor to *Alternaria solani* with little or no effect on *F. oxysporum* as test pathogens of tomato.

Srivastava and Bihari (1997) showed the fungicidal properties of aqueous leaf extracts of *A. indica* against *Alternaria alternata* from pear fruits with 85 % control of fruit rot *in vivo*.

Shivpuri *et al.* (1997) noticed that ethanol extracts of *A. indica* showed fungitoxic properties against *Alternaria brassicola, Colletotrichum capsici, F. oxysporum, R. solani* and *Sclerotinia sclerotiorum*. They reported that all extracts were found growth inhibitory of test pathogens under laboratory conditions at 500 and 1000 µg/ml.

Mariappan (1995) obtained the leaf extracts (10%) of five plant species by using water, ethanol, methanol, benzene and acetone against the *Magnaporthe oryzae*. They recorded that leaf extracts prepared with the solvents were more inhibitory effective than water extracts.

Ganguly (1994) reported that aqueous neem leaf extract inhibited mycelial growth and spore germination of *Helminthosporium oryzae* and *Pyricularia oryzae* responsible for blast and brown spot of rice plant, respectively.

Pandey (1993) showed the stem extracts of *Acacia catechu* against the growth of *Fusarium solani*. He found that stem extract has antifungal activity which might be due to the presence of some antimicrobial phytochemicals in the plant such as catechin andcatechutannic acid, taxifolin, tannins, gambrine and fisetinand.

Hossain *et al.* (1993) reported the leaf extracts of *M. scendens* and *P. orientale* were found to possess antifungal activity on a number of pathogens including *Alternaria* sp. and *Rhizopus* sp.

Singh *et al*. (1993) reported that aqueous leaf extract of *A. indica* gave good control of *F. oxysporum.* They showed the disease development with minimum percentage loss in fruit weight.

Meena and Mariappan (1993) reported the inhibitory effect of neem leaf extract on growth and spore germination of seed mycoflora including *Alternaria tenuis*, *A. flavus*, *Curvularia lunata*, *F. moniliforme* and *Rhizopus stolonifer*.

2.4 Eessential oils against the pathogens :

Guerra *et al.* (2014) evaluated the essential oils of bergamot, lemongrass, copaiba, *Eucalyptus citriodora*, blue gum, fennel, ginger, spearmint, sweet orange, lemon and clary sage (0.5%) and also the antibiotic mycoshield® (3 g l^{-1}) on reducing soft rot in chinese cabbage in the greenhouse and plants were inoculated with *Pectobacterium carotovorum* subsp. *carotovorum* (pcc-c) at 72 hours later. The oils of bergamot, copaiba, *E. citriodora*, spearmint and Sweet orange were then tested for the stability of their effectiveness in the control of three isolates of *P. carotovora* subsp. *carotovorum*. These oils reduced the sev (30.5 to 38.6%) and the audpc (23.1 to 26.6%) with no differences between them or the mycoshield® (sev 45.2 and audpc 32.8%), except for the copaiba (20.3%) which was less effective than the antibiotic in the reduction of the audpc. Therefore, spraying with the oils of bergamot, copaiba, *e. Citriodora*, spearmint and sweet orange has potential in the control of this disease.

Tabassum and Vidyasagar (2013) reported that the essential oils and extracts have antibacterial, antifungal, antiviral, antiparasitic and antidermatophytic properties. It is now considered as a valuable source of natural products for development of medicines against various diseases and also for the development of industrial products.

Mikicinski *et al.* (2012) evaluated the fungicides and essential oils: lavender, sage, lemon balm, clove and thyme oil (biozell) against *Erwinia amylovora*, *Xanthomonas arboricola* pv. *corylina*, *X. arboricola* pv. *juglandis*, *Pseudomonas syringae* pv. *syringae*, *Agrobacterium tumefaciens* (presently *Rhizobium radiobacter*). They observed that the fungicides, metalaxyl-m with mancozeb, mancozeb alone and copper oxychloride inhibited the growth of bacteria. Whereas, essential oils of sage, cloves and biozell were the strongest inhibitors of bacteria.

Lucas *et al.* (2012) assessed the potential of Indian clove essential oil in the reduction of *Xanthomonas vesicatoria* causing tomato bacterial spot. Acibenzolar s-methyl (0.2 mg ml^{-1}) was used as control. They reported that the Indian clove essential oil and acibenzolar-s-methyl gave a control of 53.0% and 89.0%, respectively.

Quereshi *et al.* (2011) carried out the essential oils from *O. gratissimum, O. sanctum* and *O. americanum* with oil yield of 2.0%, 2.5% and 2.25%, respectively. Gc analysis of essential oil indicates the presence of important constituents. Tlc of leaf pigments represents the most important pigments followed by tlc of essential oils for determining the presence of alcohols, esters and carbonyl compounds.

Bajpai *et al.* (2011) discussed of natural products such as essential oils for application in agriculture as biocontrol measures against *Xanthomonas* species. The current knowledge on the use of essential oils to control *Xanthomonas* bacteria in vitro and in vivo models. A brief description on the legal aspects on the use of essential oils against bacterial pathogens has also been presented.

Altundag and Aslim (2011) tested *Origanum minutiflorum, Sideritis erytrantha, Satureja wiedemanniana, Salvia tchihatcheffii* and *Thymus sipyleus* subsp. *sipyleus* against *Xanthomonas vesicatoria*. They reported that the essential oil of *Origanum minutiflorum* was maximum inhibition zones in the range of 4-43 mm. While the essential oil of *O. minutiflorum* reduced significantly bacterial spot severity (82.25%).

Gorski *et al.* (2010) investigated the selected natural essential oils of coriander (*Coriandrum sativum*), manuka (*Leptospermum scoparium*), lavender (*Lavandula officinalis*) and peppermint (*Mentha piperita*) on the mycelium growth and sporulation of *Trichoderma harzianum* found in common mushroom (*Agaricus bisporus*) at concentrations of 250, 500 and 1000 $\mu g/cm^3$. They recorded that the manuka oil was statistically significant maximum inhibition of mycelial growth of *Trichoderma harzianum* amounted to 67.4 and 64.7% at a concentration of 500 and 1000 $\mu g/cm^3$.

Nehal (2009) evaluated essential oils of carnation, caraway, thyme oils and the chemical fungicide ridomil MZ-72 against *Alternaria solani*. He reported that the Carnation oil was

strongest and had most extensive inhibitory effect on fungal growth. Whereas, caraway and thyme oils were slightly less effective followed by the chemical fungicide. Further applied of essential oils in the field and proved that essential oils twice as foliar spray had a superior effect to the fungicide treatment for reducing the early blight incidence comparing with untreated control. The highest reduction in disease incidence and yield increase was recorded in treatments with 1% of carnation, caraway and thyme oils in descending order.

Udomsilp *et al.* (2009) applied the essential oils of frankincense (*Boswellia carteri*) and cassia (*Cacia farnesiana*) against *Alternaria brassicicola, Aspergillus flavus, Bipolaris oryzae, Fusarium moniliforme, Fusarium proliferatum, Pyricularia oryzae* and *Rhizoctonia solani*. They recorded that the maximum mycelium growth inhibition was recorded of *F. monoliform* (62.11%), *F. proliferratum* (16.66%), *P. grisea* (33.33%), *P. oryzae* (33.33%), *R. solani* (44.44%), *A. brassicicola* (72.29%) and *A. flavus* (12.11%) at concentration 2.0%v/v of 7 days after inoculation. While Cassia oil could inhibit mycelium growth of all pathogenic fungi when applied at a concentration over 2.0 %v/v.

Hadizadeh *et al.* (2009) studied the essential oils of nettle (*Urtica dioica*), thyme (*Thymus vulgaris*), eucalyptus (*Eucalyptus* spp.), rue (*Ruta graveolens*) and common yarrow (*Achillea millefolium*) against *Alternaria alternata.* They observed that the thyme oil was exhibited a lower degree of inhibition 68.5 and 74.8% at 1500 and 2000 ppm, respectively. Whereas, strongly reduced the spore germination and germ tube elongation of the pathogens in the presence of 1500 ppm of the nettle oil.

Mironescu *et al.* (2009) investigated the essential oils of juniper, common thyme, pine, peppermint, silver fir, eucalyptus, fennel, tarragon, caraway and wild thyme against *Aureobasidium* sp. and *Alternaria* sp. The antifungal activity of thyme, common thyme and fennel essential oil appeared the most interesting, with strong fungicidal effect on both moulds tested followed by tarragon, caraway and eucalyptus. While, peppermint, juniper and silver fir seem to have very small or no inhibitory action on moulds.

Siripornvisal *et al.* (2009) evaluated that the essential oils of four medicinal plants against *Botrytis cinerea*. They observed that the vapors of clove oil, cinnamon oil and lemongrass oil exhibited strong inhibitory effects on *B. cinerea*, with a MIQ (minimal inhibitory quantity) equal

to 15 µl. However the headspace vapors of galingale oil exhibited weaker inhibitory effect (miq = 25 µl). While, galingale oil was exhibited some fungistatic properties.

Piyo *et al*. (2009) evaluated the essential oils of Basil (*Ocimum basilicum*) and Sweet fennel (*Ocimum gratissimum*) against *Alternaria brassicicola, Aspergillus flavus, Bipolaris oryzae, Fusarium moniliforme, Fusarium proliferratum, Pyricularia aryzae* and *Rhizoctonia solani*. Basil oil at a concentration of 0.6%v/v was the strongest mycelium growth inhibition of *F. moniliforme* (100%), *F. proliferratum* (49.6%) and *P. grisea* (100%). Whereas, *B. oryzae, A. brassicicola* and *A. flavus* was inhibited by 97.40, 94.62 and 59.25%, respectively at 2.0%v/v. Further, recorded the data of spore germination inhibition in the treatment of basil essential oil against *F. monoliforme* (92.31%) and *A. brassicicola* (99.74%) at 0.8%v/v. While, sweet fennel oil has completely inhibit (100%) spore germination of all pathogenic fungi at 0.8 %v/v.

Sitara *et al.* (2008) evaluated the essential oils from the seeds of neem (*Azadirachta indica*), mustard (*Brassica campestris*), black cumin (*Nigella sativa*) and asafoetida (*Ferula assafoetida*) against *Aspergillus niger, A. flavus, Fusarium oxysporum, F. moniliforme, F. nivale, F. semitectum, Drechslera hawiinesis* and *Alternaria alternata* @ 0.5, 0.1 and 0.15%. Ridomyl gold (MZ 68% wp) was used for comparison. The extracted oils except mustard were found fungicidal activity of varying degree against test species. Whereas, asafoetida oil @ 0.1% and 0.15% significantly inhibited the growth of all test fungi except *A. flavus* and *Nigella sativa* oil @ 0.15 was also effective but little fungicidal activity against *A. niger* followed by neem, ridomyl gold and mustard oils.

Luqman *et al.* (2008) evaluated the essential oil of *Eucalyptus citriodora* against C*andida albicans, Escherichia coli* and *Mycobacterium smegmatis.* They found the essential oil of *E. citriodora* was to be active against *Trichophyton rubrum* followed by *Histoplasma capsulatum, Candida albicans* (mtcc) and *Cryptococcus neoformans*. Similarly, it was found active toward gram-positive bacteria compared to gram-negative and showed activity towards drug-resistant mutants of *C. albicans* and *E. coli*.

Tobias *et al.* (2007) reported the essential oils of cinnamon found to be most effective against *Xanthomonas campestris* pv. *vesicatoria*, but all oils decreased the germination ability. Thyme and savory teas were effective against *Pseudomonas syringae* pv. *tomato.* Other examined

materials had insufficient bactericide impact (sucrose, NaCl, ethanol, valerian extract, pepper mint).

Vuida (2007) reported the antifungal potential of essential oils of oregano, thyme and clove presented inhibitory effects on food spoilage, *Aspergillus niger* and *Aspergillus flavus*. They showed among essential oils, Oregano was found highest inhibition of mold growth. Whereas, clove essential oil was a stronger inhibitor against *A. niger* than against *A. flavus*.

Parajuli *et al.* (2005) assessed the essential oils of *Thymus linearis, Artemisia gmelinii, A. dubia, Juniperus recurva, Nardostachys grandiflora* and *Zanthoxylum armatum* for their fungitoxicity against *Alternaria brassicicola. s*Further data analysis showed species level efficiency in order of *Nardostachys grandiflora = Thymus linearis Aartemisia dubia > A. gmelinii > Zanthoxylum armatum*.

David and Halland (2004) evaluated the essential oils of mint, citrus, cinnamon, clove, rosemary, geranium, wintergreen, cumin against *Penicillium digitatum*. They reported that the oils of cinnamon leaf, clove leaf, cumin and spearmint displayed larger than average zones of inhibition while the oils of *Citrus* sp. and wintergreen displayed minimal inhibition.

Iscan *et al.* (2002) investigated essential oils of peppermint (*Mentha piperita*) against 21 human and plant pathogenic microorganisms. The bioactivity of the oils menthol and menthone was compared using the combination of *in vitro* techniques such as microdilution, agar diffusion, and bioautography. It was shown that all of the peppermint oils screened strongly inhibited plant pathogenic microorganisms, whereas human pathogens were only moderately inhibited. Chemical compositions of the oils were analyzed by GC and GC/ms. Using the bioautography assay, menthol was found to be responsible for the antimicrobial activity of these oils.

Babu *et al.* (2000) showed that spraying with 3 % of neem oil in tomato pot cultures resulted in 53 % reduction in disease incidence over the control**.**

Denys and and James (1990) extracted essential oils from leaves, flowers and stems of *Ocimum basilicurn, O. kilimandscharicum* and *O. micranthum* by solvent extraction, hydrodistillation, and steam distillation for essential oil content and the oil analyzed by GC and GC/MS for composition. They observed the essential oil content was highest in flowers for *O. basilicum* and

in leaves for *O. micranthum.* While, no significant differences were found in essential oil yield and relative concentration of major constituents using fresh or dry samples and using samples from 75 g to 10 g of dry plant tissue.

2.5 Botanical extracts and essential oils against the pathogens :

Bhardwaj and Sahu (2014) evaluated the different botanicals, fungicides and essential oils against *Colletotrichum falcatum* in *in-vitro*. Amongst botanicals, they recorded maximum inhibition in mycelial growth (92.59%) in *Ocimum* followed by turmeric (79.25%), ginger (75.92%) and onion (72.41). While, minimum inhibition of mycelia growth (64.44%) was recorded in garlic at 15% concentration. Further, they recorded complete inhibition of mycelial growth in peppermint oil and mentha oil, followed by geranium oil (75.83%), patchouli oil (70.00%) while minimum inhibition in mycelial growth was recorded in palmaroza oil (63.89%). Out of four fungicides, bavistin showed complete inhibition of mycelial growth followed by folicur (88.89 %), contaf (82.89%) while least inhibition (80.78%) at 20 ppm concentration.

Chethana *et al.* (2012) evaluated the clerodendron, cinnamon, garlic, neem oil, pongamia oil and turmeric against *Alternaria porri* (Ellis). They reported that the fresh aqueous extract of garlic (20%) was effective in causing 100 per cent inhibition of mycelial growth. Whereas, neem oil and pongamia oil (20%) caused 76.94% and 69.94 % inhibition.

Sasode *et al.* (2012) evaluated the botanicals of neem, eucalyptus, datura, pudina, tulsi, lantana against *Alternaria brassicae* at 10%. Whereas, neem and eucalyptus were also used in the oil forms. They reported that the minimum growth was recorded in neem followed by eucalyptus, tulsi, lantana, datura and pudina. Whereas, neem was significantly superior over tulsi, lantana, datura and pudina but at par with eucalyptus.

Bhadouria and Tomar (2012) evaluated the leaf extracts @ 5%; oil, bulb and rhizome extract @ 1% against *Alternaria tagetica.* They recorded that the neem oil absolutely inhibited fungal growth of *Alternaria tagetica* and it was significantly superior over other plant extracts except eucalyptus oil in which only 3.33 mm including with control (82.67 mm). Under higher concentration (leaf extracts @ 20%; oil, bulb and rhizome extracts @ 10%). Further, they observed that the higher concentration of neem oil and eucalyptus oil absolutely inhibited the

fungal growth and these two were significantly superior over iopmia leaf extract (50.33 mm), lantana leaf extract (42.67mm), parthenium leaf extract (36.00 mm), calotropis leaf extract (27.67 mm), onion bulb extract (17.67 mm) and datura leaf extract (16.33 mm). Neem oil and eucalyptus oil were statistically at par with *ginger* bulb extract (4.00 mm), neem leaf extract (5.00 mm), eucalyptus leaf extract (6.33), tulsi leaf extract (7.00 mm), garlic bulb extract (7.00 mm) and pudina leaf extract (9.00 mm). The maximum radial growth (76.33 mm) was recorded in control.

Sehajpal *et al.* (2009) investigated 44 plant extracts and 8 plant oils against the pathogen of *Rhizoctonia solani.* They showed that the 36 plant extracts were varied degree of antimicrobial effect at different concentrations against the pathogen whereas *Abrus precatorious*, *Acacia auriculiformis*, *Bougainvillea glabra*, *Convolvulus arvensis*, *Hibiscus rosa-sinensis*, *Morus alba*, *Thevatia peruviana* and *Withania somnifera* did not exert any effect. Among all the plant extracts, *A. sativum* exhibited strong fungitoxicity even at the lowest concentration, i.e. 100 ppm, with relative magnitude of inhibition 2.0 mm against the pathogen *R. Solani*.

Wolf *et al.* (2008) tested the various essential oils, organic acids, biosept, (grapefruit extract), tillecur and extracts of stinging nettle and golden rod against *Xanthomonas campestris* pv. *campestris*, *Clavibacter michiganensis* subsp. *michiganensis*, *Alternaria dauci* and *Botrytis aclada*. They reported that the thyme oil, oregano oil, cinnamon oil, clove oil and biosept had the highest activity against the seed borne pathogens *Xanthomonas campestris* pv. *campestris*, *Clavibacter michiganensis* subsp. *michiganensis*, *Alternaria dauci* and *Botrytis aclada*. While, least antimicrobial activity was found in the organic acids.

2.6 Botanical extracts and oils on the yield of crops :

Tuan *et al.* (2014) revealed that garlic and chili combination solution effectively reduced cabbage insect pests. On other hand, the spray solution not only reduced the number of days required for the cabbage growth but also greatly enhanced the leaf number, head diameter, head weight and quality of cabbage. Garlic and chili combination solution have positive effects on pests reduction and improve growth, yield and quality of cabbage vegetable.

Baidoo and Adam (2012) studied the effects of ethanolic extract of neem, *Azadirachta indica* (meliaceae), seeds and petroleum ether extract of *Lantana camara* leaves (verbenaceae) on *Plutella xylostella*, *Brevicoryne brassicae* and *Hellula undali.* They reported that the mean weight of cabbage heads on the sprayed plots was significantly heavier than that of the control unsprayed plots. Further, also reported the leaf extracts increased yield by 37.05% and 25.80%, respectively. Spraying the cabbage plants with the plant extracts significantly reduced the numbers of pests compared with the control plants.

Patil *et al.* (2003) recorded the neem treated cabbage plant and found significantly higher yield of marketable heads of cabbage and significantly better control of pest.

Patil *et al*. (2001) showed that neem leaf extract was effective in reducing early blight incidence with increased yield of tomato infected by *Alternaria solani*.

Patil *et al.* (2001) found that incidence of tomato early blight caused by *Alternaria solani* was affected by a botanical like neem seed extract with increased fruit yield between 156.43 and 168.56q / ha.

Khan *et al.* (2000) evaluated that the leaf extract of *Datura alba,* seed oil of neem *(Azadirachta indica),* neem seed bitter and nimbokil 60 EC at 2. 2 and 3% concentrations on the growth of *Xanthomonas campestris* pv. *malvacearum in vitro* and on the greenhouse grown cotton varieties/lines. They showed the results, *Datura alba* significantly retarded the growth of bacterium followed by nimbokil, neemseed bitter and neemseed oil at 3% concentration, respectively. Whereas, significantly less number of leaf shedding, less number of bare nodes and more number of bolls, increased boll weight and yield of seed cotton of varieties sprayed with standard concentrations of *Datura alba* and nimbokil 60 EC as compared to untreated control under green house.

Chattopadhyay (1999) found that foliar spray of *A. indica* leaf extract and azadirachtin reduced mycelia growth of *Alternaria alternata* (causing loss of sunflower and tomato), decreased disease severity and increased yield over control.

CHAPTER - III

MATERIALS AND METHODS

The material used and methods adopted for studies on **"Botanical pesticides in the management of *Xanthomonas campestris* pv. *campestris* and *Alternaria brassicae* of cabbage"** are described here under appropriate sub-headings.

3.1 Experimental site :

The present experiments were conducted in the field and research laboratory of Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, during two *rabi* seasons of 2012-2013 to 2013-2014. The field experiment was planned in Randomized Block Design (RBD) with three replications for each treatment and Complete Randomized Design (CRD) in laboratory with four replications for each treatment. The site selected for experimentation was uniform, cultivable with typical sandy loam soil having good drainage.

3.2 Topography :

Allahabad is situated at 25.27 North latitude 80.50 East longitude and at an altitude of 98 m above sea level. The climate is typically semi arid and subtropical. The maximum temperature reaches up to 47 ^{o}C in summer and drops down to 1.5 ^{o}C in winter.

3.3 Cleaning and sterilization of glass wares :

The Petri plates, pipettes conical flasks, test tubes, beaker *etc*. used in the experiment were thoroughly washed with tap water and dried. The Petri plates and pipettes were wrapped in an aluminum foil sterilized in a hot air

oven at 160 ^{o}C for 2 hrs. All the media and glass wares required for isolation were sterilized in autoclave at 121 ^{o}C for 15 lbs pressure/ inch^{2} kept constantly for 20 min **(Aneja, 2004).**

3.4 Preparation of culture media :

Potato dextrose agar and nutrient agar media were used for isolation of *Alternaria brassicae* and *Xanthomonas campstris* pv. *campestris,* and there mass multiplication in the maize-sand and nutrient broth, respectively.

3.4.1 Preparation of Potato Dextrose Agar medium -

Composition :

Peeled Potato	:	200 gm
Dextrose	:	20 gm
Agar	:	20 gm
Distilled water	:	1000 ml
pH	:	6.0

Procedure :

Two hundred gram washed, peeled and sliced potatoes were boiled in 500 ml of distilled water in a sauce pan till they were easily penetrated by glass rod. The extract obtained was filtered through muslin cloth and all the liquid was squeezed in a beaker. 20 gm agar was added bit by bit to the rest of 500 ml hot water to dissolve. Then 20gm of dextrose was added. Volume of content was made up to 1000 ml by adding more distilled water. Then 200ml

of this solution was dispensed in conical flasks. These conical flasks were plugged with non absorbent cotton and sterilized at 121 ºC at 15 lbs for 15 minutes in an autoclave**.**

Preparation of Nutrient Agar		**Nutrient broth media**	
Agar	: 15 gm	Beef extract	: 3 gm
Beef extract	: 3 gm	Peptone	: 5 gm
Peptone	: 5 gm	Nacl	: 8gm
Nacl	: 8gm	Distilled Water	: 1000 ml
Distilled Water	: 1000 ml	pH	: 7.3
pH	: 7.3		

3.4.2 Procedure for nutrient agar/nutrient broth media :

Dissolved all the ingredients, except agar powder. Added 15 gm agar bit by bit in hot solution. Dispensed 200 ml each to five conical flasks and plug the flask with nonabsorbent cotton. Sterilized at 121 ^{o}C, 15 lbs pressure for 20 minutes in an autoclave. Nutrient Broth was prepared in same way, except agar was not added to it.

3.5 Leaf spot symptom of cabbage (*Alternaria brassicae*) :

The diseased leaves were collected in a clean polythene bag from the Central Research Field, SHUATS, Allahabad. These leaves were brought to the laboratory of Department of Plant Pathology for isolation of pathogen. Leaf spot symptoms (Plate 1) may first develop on young plants in seedbeds of cabbage. Dark brown to black spots may appear on tissues of any age and vary in size from pinpoint to 2 inches in diameter. The leaf spots enlarge in

concentric circles and mature lesions have a bull's eye type appearance. They produce black sooty colored spores within the leaf spots. The black spores easily detach from the leaf if touched and are visible on the leaf surface.

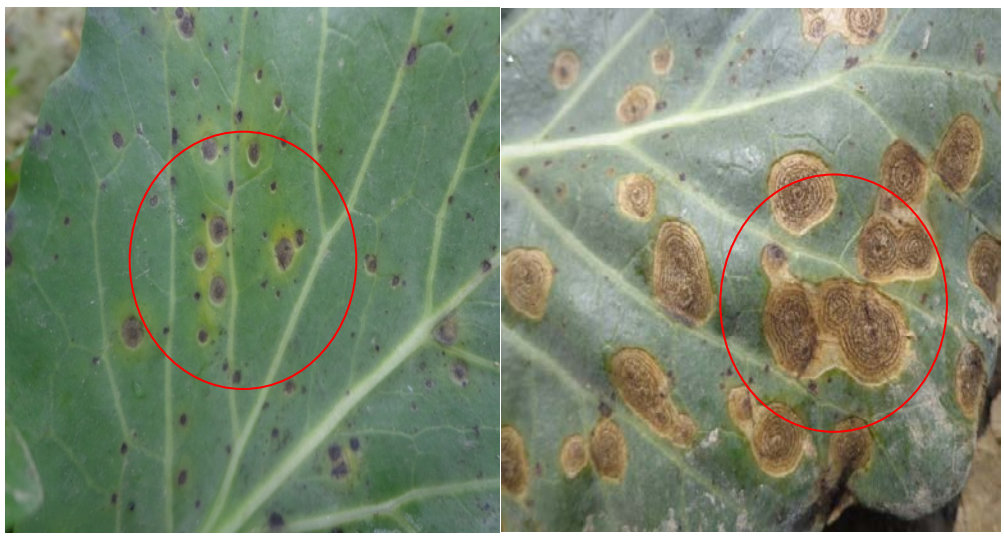


Plate: 1. (a) Symptoms of Alternaria leaf spot (b) Characteristic concentric lesions on the leaf side of cabbage.

3.5.1 Isolation of *Alternaria brassicae* :

This method was followed as described by **Aneja (2004)**. The infected leaf was washed in tap water and cut into small pieces (2 mm), surface sterilized with 0.1% mercuric chloride solution, thrice rinsed with sterilized distilled water, transferred aseptically into petri plates containing melted lukewarm (45 °C) PDA medium and then small pieces of infected leaf were kept aseptically on media inside Petri plates. These Petri plates were incubated in BOD incubator at 28 ± 1°C. After 48 hrs of inoculation, whitish colony growth of mycelium was observed in petri plates (Plate 2). From the periphery of this whitish colony growth a small bit was taken and slide was prepared using lacto phenol. The slide was observed under microscope for the further identification of pathogen.

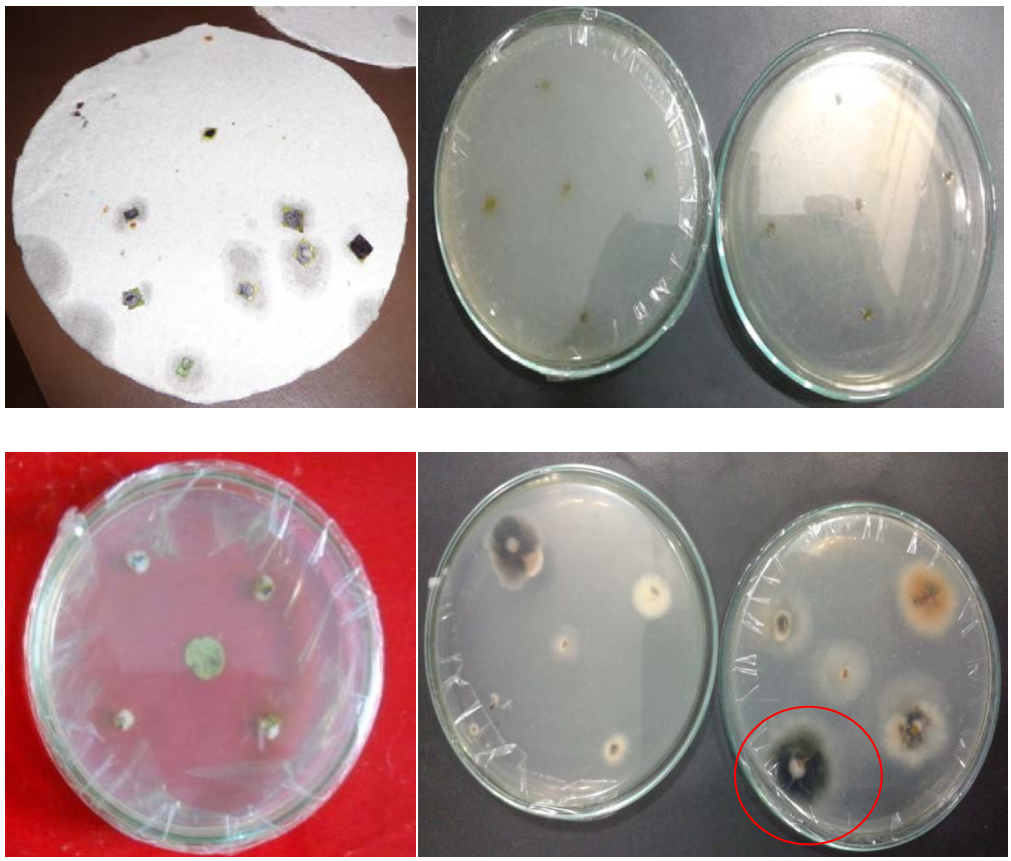


Plate: 2. Isolation procedure of *Alternaria brassicae.*

3.5.2 Identification of *Alternaria brassicae* :

The pathogen was identified on the basis of following characteristics **(Meena *et al.*, 2010) –**

Mycelium: Septate, brownish grey

Conidiophore: Dark, septate, arise in fascicles, 14-74µ × 4-8µ

Conidia: Brownish black, obclavate, muriform, produced singly or in chains

Spore body: 96-114 × 17-24 µ

Spore beak length: 45-65 µ

Spore: Transverse septation 10-11, longitudinal septation 0-6.

Plate: 3. Microscopic view and measurement of conidia and conidiophores of *Alternaria brassicae* (40x).

The fungus isolated from infected cabbage leaf was identified as *Alternaria brassicae* (Plate 3) and it is systemic position according to **Ainsworth *et al.* (1973)** is as follows -

3.5.3 Systematic position :

Kingdom	:	Fungi
Division	:	Eumycota
Sub-division	:	Deuteromycotina
Class	:	Hyphomycetes
Order	:	Moniliales
Family	:	Dematiaceae
Genus	:	*Alternaria*
Species	:	*brassicae*

3.5.4 Purification and maintenance of *Alternaria brassicae* :

The culture of *Alternaria brassicae* was purified and maintained on PDA petri plates and in slants (Plate: 4). These were incubated at 25 ± 2 °C temperature for further studies.

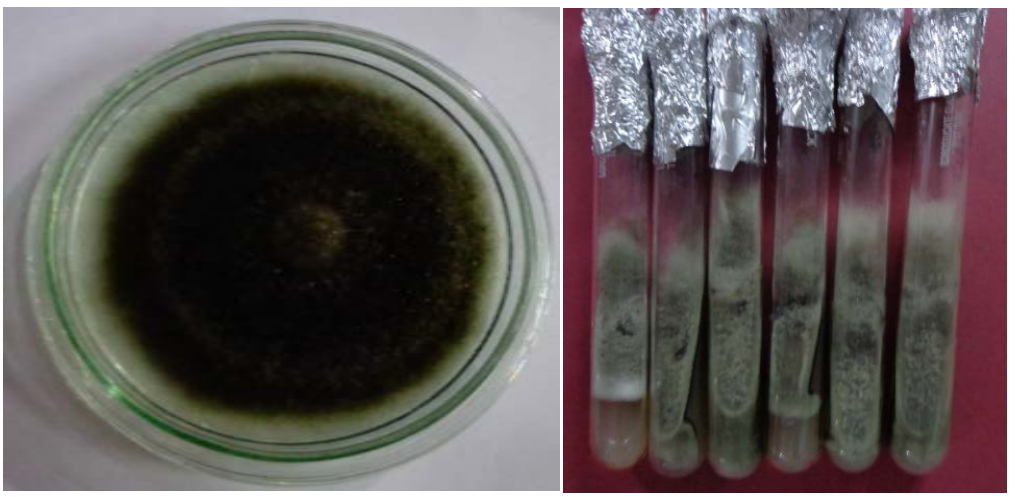


Plate: 4. Pure culture of *Alternaria brassicae* in petri plates and slants.

3.5.5 Mass multiplication of *Alternaria brassicae :*

The mass culture of *A. brassicae* was prepared on maize-sand medium using the method of **Whitehead (1957).** 60 g of washed sand, 140 g maize and 60 ml distilled water were mixed and boiled for about 1 hr, and then 200 gm of such mixture was filled in 500 ml Erlenmeyer flasks. The flasks were plugged with non-absorbent cotton and sterilized in an autoclaved at 15 lbs for 20 minutes for two successive days. After cooling at room temperature, the flasks were inoculated with 5 mm mycelial disc of *Alternaria brassicae* and incubated in B.O.D. incubator at 28$\pm$1 ^{o}C for 10 days (Plate: 5). For soil application in pots and field experiments, the grains colonized by pathogen were mixed in soil as such.

Plate: 5. Maize-sand medium inoculated with *Alternaria brassicae* culture.

3.6 Pathogenicity test of *A. brassicae* on cabbage crop :

Associated pathogen with leaf spot of cabbage was isolated and studied for its pathogenic ability to induce leaf blight, following the method used by **Sharma *et al.* (2013)**. Earthen pots (30 cm in diameter) containing sterilized sand loam soil, infested with inoculum of *A. brassicae* @ 10, 15 and 20 g were used for pathogenic test. Check treatments (control) was prepared without the addition of tested fungi. The seeds of highly susceptible variety, **"Golden Acre"** were sown @ 10 seeds/pot in the earthen pots. The pots were then watered lightly and kept under careful observation in the open field of Biological Science Dept., SHUATS, Allahabad. Each treatment was replicated four times. After thirty days of sowing seeds, leaf blight symptoms appeared and the pathogen was re-isolated from the artificially diseased plants to fulfill Koch's postulations and the characters of the fungi were confirmed with the original isolates (Plate 6).

Plate: 6. Proved the pathogenicity of *A. brassicae.*

3.7 Disease symptom of black rot of cabbage :

Symptoms of infected leaves occur through water pores at the margins or occasionally through wounds caused by chewing insects. The tissue turns yellow and the chlorosis progresses towards the centre of leaf, forming a V-shaped area with base of V towards the midrib. The veins show brown to black discoloration (Plate 7). Leaves so invaded may be stunted unilaterally.

3.7.1 Isolation of *Xanthomonas campestris* pv. *campestris* :

Black rot causing bacterium was isolated from naturally infected cabbage plant leaves showing typical 'V' shaped symptoms. The diseased leaves were collected in a clean polythene bag from the field and brought to the laboratory of Department of Plant Pathology for isolation of pathogen. The infected samples were washed thoroughly and the infected areas were cut into small bits, surface sterilized with 70% ethyl alcohol for a minute. These bits were then washed with three changes of sterile water and crushed on sterilized glass slide to get bacterial suspension. This suspension was streaked on poured petri plates that contained nutrient agar medium to get single

isolated colonies of the bacterium. The plates were incubated for 48hrs at room temperature. On 3rd day observed pure glistering yellow droplets like colonies in the incubated petri plates. It was are observed yellow colour colonies and made the slide through gram staining. The slide was observed under oil emersion microscope for identification of black rot pathogen **(Didwania *et al.*, 2013).**

Plate: 7. a) Black rot symptom b) Isolation process of *X. c.* pv. *campestris*

3.7.2 Identification of *Xanthomonas campestris* pv. *campestris* :

Isolated bacterium was identified on the basis of morphological and biochemical tests - The cultural, morphological, physiological and biochemical characters of the pathogen using 24-48 hrs old cultures were studied.

3.7.2.1 Culture characters :

The colony morphology was studied from 48 hrs old culture of the bacterium grown on NA medium. Colonies were observed for their colour, shape, size, elevation, margin and fluidity.

3.7.2.2 Pigment production :

Production of water soluble and insoluble pigments by the isolates was studied by streaking on nutrient agar medium.

3.7.2.3 Cell shape and arrangement :

The young cultures were Gram stained by smearing loopful culture on grease free clean slides stained accordingly and examined microscopically for shape, arrangement and Gram reaction.

Gram staining :

Actively growing *Xanthomonas* sp. culture was taken for preparing a thin smear on clear slide. The smear was then air dried on flame gently. Now the smear was passed through crystal violet stain solution for 30 sec. Slide was rinsed with running tap water to remove excess of stain adhered on slide. Then slide was passed through iodine solution for 60 sec. Slide was again rinsed with using tap water. Further the slide was dipped in alcohol for 2 seconds and then placed to the safranin for 3min. The slide was again rinsed with water and air dried. The slide was examined under oil-immersion objective using one drop of cedar wood oil. Further strain was identified as *Xanthomonas campestris* according to Bergey's Manual of Systematic Bacteriology **(Kreig and Halt, 1984).**

3.7.2.4 Solubility in 3% KOH :

A loopful of bacterial culture was placed on a clear glass slide. One drop of 3% KOH solution was added, thoroughly mixed with the help of inoculation needle and moved up and down to know the solubility in KOH.

3.7.2.5 Endospore staining :

A loopful of bacterial culture was taken and smeared on glass slide and fixed by heating, and then a few drops of 1.5% amidoblack was added and allowed to stay for 70 sec. Then the slide was washed under gentle stream of running water, stained for 20 sec with 1% carbol fuschin and washed

thoroughly under tap water. Finally the slide was blot dried and observed under microscope for endospore.

3.7.3 Biochemical Test :

Biochemical Tests are described under the following headings and these methods were followed by **Schaad (1980, 1988).**

3.7.3.1 Citrate test :

Some bacteria can utilize citrate as the only carbon source and the citrate test shows if the actual bacterium has this capability.

Method :

1. Inoculated a tube containing citrate medium with a small amount of bacteria. It is also possible to streak or perform a deep inoculation into "Simmons citrate tube".
2. Incubate at 30-37 ºC during 24-48 hrs.

Positive test result : Growth in citrate medium or growth with colour change to blue in Simmon's citrate tube.

Negative test result : No growth in citrate medium yeller growth but no colour change (still green colour) in Simmon's citrate tube.

3.7.3.2 Hydrogen sulfide production :

Some bacteria can metabolize certain sulfur containing compounds under production of hydrogen sulfide (H_2S). Hydrogen sulfide is a toxic, flammable and badly smelling gas (smells like rotten eggs). If soluble iron or lead salts (for instance ferric citrate) is used in a so called H_2S medium, which should also contain sodium thiosulfate ($Na_2S_2O_3$), they can react with H_2S, if present, under formation of black insoluble iron and lead sulfide, respectively (Plate 8).

Method :

1. Perform a deep inoculation in the H_2S medium with bacteria from one colony.

2. Incubate the tube at 30-37 ºC during 24-48 h.

Positive test result : a black precipitate in the medium.

Negative test result : no precipitate in the medium.

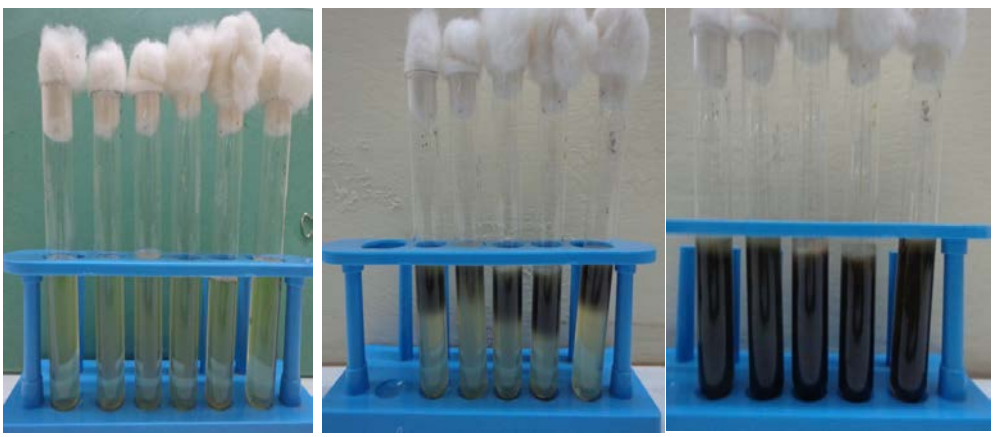


Plate: 8. Black precipitate showing the positive result in production Hydrogen sulfide.

3.7.3.3 Indole test :

Bacteria, which express the enzyme tryptophanase can hydrolyze the amino acid tryptophan to indole, pyruvic acid and ammonia. Presence of indole can be shown by means of Kovác's reagent in the socalled indole test. Kovác's reagent contains dimethyl aminobenzaldehyde, which forms a red complex with indole.

Method :

1. Suspend one colony from a pure culture of the bacterium to be investigated, in a suitable medium (for instance LTLSB or tryptophan medium).

2. Incubate the medium at 37 ºC during 20-28 h.

3. Add a few drops of Kovác's reagent.

Positive test result : The indole reagent change colour to cerise red.

Negative test result : The indole reagent remains pale yellow.

3.7.3.4 Oxidase test :

Method :

1. Keep the plastic ampoule (which contains a glass ampoule) between your fingers and with the opening upwards and away from your face. Press the ampoule with your fingers until the glass ampoule breaks.
2. Apply two drops of the oxidase reagent onto a piece of filter paper.
3. Transfer bacteria from one colony with a plastic or platinum loop onto the spot with the oxidase reagent. The colonies should have been incubated at the appropriate temperature for 18-24 h.

Positive test result : Dark blue purple colour change within 10-30 sec.
Negative test result : No colour change or colour change after more than 30 sec.

3.7.3.5 Urease test : Some bacteria have the enzyme urease, which in the presence of H_2O converts urea (carbamide) to NH_3 (ammonia) and CO_2 (carbon dioxide), which forms ammonium carbonate in the presence of water.

Method :

By growing the bacteria in urease medium containing a pH indicator, it can be determined if the bacteria express urease. If the bacteria have an urease, urea will be converted to ammonium carbonate and the medium will turn alkaline. Thus, the colour will change to red (cerise).

The bacterium isolated from black rot of cabbage leaf was identified as *Xanthomonas campestris* pv. *campestris* and it systemic position according **(Dowson) Dye *et al.* (1980)** is as follows -

3.7.4 Systematic position :

Kingdom	:	Bacteria
Phylum	:	Proteobacteria
Class	:	Gamma proteobacteria
Order	:	Xanthomonadales
Family	:	Xanthomonadaceae
Genus	:	*Xanthomonas*
Species	:	*campestris* pv. *campestris*

3.7.5 Purification and maintenance of *Xanthomonas campestris* pv. *campestris*:

The single fresh and pure glistering yellow droplets like colonies were purified on other petriplates having nutrient agar medium. Such culturally identical single colony was then transferred in containing nutrient agar slants and maintained by preserving in a refrigerator for all future experiments, and making periodic transfers after every fortnight (Plate 9).

Plate: 9. Pure culture of *X. campestris* pv. *campestris*.

3.7.6 Mass multiplication of *X. campestris* pv. *campestris* :

Test pathogen was multiplied in nutrient broth medium. The flasks containing nutrient broth were plugged with non-absorbent cotton and sterilized in an autoclaved at 15 lbs for 20 min. After cooling at room temperature, the flasks were inoculated with mycelium of *X. campestris* and incubated in B.O.D. incubator at 28±1 ^{o}C for two days.

3.7.7 Pathogenicity test of black rot pathogen :

Pathogenicity of the bacterium was proved on cauliflower plants by following Koch's Postulate on one-month-old plants, raised in 25 cm earthen pots in green house by using 48hrs old bacterial growth cultures. Inoculation was done by the method of **Chitarra *et al*. (2002)** who worked the major veins of the first two true leaves were stabbed at 5-10 points with a sterile toothpick contaminated with *X. campestris* pv. *campestris* cells scraped directly from a culture of nutrient broth medium. Tap water was used as a negative control. Pots with inoculated plants were kept in poly house for favourable temperature and relative humidity, with a 12 hrs life cycle. The appearance of typical V-shaped, yellow lesions with blackened veins after 7-10 days was considered to be a positive response (Plate 10)**.**

Plate: 10. Pathogenicity of *X. campestris* pv. *campestris.*

3.8 *In-vitro* screening of botanical extracts selected for antifungal and antibacterial activities:

Table: 3.1 List of the botanicals screened *in-vitro* for their antimicrobial activities -

S. No.	Botanicals name	Common name	family
1.	*Allium sativum*	Garlic	Amaryllidaceae
2.	*Ocimum sanctum*	Tulsi	Lamiaceae
3.	*Lantana camara*	Wild sage	Verbenaceae
4.	*Datura stramonium*	Thorn apple	Solanaceae
5.	*Eucalyptus globules*	Fever tree	Myrtaceae
6.	*Calotropis procera*	Madar	Apocynacae
7.	*Azadirachta indica*	Neem	Meliaceae
8.	*Emblica officinalis*	Amla	Phyllanthaceae
9.	*Argemone maxicana*	Satyanashi	Papaveraceae
10.	*Bambusoideae*	bamboo	Poaceae
11.	*Ficus religiosus*	Cluster fig	Moraceae
12.	*Agele marmelos*	Bael	Rutaceae
13.	*Acyranthus aspera*	Lat zira	Amaranthaceae
14.	*Canna indica*	Canna	Cannaceae
15.	*Cassia fistula*	Amaltas	Fabaceae
16.	*Croton boplandianum*	Croton	Euphorbiaceae
17.	*Cannabis sativa*	Bhang	Cannabaceae
18.	*Ricinus communis*	Castor	Euphorbiaceae
19.	*Morus alba*	Shahtoot	Moraceae
20.	*Ipomea palmata*	Railway creeper	Convolvulaceae
21.	*Hibiscus*	China rose	Rosaceae
22.	*Calendula*	Pot marigold	Asteraceae
23.	*Bougainvillea glabra*	Bougainvillea	Nyctaginaceae
24.	*Chrisanthimum coronarium*	Gul-e-duadi	Asteraceae
25.	*Withania somnifera*	Ashwagandha	Solanaceae
26.	*Chenopodium album*	Bathua	Amaranthaceae
27.	*Turmeric longa*	Turmeric	Zingiberaceae
28.	*Mentha piperita*	Mint	Lamiaceae
29.	*Millettia pinnata*	Karanj	Fabaceae
30.	*Syzygium aromaticum*	Clove	Alphaflexiviridae
31.	*Mentha arvensis*	Mentha	Lamiaceae
32.	*Polyalthia longifolia*	Ashok	Fabaceae
33.	*Dalbarjia siso*	Shisham	Fabaceae
34.	*Carica papaya*	Papaya	Alphaflexiviridae
35.	*Tagetes tenuifolia*	Marigold	Asteraceae

36.	*Cuminum cyminum*	Cumin	Apiaceae
37.	*Trigonella foenum*	Methi	Fabaceae
38.	*Cynodon dactylon*	Bermuda grass	Poaceae
39.	*Morraya koengi*	Curry leaf	Rutaceae
40.	*Ficus glomerata*	Goolar	Moraceae
41.	*Ixora coccinea*	Rokmini	Rubiaceae
42.	*Vinca rosea*	Sadabahar	Apocynacae
43.	*Callistemon lanceolatus*	Bottle brush	Mrtyaceae
44.	*Asparagus racemosus*	Shatamuli	Liliaceae
45.	*Solanum nigrum*	Mokoi	Solanaceae
46.	*Ageratum cozyodies*	Neela phulna	Compositeae
47.	*Annona squamosa*	Sharifa	Annonaceae
48.	*Euphorbia tricalli*	Milk bush	Euphorbiaceae
49.	*Acacia nilotica*	Babool	Mimosaceae
50.	*Lawsonia inermis*	Mehndi	Lythraceae
51.	*Ceasalpinia pulcherrima*	Peacock flower	Ceasalpiniodeae
52.	*Parthenium hysterophorus*	Congress grass	Compositea
53.	*Bryophyllum pinnatum*	Bryophyllum	Crassulaceae
54.	*Cereus peruvianus*	Peruvian apple	Cactaceae
55.	*Mussaenda acuminata*	Mussaenda	Rubiaceae
56.	*Mimosa pudica*	Chui mui	Mimosceae
57.	*Leucas aspera*	Chota halkusa	Mimosceae
58.	*Ziziphus jujuba*	Ber	Rhamnaceae
59.	*Tamarindus indica*	Tamarind	Fabaceae
60.	*Nyctanthes arbortristis*	Harshringar	Oleaceae
61.	*Ficus bengalensis*	Sacred fig	Moraceae
62.	*Hamelia patens*	Fire bush	Rubiaceae
63.	*Amaranthus spinosus*	Jungle chulai	Amaranthaceae
64.	*Moringa*	Saijan	Moringaceae
65.	*Zingiber officinale*	Ginger	Zingiberaceae
66.	*Plumeria* sp.	Plumeria	Apocynaceae
67.	*Urtica diocea*	Doob grass	Poaceae
68.	*Nerium*	Kaner	Apocynaceae
69.	*Sida spinosa*	Bariyara	Malvaceae
70.	*Albizia lebbeck*	shirish	Fabaceae
71.	*Coccinia cardifolia*	Kanduri	Cucurbitaceae
72.	*Punica granatum*	Pomegranate	Lythraceae
73.	*Chlorophytum borivilianum*	Safed musli	Asparagaceae
74.	*Syzygium cumini*	Jamun	Myrtaceae
75.	*Convolvulus microphyllus*	Pushpashankhi	Convolvulaceae

Screening of seventy five botanical extracts:

1. *Allium sativum*

2. *Ocimum sanctum*

3. *Lantana camara*

4. *Datura stramonium*

5. *Eucalyptus globules*

6. *Calotropis procera*

7. *Azadirachta indica*

8. *Emblica officinalis*

9. *Argemone maxicana*

10. *Bambusoideae*

11. *Ficus religiosus*

12. *Agele marmelos*

13. *Acyranthus aspera*

14. *Canna indica*

15. *Cassia fistula*

16. *Croton boplandianum*

17. *Cannabis sativa*

18. *Ricinus communis*

19. *Morus alba*

20. *Ipomea palmata*

21. *Hibiscus* sp.

22. *Calendula*

23. *Bougainvillea glabra*

24. *Chrisanthimum coronarium*

25. *Withania somnifera*

26. *Chenopodium album*

27. *Turmeric longa*

28. *Mentha piperita*

29. *Millettia pinnata*

30. *Syzygium aromaticum*

31. *Mentha arvensis*

32. *Polyalthia longifolia*

33. *Dalbarjia siso*

34. *Carica papaya*

35. *Tagetes tenuifolia*

36. *Cuminum cyminum*

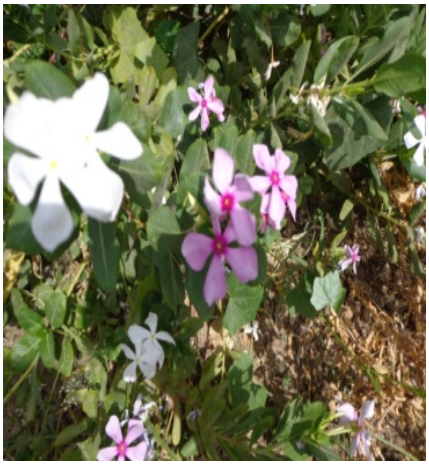
37. *Trigonella foenum*

38. *Cynodon dactylon*

39. *Morraya koengi*

40. *Ficus glomerata*

41. *Ixora coccinea*



42. *Vinca rosea*

43. *Callistemon lanceolatus*

44. *Asparagus racemosus*

45. *Solanum nigrum*



46. *Ageratum cozyodies* **47.** *Annona squamosa* **48.** *Euphorbia tricalli*




49. *Acacia nilotica* **50.** *Lawsonia inermis* **51.** *Ceasalpinia pulcherrima* **52.** *P. hysterophorus*



53. *Bryophyllum pinnatum* **54.** *Cereus peruvianus* **55**. *Mussaenda acuminate*

56. *Mimosa pudica*

57. *Leucas aspera*

58. *Ziziphus jujuba*

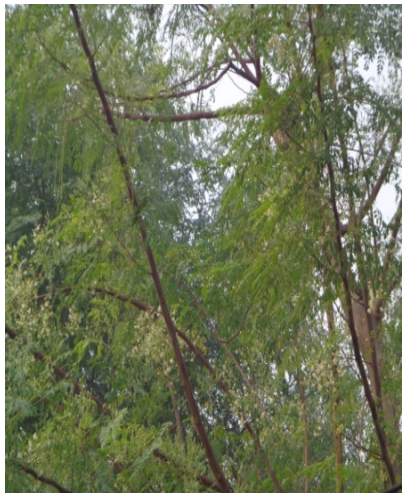
59. *Tamarindus indica*

60. *Nyctanthes arbortristis*

61. *Ficus bengalensis*

62. *Hamelia patens*

63. *Amaranthus spinosus*



64. *Moringa*

65. *Zingiber officinale*

66. *Plumeria* sp.

67. *Urtica diocea*

68. *Nerium*

69. *Sida spinosa*

70. *Albizia lebbeck*

71. *Coccinia cardifolia*

72. *Punica granatum* **73.** *Chlorophytum borivilianum* **74.** *Syzygium cumini* **75.** *C. microphyllus*

3.8.1 Collection of plant materials and preparation of extracts :

Fresh plant leaves were collected from Sam Higginbottom University of Agriculture, Technology & Sciences, Allahabad campus and surrounding area of the campus. The plant samples were first washed with tap water, surface sterilized with 2% sodium hypochloride and kept in sterilized covered beaker. All fresh plant materials were weighed 50 g, crushed with 50 ml sterilized distilled water (1:1 W/V) and homogenized for five minutes in mortar and pestle. The macerate was first filtered through double-layered muslin cloth, and then centrifuged at 4000 rpm for 30 min. The supernatant was filtered through Whatman No. 1 filter paper and sterilized at 120 ^{0}C for 30 min (Plate: 11). The extracts were preserved aseptically in a brown bottle at 5 ^{0}C until further use. This method was followed by **Satish *et al.* (1999)**. The obtained extracts served as the crude extract (100 % concentration).

Plate: 11. Procedure for preparation of aqueous leaf extracts

3.8.2 Screening of botanical for their antimicrobial activities :

Poison food technique was used accordingly **Dhingra and Sinclair (1985)** to test 50% concentration of the aqueous leaf extracts against *X. campestris* and *A. brassicae*. 50 ml of aqueous extracts were mixed with 50 ml of NA and PDA media before pouring. Each treatment was replicated three times. One treatment where without extracts were maintained as control. After, solidification of the medium, one ml of bacterial (*X. campestris*) suspension, prepared in

sterilized distilled water by 48hrs old bacterial culture was incorporated in containing a mixture of nutrient agar and aqueous leaf extracts petriplates.

After, solidification of the medium, pure culture of *A. brassicae* from 7 days old culture cut with a cork borer (4 mm) was placed at the center of each Petri plates that containing a mixture of potato dextrose agar and aqueous leaf extracts. These methods were followed by **Singh *et al.* (2013).**

The petriplates were incubated at ambient temperature (28 ± 2^0 C) in BOD incubator. At the end of incubation period, radial colony growth (cm) and no. of bacterial colonies were recorded for each treatment by using the following formula **(Bliss, 1934).**

$$\text{Inhibition Per cent} = \frac{\text{Colony diameter in control (mm)} - \text{Colony diameter in treatment (mm)}}{\text{Colony diameter in control (mm)}} \times 100$$

3.8.3 Antimicrobial activities :

Eleven botanicals were found least mycelial radial growth and maximum inhibition % among 75 screened botanicals which have more inhibition of bacterial population of tested pathogens. These botanicals (listed table: 2.2) were selected for the further studies.

3.9 Assay of secondary metabolites and active compound from the selected botanicals :

3.9.1 Phytochemical Analysis :

Phytochemical analysis for major phyto-constituents of the plant extracts was undertaken using standard qualitative methods as described by **Rizk and Bashir (1980) and Brindha *et al.* (1981).** The plant extracts were screened for the presence of biologically active compounds like glycosides, phenolics, alkaloids, tannins, flavonoids, saponins and steroids.

3.9.2 Identification test :

The test were carried out to find the presence of the active chemical constituents such as glycosides, phenolics, alkaloids, tannins, flavonoids, saponins, phlobotannins, anthraquinones, terpenoids, carbohydrates and steroids by the following tests-

3.9.2.1 Test for alkaloids :

2 ml filtrate of the plant extract was mixed with 2ml of conc.HCl and about 6 drops of Mayor's reagents. A creamish or pale yellow precipitate indicates the presence of alkaloids (Plate : 12).

3.9.2.2 Test for steroids :

One ml of the extract was dissolved in 2 ml of acetic anhydride which is then added to 2 ml of H_2SO_4. The colour changes from violet to blue or green in some samples indicating the presence of steroids (Plate : 13).

3.9.2.3 Test for tannins:

One ml of the extract was treated with few drops of 1% ferric chloride solution and observed for brownish green or a blue-black coloration, confirming presence of tannins (Plate : 14).

3.9.2.4 Test for flavonoids :

Four ml of filtrate was added to 5-6 drops of conc. HCl and 1.5 ml of methanol solution. Pink tomato red color indicates the presence of flavonoids (Plate : 15).

3.9.2.5 Test for glycosides :

Two ml of extract is mixed with 2 ml chloroform. Then 2 ml acetic anhydride and 2 drops of conc. H_2SO_4 were added from the side of test tube. First red, then blue and finally green colour appears indicating presence of glycosides (Plate : 16).

3.9.2.6 Test for Phenolics :

Two ml of ethanol was added to the test solution and few drops of ferric chloride solution. Blue coloration indicates the presence of phenolics.

3.9.2.7 Test for saponins :

Two ml of distilled water was added to 2 ml of the test solution and shaked well and observed for frothing.

3.9.2.8 Tests for anthraquinones :

(a) Borntrager's test: 3ml of aqueous extract was shaken with 3 ml of benzene, filtered and 5 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of a pink, red or violet colour in the ammonical (lower) phase indicates the presence of free anthraquinones.

(b) 3 ml of the aqueous extract was boiled with 3ml of aqueous sulphuric acid and filtered while hot. 3 ml of benzene was added to the filtered and shaken. The benzene layer was separated and 3 ml of 10% HN3 added. A pink, red or violet coloration in the ammonical (lower) phase indicates the presence of anthraquinone derivatives (Plate : 17).

3.9.2.9 Test for terpenoids :

2ml of the organic extract was dissolved in 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated sulphuric acid was then added and heated for about 2 min. A grayish colour indicates the presence of terpenoids (Plate : 18).

3.9.2.10 Tests for carbohydrates :

(a) Molisch's test: 3 ml of the aqueous extract was added to 2 ml of Molisch's reagent and the resulting mixture shaken properly, then 2 ml of concentrated H_2SO_4 was poured carefully down the side of the test tube. A violet ring at the interphone indicates the presence of carbohydrate.

(b) 3 ml of the aqueous extract was measured into test tube and1 ml of iodine solution was added. A purple coloration at the interphone indicates the presence of carbohydrates (Plate : 19).

3.9.2.11 Test for gums and mucilage :

1ml of extract was mixed with 2.5 ml of absolute alcohol under constant stirring. The formation of precipitates indicated the presence of gums and mucilage **(Sofowora, 1993; Trease and Evans, 1989)** (Plate : 20)**.**

3.9.2.12 Test for phlobatannins :

1ml of extract was treated with few drops of 2% aqueous HCl and boiled for few minutes. The appearance of red precipitates indicated the presence of phlobatannins **(Ajayi, 2013)** (Plate : 21)**.**

3.9.2.13 Test for reducing sugars :

2-3 ml of Fehling solution A and B were heated gently and allowed to cool. Then 1ml of extract was added to it. The mixture was boiled for 5-10 minutes. Brownish red precipitates indicated the presence of reducing sugars **(Evans, 2002).**

3.9.2.14 Test for Proteins :

Biuret Test – Test solution was treated with 10% sodium hydroxide solution and two drops of 0.1% copper sulphate solution and observed for the formation of violet/pink color. Test for Free Amino Acids: Ninhydrin Test – Test solution when boiled with 0.2% solution of Ninhydrin, would result in the formation of purple color suggesting the presence of free amino acids (Plate : 22).

3.9.2.15 Test for Coumarins :

0.5 g of the moistened various extracts was taken in a test tube. The mouth of the tube was covered with filter paper treated with 1 N NaOH solution. Test tube was placed for few minutes in boiling water and then the filter paper was removed and examined under the UV light for yellow fluorescence indicated the presence of coumarins.

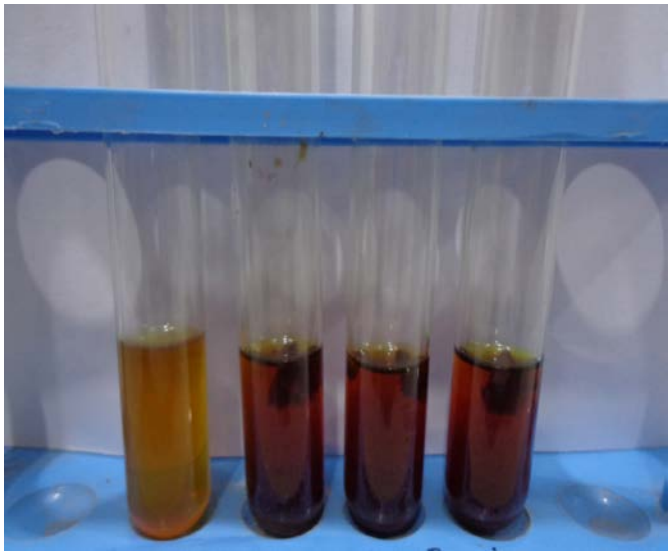


Plate : 12. Test of Alkaloids

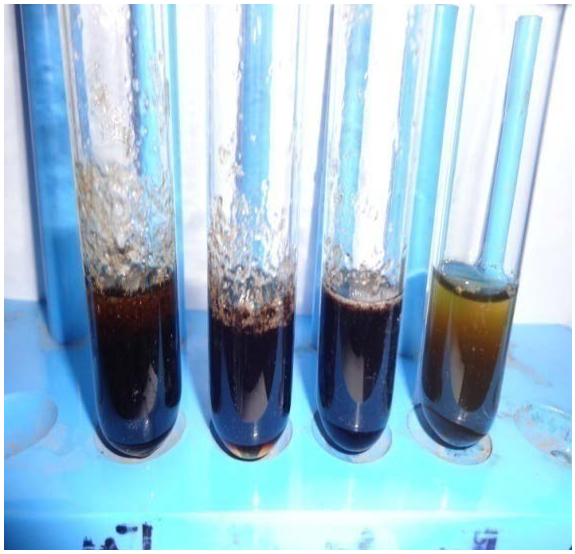


Plate : 13. Test of Steroids

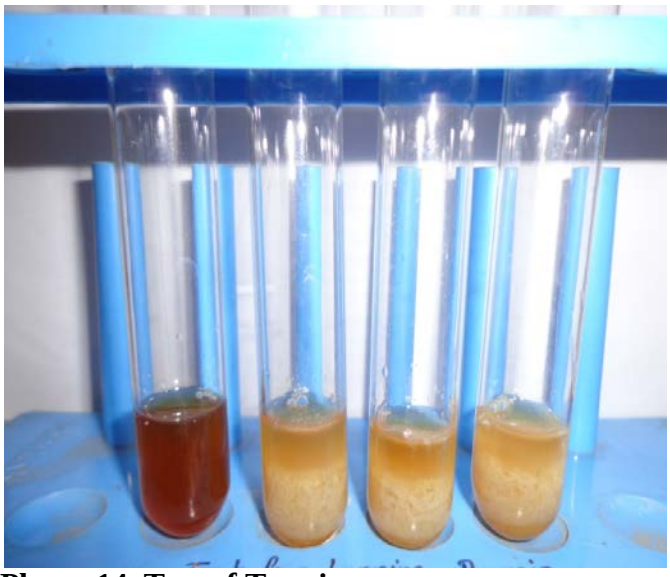


Plate : 14. Test of Tannins

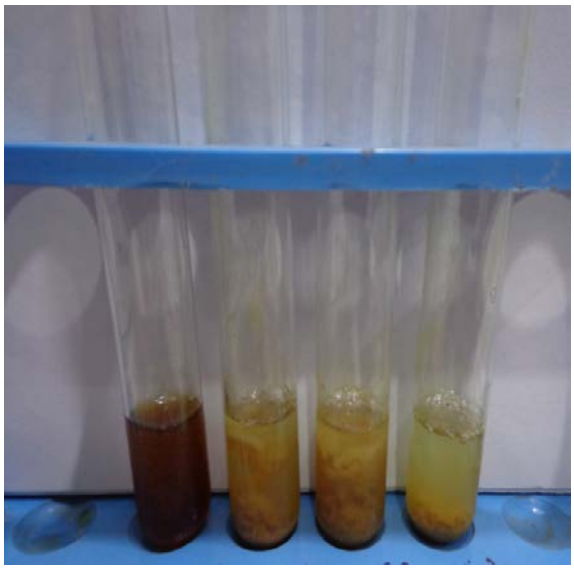


Plate : 15. Test of Flavonoids

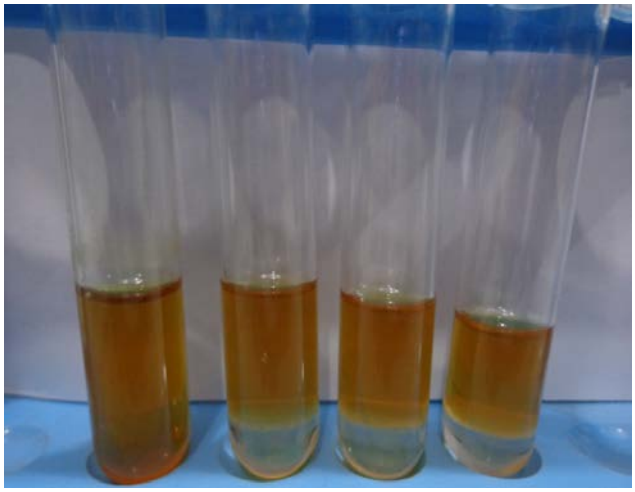


Plate : 16. Test of Glycosides

Plate : 17. Test of Anthroquinones

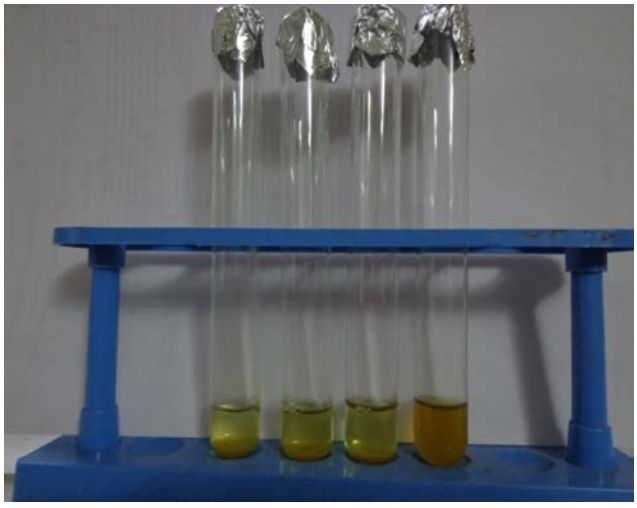


Plate : 18. Test of Terpinoids

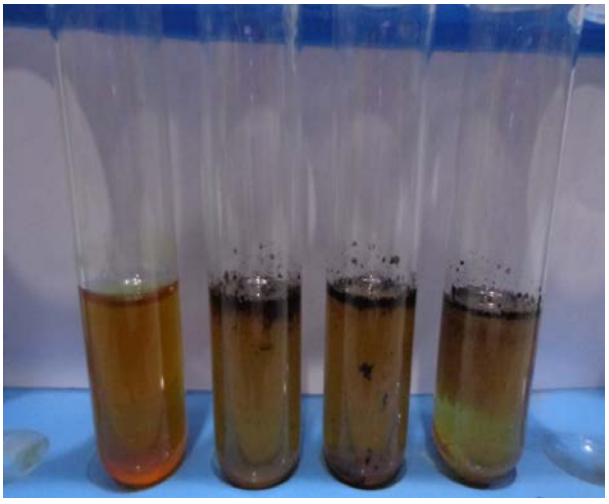


Plate : 19. Test of Carbohydrates

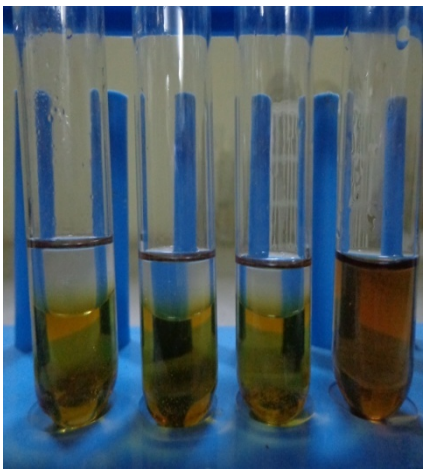


Plate : 20. Test of Gums **Plate : 21. Test of Phlobatanins** **Plate: 22. Test of Proteins**

3.10 *In-vitro* efficacy of selected plant extracts on the radial growth of *A. brassicae* and number of colony of *X. campestris* pv. *campestris* :

For growth inhibition tests stock solutions were prepared by crushing known weight of fresh leaves with distilled water (1:1 W/V). The pulverized mass of a plant part was passed through a three-fold fine cloth and was centrifuged at 3000 rpm for 15 min. The supernatant was filtered through Whatman's No. 1 filter paper and the filtrate was collected in 250 ml Erlenmeyer flask. The filtrate of each plant extract was mixed with PDA and NA media to make 25, 50 and 75% concentrations. Bordeaux mix was used at 0.2, 0.3 and 0.4% for the check treatment. After autoclaving, a plant extract supplemented media were poured in sterilized petri plates and allowed to solidify. These petri plates were inoculated at the centre with a 5 mm agar disc, cut from the margin of actively growing culture of the *A. brassicae,* and 1 ml bacterial suspension was poured in the containing of nutrient agar petri plates. In the control, a petri plate containing PDA and NA media with requisite amount of sterilized water instead of a plant extract was also inoculated with plant pathogens. This experiment was conducted during 2012 with thirteen treatments were replicated four times and followed the design of CRD. Inoculated petri plates were kept at 28°C in BOD.

3.11 Antifungal and Antibacterial Activity of Essential Oils from the Selected Botanicals

3.11.1 Extraction of oils from botanical extracts :

Fresh leaves of *Eucalyptus globules*, *Azadirachta indica*, *Lantana camera*, *Buchnera hispida*, *Syzygium aromaticum*, *Carica papaya*, *Allium sativum*, *Zingiber officinale*, *Psidium guajava*, *Mentha peperata*, *Mentha arvensis* and *Ocimum sanctum* were obtained from the gardens of campus and surrounding area of the Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad. The leaves were taken to laboratory and cut out by a pair of scissors to small part. The Clevenger consists of a main tube combined with condenser and graduated tube with glass stopcock. A return tube for the aqueous part of the distillate connects the bottom of the measuring tube and the main tube. One kg of fresh and clean leaves were placed into the three necked round extraction flask and soaked with water. The flask was heated using heating mantel. Water and leaves, were mixed and allowed to boil. Water and extracted leaves oil evaporate. The vapour mixture condensed using reflux condenser, from condenser distillate water and oil flow in to gradated tube; as the oil is not miscible with water it may be easily separated and started accumulating and distillate water returning to the flask, after the oil has been separated from it, so that can be re-boiled (Plate : 23). The oil was allowed to stand for sufficient time, to be clear and then it was collected and stored in dark glass vial in a refrigerator until it has been tested **(Cassel and Vargas, 2006).**

Plate : 23. During the extraction of essential oils by the help of Clevenger and Soxhlet apparatus.

3.11.2 *In-vitro* efficacy of essential oils on the radial growth of *A. brassicae* and number of colony of *X. campestris* pv. *campestris* :

Extracted essential oils through soxhlet apparatus which are listed in Table: 2.3; to evaluate the antifungal and antimicrobial properties of extracted essential oils. Each essential oil was mixed with PDA (for *A. brassicae*) and NA (for *X. campestris* pv. *campestris*) media to make 2.0, 4.0 and 6.0 % (v/v) concentrations. Bordeaux mix was used at 0.2, 0.3 and 0.4% for the check treatment. After autoclaving, an oils supplemented media were poured in sterilized Petri plates and allowed to solidify. Each Petri plate received 20 ml of oil supplemented media. PDA poured petri plates were inoculated at the centre with a 5 mm agar disc, cut from the margin of actively growing culture of the *Alternaria*, and one ml of bacterial suspension inoculated in sterilized petri plates after that lukewarm NA medium was poured in inoculated petri plates. In the control, a Petri plate containing PDA and NA media with requisite amount of sterilized water instead of oil was also inoculated with a plant pathogens. This experiment was conducted during 2013 with fourteen treatments were replicated four times and followed the CRD. Inoculated petri plates were kept at 28°C in BOD.

After incubation, the mycelial radial growth (mm) of *A. brassicae* and per cent inhibition over control were recorded for each treatment by using the following formula **(Bliss, 1934).**

$$\text{Inhibition Per cent} = \frac{\text{Colony diameter in control (mm)} - \text{Colony diameter in treatment (mm)}}{\text{Colony diameter in control (mm)}} \times 100$$

3.12 Detail of treatments :

Table: 3.2. Treatments detail of selected botanicals leaf extracts against *X. campestris* pv. *campestris* and *Alternaria brassicae* in *in-vitro*

Treatments	**Selected botanical extracts**	**Concentration %**
T_1	*Ocimum sanctum*	25, 50 and 75
T_2	*Calotropis procera*	”
T_3	*Mentha peperata*	”
T_4	*Allium sativum*	”
T_5	*Lantana camara*	”
T_6	*Datura stramonium*	”
T_7	*Azadirachta indica*	”
T_8	*Eucalyptus globules*	”
T_9	*Zingiber officinale*	”
T_{10}	*Mentha arvensis*	”
T_{11}	*Croton boplandianum*	”
T_{12}	Bordeaux mixture	0.2, 0.3 and 0.4
T_0	*Alternaria brassicae* / *X. campestris*	-

3.13 Detail of treatments :

Table: 3.3 Treatments details of selected essential oils against *X. campestris* pv. *campestris* and *Alternaria brassicae* in *in-vitro*

Treatments	Selected essential oils	Concentration %
T_1	*Eucalyptus globules*	2, 4 and 6
T_2	*Azadirachta indica*	”
T_3	*Lantana camera*	”
T_4	*Buchnera hispida*	”
T_5	*Syzygium aromaticum*	”
T_6	*Carica papaya*	”
T_7	*Allium sativum*	”
T_8	*Zingiber officinale*	”
T_9	*Psidium guajava*	”
T_{10}	*Mentha peperata*	”
T_{11}	*Mentha arvensis*	”
T_{12}	*Ocimum sanctum*	
T_{13}	Bordeaux mixture	0.2, 0.3 and 0.4
T_0	*Alternaria brassicae* / *X. campestris*	-

3.13.1 Observations recorded :

- Mycelial radial growth of *A. brassicae* of each concentration at 48, 72, 96 and 120 hrs after incubation.
- Number of colonies ml^{-1} of *X. campestris* of each concentration at 48 and 72 hrs after incubation.
- Per cent growth inhibition over control of both pathogens for each concentration.

3.14 Cultural operations :

3.14.1 Seedbed preparation :

Before sowing of the cabbage seed in the departmental field of Plant Pathology, seedbed was prepared, size of seedbed was 1.0 m x 1.0 m wide and 10 cm high. Applied a thin layer of compost on the seedbed in a square meter seedbed, incorporated 40 gm ammonium sulfate, 50 gm super phosphate, 30 gm potassium chloride and 2 kg compost. To protect the seedbed, covered by net to prevent the seedlings from early pest infestation. 20 seeds per 30 cm (1 foot) at a depth of 1.0 cm were placed. Sowing dates were 02/10/2012 and 24/09/2013 (Plate : 24).

Plate : 24. View of raising nursery plant of cabbage crop.

3.14.2 Field preparation :

Before transplanting of seedling, field was thoroughly ploughed and pulverized with tractor drawn cultivator to attain desirable tilt. Leveling and formation of plots were done manually. The field was then cleaned by picking the stubble of previous crop etc. one harrowing was applied to field before seedling.

3.14.3 Hardening :

Five (5) days before transplanting, gradually expose seedlings daily to strong sunlight and also reduce the water supply to lessen stress of the seedlings after transplanting.

3.14.4 Transplantation :

Plots were laid out as per experimental design lines, marked with the help of manually operated marker. The seedling of variety ***Golden acre*** of cabbage was planted by dibbling method with spacing of 60 cm row to row and 60 cm between plant to plant by placing one seedling per hill at depth of 10 cm. seedling was done on 05/11/2012 and 27/10/2013.

Plate : 25. View of Experimental field.

3.14.5 Fertilizer management :

For Cabbage, the general fertilizer recommended doze of NPK = 100:50:100 kg ha^{-1}. Splited the recommended fertilizer amount into 3 and applied it as basal, 3 weeks after transplanting and during heading.

3.14.6 Intercultural operations :

As and when needed weeding operations were carried out to conserve soil moisture and to keep the experimental field free from the weeds. Two weedings were done at 16 days interval.

3.14.7 Irrigation and harvesting :

Cabbage needs sufficient amount of water after transplanting and during head formation. In Allahabad condition was raining. And also Irrigations were given at 15 days interval.

3.15 Details of Experiment :

Experimental Design	:	Randomized Block Design
Number of Replications	:	3
Number of Treatments	:	13 (for botanical extracts)
Crop	:	Cabbage
Variety	:	*Golden acre*
Total Plots	:	39
Plot Size	:	3 x 3 m^2
Seed rate	:	500 g/ha
No. of rows / plot	:	5
No. of plants / row	:	5
Total No. of Plants / plot	:	25
Date of seed sowing	:	02.10.2012 and 24.09.2013
Date of transplanting	:	05.11.2012 and 27.10.2013

3.16 Field efficacy of selected plant extracts on Cabbage crop :

The field experiment was conducted during *rabi* season of 2012-13. The experiment was conducted following randomized block design with three replications in a treatment and having plot size 3 x 3 m^2. Treatments included in the laboratory experiment that screened leaf extracts are listed in Table : 2.2.

A. brassicae culture was raised on PDA and spore suspension was prepared in water and adjustment to 10-15 spores/ microscopic field at 10x. The spore suspension was sprayed at 35 days after sowing of cabbage crop.

Leaf extracts @ 75% were sprayed 5 days after inoculation. The fungicide Bordeaux mix @ 0.4% commonly recommended for controlling this disease was also included. Second and third spray with plant extracts and fungicide were given after 15 days intervals.

3.17 Field efficacy of essential oils on Cabbage crop :

The field experiment was conducted during *rabi* season of 2013-14. The experiment was conducted following randomized block design with three replications in a treatment and having plot size 3 x 3 m^2. Treatments included in the laboratory experiment that screened essential oils are listed in Table: 2.3.

A. brassicae culture was raised on PDA and spore suspension was prepared in water and adjustment to 10-15 spores/ microscopic field at 10x. The spore suspension was sprayed at 35 days after sowing of cabbage crop.

Extracted oils @ 6% were sprayed 5 days after inoculation. The fungicide Bordeaux mix @ 0.4% commonly recommended for controlling this disease was also included. Second and third spray with oils and fungicide were given after 15 days intervals.

Observation on disease severity was recorded after 45, 60 and 90 days after sowing. The disease severity of black leaf spot was assessed on 0-5 scale **(Karunanithi, 1996)**, where 0=No visible symptoms, 1=1-10% leaf area infected (LAI), 2=11-25% LAI, 3=26-50% LAI, 4=51-75 % LAI, 5=76-100 % LAI.

The disease severity of black rot was assessed on a six point scale of 0-9 based on the relative lesion size appeared only on injured points as: 0 = no symptoms, 1 = small necrosis or chlorosis surrounding the infection point, 3 = typical small V- shaped lesion with black veins, 5 = typical lesion half way to the middle vein, 7 = typical lesion progressing to the middle vein and 9 = lesion reaching the middle vein **(Singh *et al.*, 2010)**.

Disease severity (%) was calculated by using the following formula:-

$$\text{Disease severity (\%)} = \frac{\text{Sum of all disease rating}}{\text{Total no.rating x Max.disease grade}} \text{ x } 100$$

3.17.1 Observations recorded :

1. Per cent disease intensity of black leaf spot of cabbage at 45, 60, 75 and 90 DAS.
2. Per cent disease intensity of black rot of cabbage at 45, 60, 75 and 90 DAS.
3. Measurement of edible head diameter (cm) at 90 and 120 DAS.
4. Weighed (gm) of edible head of cabbage after harvest.

3.18 Statistical Analysis :

The present laboratory experiments were carried out under complete randomized design (CRD) and Field experiments were carried out under randomized block design (RBD). The analysis of variance technique was applied for drawing conclusions from the data **(Fisher and Yates, 1968).**

SKELETON OF ANOVA TABLE (CRD)

Table: 3.4

Source of variation	**d.f.**	**S.S.**	**M.S.S**	**F cal.**	**F tab. (5%)**	**Result**
Due to treatment	(t-1)	TSS	TSS/(t-1)	MTSS/EMSS	F (t-1), t(r-1)	**S/NS**
Due to error	t(r-1)	ESS	E.S.S/t(r-1)			
Total	(rt-1)	TSS				

SKELETON OF ANOVA TABLE (RBD)

Table: 3.5

Source of variation	**d. f.**	**S.S.**	**M. S. S.**	**F (cal)**	**F (tab)at 5%**	**Results**
Due to replications	(r-1)	R. S. S.	$\frac{\text{R.S.S}}{\text{(r-1)}}$ =M.S.S.R	$\frac{\text{M.S.S.R}}{\text{M.E.S.S}}$	F (r-1)	S/NS
Due to treatments	(t-1)	T. S. S.	$\frac{\text{T.S.S}}{\text{(t-1)}}$ =M.T.S.R	$\frac{\text{M.T.S.S}}{\text{M.E.S.S}}$	E(r-1) (t-1)	S/NS
Due to error	(r-1) (t-1)	E.S. S.	$\frac{\text{E.S.S}}{\text{(r-1) (t-1)}}$ =M.E.S.S	-	F (t-1), (r-1) (t-1)	-
Total	(r t-1)	T.S.S	-	-	-	-

To calculate the critical difference, standard error of difference between means (S. Ed.) has to be calculated, with the help of the following formula:

$S.E\ (d)\ \ for\ treatment = \sqrt{\frac{2 \times MESS}{r}}$, C.D. at 5% = S.E.d × $t_{(5\%)}$ edf.

Where,

d. f. = degree of freedom, S. S = sum of square, M.S.S = Mean sum of square

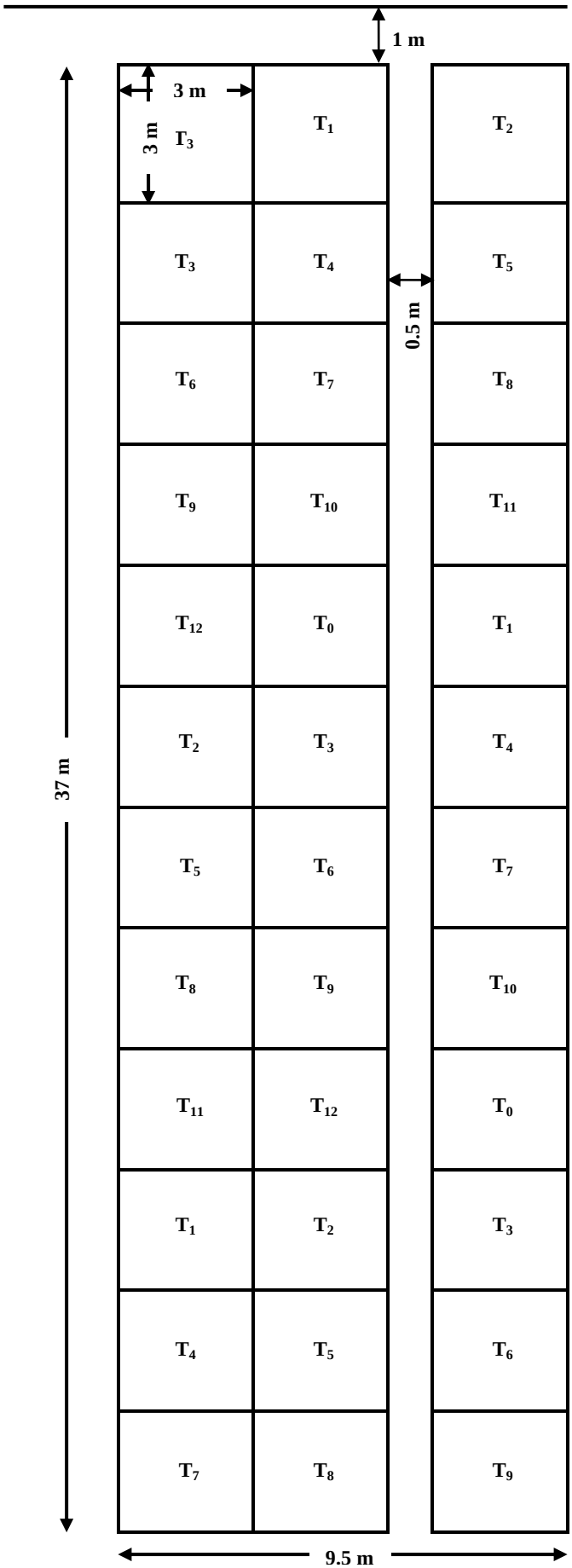
F. (cal) = Calculated value of 'F', F. (tab) = Table value of 'F'

R. S. S. = Sum of square due to replicate, E. S. S. = Error sum of squares

T. S. S. = Total sum of squares, M. R. S. S = Mean sum of squares (Replication)

S. S. T. = Sum of square due to treatment, MESS = Mean sum of square due to error

MSST = Mean sum of squares (Treatment)



Layout of Experiment

CHAPTER - IV

RESULTS AND DISCUSSION

The results of study entitled **"Botanical pesticides in the management of *Xanthomonas campestris* pv. *campestris* and *Alternaria brassicae* of cabbage",** under laboratory and field conditions were conducted in the Departmental laboratory and research plot of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad. Observations were recorded at screening of 75 plant leaf extracts and their antimicrobial activity against *Alternaria brassicae* and *X. campestris* pv. *campestris*, assayed of secondary metabolites and active compound from the selected botanicals, evaluated of selected botanical extracts and essential oils on *Xanthomonas campestris* pv. *campestris* and *Alternaria brassicae,* disease severity, diameter (cm) of edible head and weight (g) of cabbage boll.

4.1 Isolation and identification of *Alternaria brassicae* :

Alternaria brassicae was isolated from leaf spot of cabbage leaves. It was successfully carried out on PDA medium. Blackish mycelium growth of *Alternaria* was observed on PDA medium after 48 hrs of incubation. For the identification, slide was prepared and observed under the compound microscope. Conidiophres are dark, septate and muriform conidia arise from conidiophores. On the basis of microscopical observation, the pathogen was identified as *Alternaria brassicae.* Above characteristics feature of *A. brassicae* was supported by **Singh (2009).**

4.1 Isolation of *Xanthomonas campestris* pv. *campestris* :

The pathogen causing black rot of cabbage was isolated from naturally infected leaves and curds collected from the field. It was successfully carried out on NA medium and produced colonies were yellow, slimy, glistening and round in shape.

4.1.1 Identification of *Xanthomonas campestris* pv. *campestris* :

Morphological, physiological and biochemical characteristics of *X. c.* pv. *campestris*:

The results of the various morphological, physiological and biochemical tests are given in Table and Plate : 4.1. Five days after inoculating in the NA medium, shiny, convex, round colonies of yellow colour developed. The isolate was found to be rod shaped, obligately aerobic,

gram negative, oxidase negative and monotricously flagellated, which is characteristic of the genus *Xanthomonas*. It was positive for catalase reaction utilized glucose, fructose, sucrose for acid production, liquefaction of gelatin and produced hydrogen sulphide and did not produce indole, in addition, the strain failed to reduce nitrate to nitrites. In this study had the same biochemical and physiological characteristics as those described by **Obradović *et al*. (2000b)**, which are also in agreement with the characteristics of *X. campestris* pv. *campestris* reported in literature by other authors **(Lelliott and Stead, 1987; Schaad, 1980, 1988; Arsenijević, 1992)** who investigated all the isolates, including the control isolate Xcc NCPPB 1144, metabolism of glucose was oxidative, the activity of catalase was positive, and the activity of oxidase was negative.

Table : 4.1 Biochemical and physiological characteristics of *X. campestris* pv. *campestris*

S. No.	Characters	Results
A.	**Morphological**	
1.	Shape	Rode shape
3.	Cell arrangement	Scattered
4.	Gram Staining	Negative
5.	Spore	Nil
6.	Flagella	Monotricous
7.	Capsule	Nil
B.	**Cultural**	
	Colony characters after 48 h at 28 0C (on NA) and growth on solid medium	Pale yellow, mucoid, slimy, glistening and round in shape
C.	**Biochemical**	
1.	Citrate test	+
2.	Hydrogen sulfide production	+
3.	Indole test	Nil
4.	Oxidase test	Nil
5.	Urease test	Nil

4.1.1 Proved the pathogenicity of *Xanthomonas campestris* pv. *campestris* :

Pathogenicity of *X. campestris* pv. *campestris* was tested in susceptible cabbage cultivar *Golden acre* grown under controlled aseptic conditions in net house. A thick suspension of 24 h old culture of the isolate was inoculated on upper surface of cabbage leaves. Plants were made with stabbing through a toothpick by means dipped in bacterial suspension. Inoculated plants were produced a systemic black discoloration of the veins after 7–10 days of inoculation, followed by the appearance of leaf lesions and desiccation of the lesion tissue. Whereas, water

control did not show any symptoms on the cultivar tested. Pathogenecity of *X. campestris* pv. *campestris* was proved and are agreement with those of **Obradovic and Arsenijevic (1999).**

4.1.1 Proved the pathogenicity of *Alternaria brassicae* :

Pathogenicity of *Alternaria brassicae* was tested in susceptible cabbage cultivar *Golden acre* grown under controlled aseptic conditions in net house. Mass culture of *A. brassicae* was incorporated in the pots. Thirty days after transplanting of cabbage, black leaf spots are enlarge in concentric circles and mature lesions have a bull's eye type on cabbage leaf. Pathogenicity of *Alternaria brassicae* was proved and are agreement with those of **Sharma *et al.* (2013)**.

4.2 Screening of botanicals for their antimicrobial activities against test pathogens :

4.2.1 Screening of botanicals for their antimicrobial activities against *Alternaria brassicae* :

Seventy five aqueous leaf extracts at 50 % concentration were screened for their antimicrobial activity against *Alternaria brassicae* in Table : 4.2 found significantly reduced the least mean colony diameter (mm) in T_7 – *Azadirachta indica* (7.7) and T_{28} - *Mentha peperata* (8.5) as compared rest of botanical leaf extracts including over control at 48 and 96 hrs after incubation.

At 48 hrs after incubation results shows that *Azadirachta indica* significantly inhibited the mycelia growth of *Alternaria brassicae* followed by remaining leaf extracts including untreated control. The treatment of *Mentha peperata* was also significantly recorded least mean mycelial growth as comparison from T_5 (10.2), T_2 (10.4), T_4 (10.4),T_1 (11.2), T_6 (11.2), T_{31} (11.5), T_{65} (11.5), T_8 (13.2), T_{13} (13.2), T_{10} (13.3), T_{23} (13.5), T_9 (14.6), T_{15} (14.5), T_{26} (14.3), T_{27} (14.5), T_{30} (14.4), T_{55} (14.5), T_{56} (14.5), T_{71} (14.3), T_{75} (14.5), T_{14} (15.3), T_{18} (15.7), T_{20} (15.6), T_{25} (15.3), T_{35} (15.6), T_{70} (15.6), T_{17} (16.4), T_{19} (16.4), T_{21} (16.4), T_{32} (16.4), T_{36} (16.5), T_{39} (16.6),T_{43} (16.5), T_{45} (16.7), T_{52} (16.5), T_{63} (16.4), T_{67} (16.3), T_{68} (16.6), T_{22} (17.4), T_{33} (17.5), T_{34} (17.5), T_{38} (17.4), T_{40} (17.5), T_{41} (17.5), T_{44} (17.5), T_{48} (17.6), T_{59} (17.5), T_{60} (17.5), T_{37} (18.4), T_{42} (18.3), T_{47} (18.5), T_{49} (18.3), T_{51} (18.5), T_{53} (18.6), T_{57} (18.2), T_{64} (18.3), T_{66} (18.3), T_{46} (19.4), T_{50} (19.3), T_{54} (19.4), T_{62} (19.4), T_{73} (20.6), T_{74} (20.4) including with control (35.7). All the leaf extracts were found non significantly differ from one another excluding with *Azadirachta indica* and *Mentha peperata*, which were recorded significantly maximum%

inhibition of mycelial growth followed by other botanicals treatments. While, control petriplates were maximum mycelial growth of *Alternaria brassicae.*

After 96 hrs incubation of *Alternaria brassicae*, it was observed that the significantly reduction of radial growth (mm) in the treatment T_7 - *Azadirachta indica* (20.2) as compared with all the leaf extracts including with control. However, the treatments T_{28} - *Mentha peperata* (21.1) and T_6 - *Mentha arvensis* (21.2) was also non significantly recorded least mean mycelial growth as comparison from T_5 (22.1), T_{65} (22.2), T_4 (23.2), T_2 (23.7), T_1 (24.2), T_{31} (26.2), T_3 (26.3), T_{16} (31.2), T_9 (31.3), T_8 (32.3), T_{12} (32.3), T_{10} (33.3), T_{30} (35.2), T_{11} (35.3), T_{19} (35.3), T_{32} (36.3), T_{13} (37.3), T_{14} (38.2), T_{18} (38.4), T_{17} (39.2), T_{23} (39.4), T_{17} (40.2), T_{75} (40.2), T_{58} (40.3), T_{22} (41.4), T_{63} (42.2), T_{20} (40.3), T_{61} (43.2), T_{52} (45.2), T_{55} (45.2), T_{21} (45.3), T_{27} (45.3), T_{24} (46.1),T_{56} (46.2), T_{70} (49.2), T_{36} (50.2), T_{33} (50.3), T_{34} (51.2), T_{38} (52.9), T_{68} (54.5), T_{57} (55.2), T_{25} (55.3), T_{67} (55.4), T_{37} (56.3), T_{45} (57.2), T_{60} (57.4), T_{43} (58.1), T_{59} (58.2), T_{39} (60.2), T_{64} (60.2), T_{69} (60.3), T_{40} (61.5), T_{44} (62.4), T_{41} (65.2), T_{47} (65.2), T_{48} (65.2), T_{49} (65.2), T_{51} (65.2), T_{72} (65.2), T_{35} (65.3), T_{26} (65.4), T_{53} (65.4), T_{62} (68.3), T_{54} (69.1), T_{50} (70.1), T_{46} (70.2), T_{73} (70.2), T_{66} (70.3), T_{42} (71.3), T_{74} (75.2) including with control (90.0). All the leaf extracts were found non significantly differ among each other. However, *Azadirachta indica, Mentha peperata* and *Mentha arvensis* were recorded significantly maximum% inhibition of mycelial growth followed by rest of botanicals treatments. While, control petriplates were maximum mycelial growth of *Alternaria brassicae.*

4.2.2 Screening of botanicals for their antimicrobial activities against *X. campestris* pv. *campestris* :

Seventy five aqueous leaf extracts at 50 % concentration were screened for their antimicrobial activity against *X. campestris* pv. *campestris* in Table : 4.2 shows highest reduced the no. of colonies in T_7 – *Azadirachta indica* (7.0), T_5 – *Ocimum sanctum* (7.5),T_{28} - *Mentha peperata* (8.0) and T_2 - *Eucalyptus globules* (8.1) as compared rest of botanical leaf extracts including over control at 48 and 96 hrs after incubation.

At 48 hrs after incubation results shows that minimum no. of colonies were obtained in *Azadirachta indica* followed by rest botanical leaf extracts including untreated control. The treatment of *Ocimum sanctum*, *Mentha peperata* and *Eucalyptus globules* were also recoreded insignificantly reduced the colonies of *X. campestris* pv. *campestris* as comparison from T_4

(9.0), T_6 (9.5), T_1 (10.0),T_{31} (10.5), T_{65} (11.1), T_3 (11.5), T_8 (11.9), T_{13} (13.0), T_{10} (13.5), T_{16} (15.0), T_{23} (15.6), T_{26} (16.5), T_{71} (16.8), T_{61} (17.5), T_{12} (18.0), T_{30} (18.0), T_{11} (24.5), T_{15} (26.0), T_{27} (26.5), T_{29} (26.8), T_{14} (27.8), T_9 (28.0), T_{55} (28.0), T_{56} (28.5), T_{75} (29.0), T_{25} (30.5), T_{58} (31.0), T_{20} (31.5), T_{70} (31.5), T_{35} (32.5), T_{18} (34.1), T_{67} (34.5), T_{21} (39.0), T_{32} (39.3), T_{63} (39.5), T_{36} (47.0), T_{43} (47.5), T_{39} (48.5), T_{33} (59.8), T_{22} (60.0), T_{34} (61.0), T_{40} (62.0), T_{41} (62.5), T_{59} (64.0), T_{44} (64.5), T_{60} (65.0), T_{69} (65.5), T_{57} (75), T_{37} (75.5), T_{42} (76.5), T_{47} (76.5), T_{53} (76.5), T_{54} (76.5), T_{49} (77.0), T_{46} (77.5), T_{50} (77.5), T_{51} (77.5), T_{24} (77.8), T_{64} (78.5), T_{66} (78.5), T_{73} (78.5), T_{74} (78.5), T_{62} (79.5) are non significantly reduced the colonies among each other. However, treatments T_{17} (37.0), T_{19} (40.5), T_{52} (45.0), T_{68} (50.5), T_{45} (55.0), T_{38} (58.0), T_{48} (66.5) and T_{72} (68.5) were found significant effect to each other.

At 96 hrs incubation of *X. campestris* pv. *campestris*, it was observed that the T_7 - *Azadirachta indica* (20.2) was recorded highest inhibition of colonies as compared with all the leaf extracts including with control. However, the treatments T_5 - *Ocimum sanctum* (19.8), T_{28} - *Mentha peperata* (19.8), T_2 - *Eucalyptus globules* (20.2), T_4 - *Datura stramonium* (20.3), T_1 - *Zingiber officinale* (20.5), T_6 - *Mentha arvensis* (20.8), T_3 - *Calotropis procera* (21), T_{31} - *Allium sativum* (21.4), T_{65} - *Lantana camara* (21.5), T_8 - *Croton boplandianum* (21.8) and T_{13} - *Acyranthus aspera* (32.3) are non significantly reduced the colonies as comparison from rest of botanical leaf extracts including with control. However, they are found significant effect from T_{12} (32.5), T_{23} (33.7), T_{16} (34), T_{10} (35), T_{30} (36.2), T_{26} (38.2), T_{15} (39.2), T_{11} (39.8), T_{61} (40.7), T_{27} (40.8), T_{29} (43.5), T_{71} (44), T_{25} (44.3), T_{55} (44.3), T_{20} (44.7), T_{56} (45.3), T_{45} (45.5), T_{14} (46.2), T_9 (47), T_{35} (47.5), T_{70} (48.8), T_{58} (49.2), T_{75} (49.2), T_{18} (50.2), T_{67} (56), T_{17} (57.2), T_{21} (61.5), T_{32} (63.3), T_{63} (66.7), T_{19} (69), T_{49} (70), T_{52} (72), T_{36} (74),T_{43} (75.5), T_{39} (75.7), T_{68} (79.2), T_{38} (88), T_{50} (90), T_{50} (91.7), T_{22} (93.7), T_{33} (97.2), T_{24} (103.3), T_{34} (104.5), T_{62} (106.3), T_{48} (108.3), T_{54} (108.3), T_{40} (109), T_{41} (110.3), T_{59} (115.3), T_{60} (117.7), T_{47} (123.3), T_{44} (128), T_{57} (130), T_{69} (131), T_{46} (131.7), T_{42} (133.3), T_{53} (134), T_{64} (140), T_{73} (146.3), T_{72} (146.7), T_{74} (146.7), T_{66} (149) and T_{37} (153.2) are non significantly differ among each other. While, control petriplates were maximum mycelial growth of *Alternaria brassicae.*

All the leaf extracts were found non significantly differ from one another excluding with *Azadirachta indica* and *Mentha peperata*, which were recorded significantly maximum%

inhibition of mycelial growth followed by other botanicals treatments. While, control petriplates were maximum no. of colonies of *X. campestris* pv. *campestris.*

Table: 4.2. Screening of botanicals at 50 % concentration on radial growth (mm) of *Alternaria brassicae* and number of colonies (CFU/ml) of *X. campestris* pv. *campestris*

Treatments	Name of the botanicals	Colony diameter (mm)		CFU ml^{-1} of 10^{-7} dilution factor	
		At 48 hrs	At 96 hrs	At 48 hrs	At 72 hrs
1.	*Zingiber officinale*	11.2	24.2	10.0	20.5
2.	*Eucalyptus globules*	10.4	23.7	8.1	20.2
3.	*Calotropis procera*	12.3	26.3	11.5	21.0
4.	*Datura stramonium*	10.4	23.2	9.0	20.3
5.	*Ocimum sanctum*	10.2	22.1	7.5	19.8
6.	*Mentha arvensis*	11.2	21.2	9.5	20.8
7.	*Azadirachta indica*	7.7	20.2	7.0	19.0
8.	*Croton boplandianum*	13.2	32.3	11.9	21.8
9.	*Argemone maxicana*	14.6	31.3	28.0	47.0
10.	*Eclipta alba*	13.3	33.3	13.5	35.0
11.	*Ficus religiosus*	14.5	35.3	24.5	39.8
12.	*Agele marmelos*	14.4	32.3	18.0	32.5
13.	*Acyranthus aspera*	13.2	37.3	13.0	32.3
14.	*Canna indica*	15.3	38.2	27.8	46.2
15.	*Cassia fistula*	14.5	33.3	26.0	39.2
16.	*Emblica officinalis*	13.3	31.2	15.0	34.0
17.	*Cannabis sativa*	16.4	39.2	37.0	57.2
18.	*Ricinus communis*	15.7	38.4	34.1	50.2
19.	*Morus alba*	16.4	35.3	40.5	69.0
20.	*Ipomea palmata*	15.6	42.3	31.5	44.7
21.	*Hibiscus*	16.4	45.3	39.0	61.5
22.	*Calendula*	17.4	41.4	60.0	93.7

23.	*Bougainvillea glabra*	13.5	39.4	15.6	33.7
24.	*Chrisanthimum coronarium*	18.2	46.1	77.8	103.3
25.	*Withania somnifera*	15.3	55.3	30.5	44.3
26.	*Chenopodium album*	14.3	65.4	16.5	38.2
27.	*Turmeric longa*	14.5	45.3	26.5	40.8
28.	*Mentha peperata*	8.5	21.1	8.0	19.8
29.	*Millettia pinnata*	14.5	33.3	26.8	43.5
30.	*Syzygium aromaticum*	14.4	35.2	18.0	36.2
31.	*Allium sativum*	11.5	26.2	10.5	21.4
32.	*Polyalthia longifolia*	16.4	36.3	39.3	63.3
33.	*Dalbarjia siso*	17.5	50.3	59.8	97.2
34.	*Carica papaya*	17.5	51.2	61.0	104.5
35.	*Brugmansia* sp.	15.6	65.3	32.5	47.5
36.	*Cuminum cyminum*	16.5	50.2	47.0	74.0
37.	*Trigonella foenum*	18.4	56.3	75.5	153.2
38.	*Cynodon dactylon*	17.4	52.9	58.0	88.0
39.	*Morraya koengi*	16.6	60.2	48.5	75.7
40.	*Ficus glomerata*	17.5	61.5	62.0	109.0
41.	*Ixora coccinea*	17.5	65.2	62.5	110.3
42.	*Vinca rosea*	18.3	71.3	76.5	133.3
43.	*Callistemon lanceolatus*	16.5	58.1	47.5	75.5
44.	*Asparagus racemosus*	17.5	62.4	64.5	128.0
45.	*Solanum nigrum*	16.7	57.2	55.0	45.5
46.	*Ageratum cozyodies*	19.4	70.2	77.5	131.7
47.	*Annona squamosa*	18.5	65.2	76.5	123.3
48.	*Euphorbia tricalli*	17.6	65.2	66.5	108.3
49.	*Acacia nilotica*	18.3	65.2	77.0	70.0
50.	*Lawsonia inermis*	19.3	70.1	77.5	90.0

51.	*Ceasalpinia pulcherrima*	18.5	65.2	77.5	91.7
52.	*Parthenium hysterophorus*	16.5	45.2	45.0	72.0
53.	*Dianthus cayophyllus*	18.6	65.4	76.5	134.0
54.	*Oxalis*	19.4	69.1	76.5	108.3
55.	*Abutilon indicum*	14.5	45.2	28.0	44.3
56.	*Mimosa pudica*	14.5	46.2	28.5	45.3
57.	*Leucas aspera*	18.2	55.2	75.0	130.0
58.	*Ziziphus jujuba*	15.4	40.3	31.0	49.2
59.	*Narcissus*	17.5	58.2	64.0	115.3
60.	*Lilium*	17.5	57.4	65.0	117.7
61.	*Ficus bengalensis*	14.4	43.2	17.5	40.7
62.	*Hamelia patens*	19.4	68.3	79.5	106.3
63.	*Amaranthus spinosus*	16.4	42.2	39.5	66.7
64.	*Moringa*	18.3	60.2	78.5	140.0
65.	*Lantana camara*	11.5	22.2	11.1	21.5
66.	*Cymbopogon citratus*	18.3	70.3	78.5	149.0
67.	*Urtica diocea*	16.3	55.4	34.5	56.0
68.	*Nerium*	16.6	54.5	50.5	79.2
69.	*Solanum xanthocarpum*	17.6	60.3	65.5	131.0
70.	*Bauhinia vareigata*	15.6	49.2	31.5	48.8
71.	*Coccinia cardifolia*	14.3	40.2	16.8	44.0
72.	*Iberis amara*	17.6	65.2	68.5	146.7
73.	*Rheumemodi*	20.6	70.2	78.5	146.3
74.	*Atemessia annua*	20.4	75.2	78.5	146.7
75.	*Convolvulus microphyllus*	14.5	40.2	29.0	49.2
Control		35.7	90	115.0	155.0
F-test		S	S	S	S

CD at 5%	0.46	0.42	1.6	13.94

4.3 Assay of qualitative secondary metabolites from the selected botanicals :

The results of qualitative phytochemical compounds of selected following botanical leaf extracts are presented in Table 4.3.

Table : 4.3 Phytochemical analysis of aqueous extracts of selected botanicals –

Botanical extracts	Alkaloid	Flavonid	Saponin	Terpenoid	Phenols/ Polyphenol	Reducing sugar	Tannin	Phlobatannins	Steroids	Carbohydrates	Amino acid	Coumarin	Protein	Gums and Mucilage	Glycosides	Anthraquinones
O. sanctum	+	-	-	-	+	-	+	-	-	-	-	+	+	+	-	+
E. globulus	-	+	-	+	+	-	+	+	-	-	-	+	+	+	-	+
L. camara	+	-	-	+	+	-	+	+		-	-	+	+	+	-	+
A. Indica	+	+	-	+	+	+	-	-	+	+	-	+	+	+	+	-
C. procera	-	-	+	-	+		+	-	-	-	+	+	+	-	+	+
Mentha peperata	+	-	+	+	-	-	-	+	-	+	-	-	-	-	+	-
A.sativum	-	+	-	+	+	-	-	-	+	-	-	+	+	+	+	+
D.stramonium	+	-	-	+	+	-	-	+	+	-	-	+	+	+	+	+
Zingiber officinale	+		-	-	+	-	+	+	-	-	-	-	-	-	-	+
Mentha arvensis	+	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-
Buchnera hispida	-	+	-	+	+	-	+	-	-	+	-	-	-	-	-	-
Syzygium aromaticum	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	-
Carica papaya	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Psidium guajava	-	+	-	-	+	-	+	-	-	+	-	-	+	-	-	-

Where is : (+) present and **(-)** absent of phytochemical

4.4 *In vitro* efficacy of botanical extracts at 25 % concentration on mycelial growth of *Alternaria brassicae* :

Data presented in Table and Fig : 4.4ab, shows that all the selected plant extracts at 25% concentration was significantly reduced the growth of *Alternaria brassicae* at 48, 72, 96 and 120 hrs after incubation.

At 48 hrs after incubation, results shows that Bordeaux mixture was significantly inhibited the mycelial growth of *Alternaria brassicae* over untreated control. The treatment T_{12} - Bordeaux mixture was significantly recorded least mean colony diameter (8.67 mm) and highest inhibition (58.66 %) of mycelial growth as compared with T_7 – *Azadirachta indica* (10.07), T_8 – *Eucalyptus globules* (12.10), T_3 – *Mentha peperata* (13.80), T_{10} – *Mentha arvensis* (14.10), T_1 – *Ocimum sanctum* (15.65), T_6 – *Datura stramonium* (15.85), T_9 – *Zingiber officinale* (17.02), T_4 – *Allium sativum* (18.22), T_5 – *Lantana camara* (18.35), T_{11} – *Croton boplandianum* (18.45) and T_2 – *Calotropis procera* (18.65) including with control (20.97). Among plant extracts, the treatments *Azadirachta indica* and *Eucalyptus globules* were recorded significantly maximum % inhibition of mycelial growth followed by other botanicals treatments. Whereas, treatments (T_3 and T_{10}), (T_1 and T_6) and (T_4, T_5, T_{11}, T_2) were found non-significant among themselves but they are significant from each other. While, control petri plates were maximum mycelial growth of *A. brassicae*.

After 72 hrs, results shows that the significantly minimum growth (12.07) and maximum per cent inhibition (74.30) was found in the treated petriplates with Bordeaux mixture followed by all the botanical treatments. Among all the botanicals, neem leaf extract was observed significant highest inhibition of growth (10.07) as compared with T_8, T_3, T_{10}, T_1, T_6, T_9 and T_4. These botanicals are significant in results from T_5, T_{11} and T_2. Whereas, treatments (T_5, T_{11}) and (T_{11}, T_2) were found non-significant among themselves.

At 96 hrs after incubation of *Alternaria brassicae* was significantly inhibited the growth in the Bordeaux mixture treatment (20.65 mm) followed by all other botanical leaf extracts. Aqueous leaf extract of neem was showed minimum mycelium growth and maximum inhibition as compared with rest botanical treatments. Among the neem (22.87), mint (22.92) and menthe (23.82) significantly reduced the growth of *Alternaria brassicae* from other leaf extracts. Whereas, treatment (T_7, T_3, T_{10}), (T_1, T_9) and (T_{11}, T_2) were shows non-significant among themselves but they are significant to each other. While, treatments T_8, T_6, T_4 and T_5 were found significant from each other.

At 120 hrs after incubation, Bordeaux mixture (22.85 mm) and neem (23.72) were showed least mycelium growth of *Alternaria brassicae* followed by all the botanicals including with over control (90.00). However, treatments (T_{12}, T_7), (T_7, T_3), (T_8, T_{10}) and (T_1, T_9) were found non-significant among themselves but they are significant to each other. Among all the selected botanicals were showed in the least growth of mycelium and maximum in growth inhibition per

	Mycelial radial growth (mm)

cent of tested pathogen.

Table : 4.4 *In vitro* efficacy of botanical extracts at 25 % concentration on mycelial growth of *Alternaria brassicae* at different hrs of interval

	48 hrs	**% Inhibition over control**	**72 hrs**	**% Inhibition over control**	**96 hrs**	**% Inhibition over control**	**120 hrs**	**% Inhibition over control**
T_1 (*Ocimum sanctum*)	15.65d	25.37	26.82g	42.89	34.62 e	52.68	37.80f	58.00
T_2 (*Calotropis procera*)	18.65b	11.06	37.15b	20.91	50.40b	31.12	64.85b	27.94
T_3 (*Mentha peperata*)	13.80e	34.19	20.67i	55.99	22.92h	68.63	25.07i	72.14
T_4 (*Allium sativum*)	18.22b	13.11	33.55d	28.57	42.40d	42.06	56.72e	36.97
T_5 (*Lantana camara*)	18.35b	12.49	35.90c	23.57	44.22c	39.57	59.22d	34.20
T_6 (*Datura stramonium*)	15.85d	24.42	27.75f	40.92	31.20f	57.36	32.95g	63.39
T_7 (*Azadirachta indica*)	10.07g	51.97	13.82k	70.58	22.87h	68.74	23.72 ij	73.64
T_8 (*Eucalyptus globules*)	12.10f	42.29	17.10 j	63.59	25.27g	65.60	27.05h	69.94
T_9 (*Zingiber officinale*)	17.02c	18.83	32.20e	31.45	34.67e	52.62	38.07 f	57.70
T_{10} (*Mentha arvensis*)	14.10e	32.76	23.82 h	49.29	23.82h	67.45	28.42 h	68.42
T_{11} (*Croton boplandianum*)	18.45b	12.02	36.57bc	22.14	49.75b	32.00	62.30c	30.78
T_{12} (Bordeaux mixture)	8.67h	58.66	12.07 l	74.30	20.65i	71.77	22.85 j	74.61
T_0 (Control)	20.97a	-	46.97a	-	73.17a	-	90.00a	-
F-test	S		S		S		S	
S. Ed (±)	0.34		0.44		0.64		0.94	
CD (P = 0.05)	0.69		0.89		1.29		1.89	

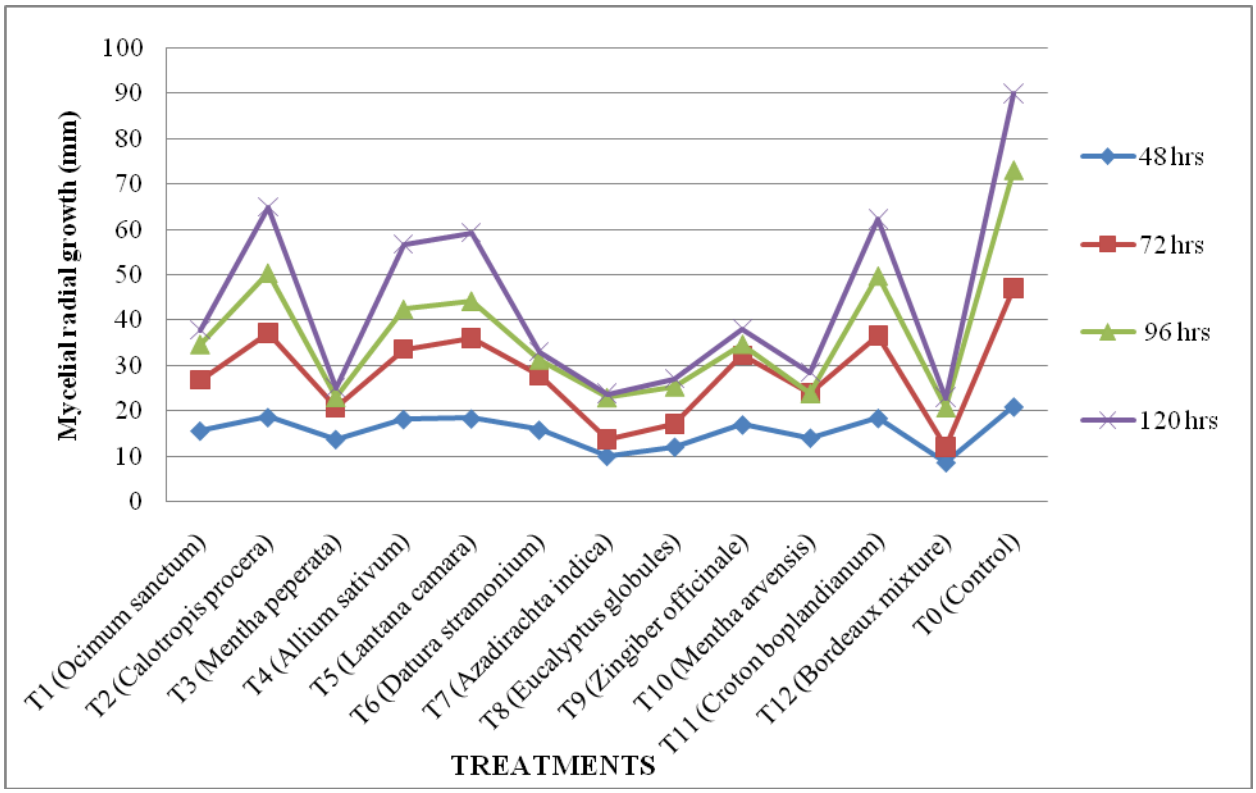


Figure : 4.4a. *In vitro* efficacy of botanical extracts at 25 % concentration on mycelial growth of *Alternaria brassicae* at different hrs of interval.

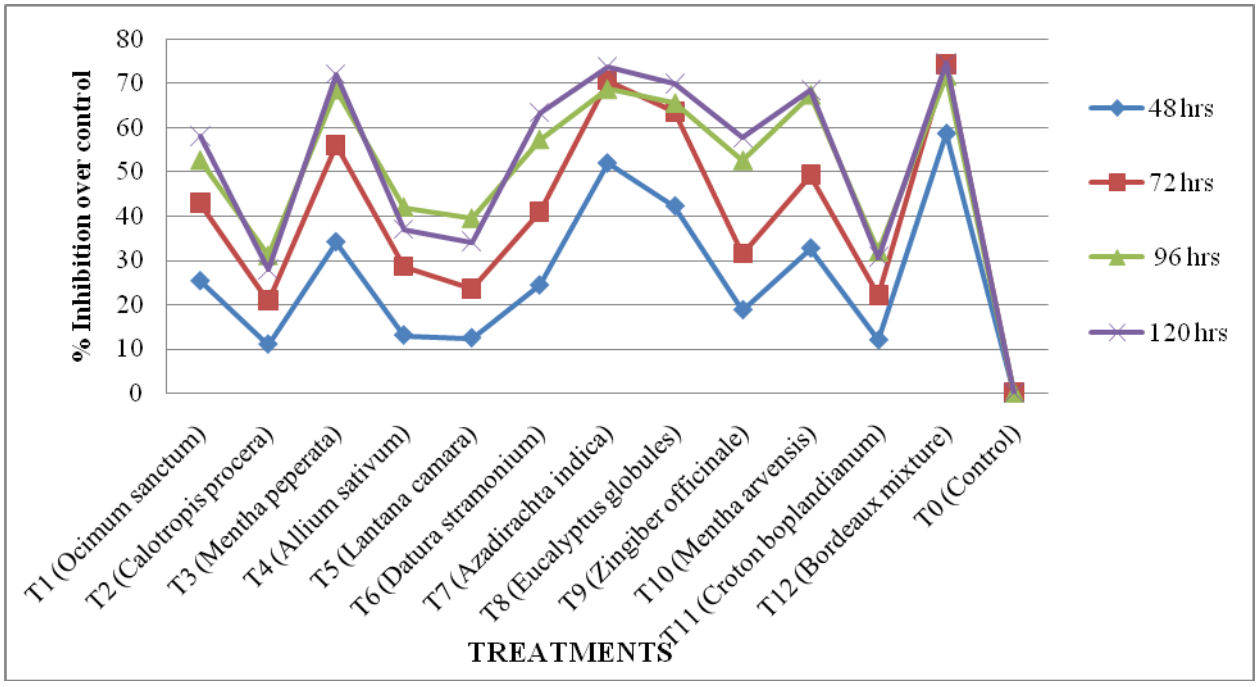
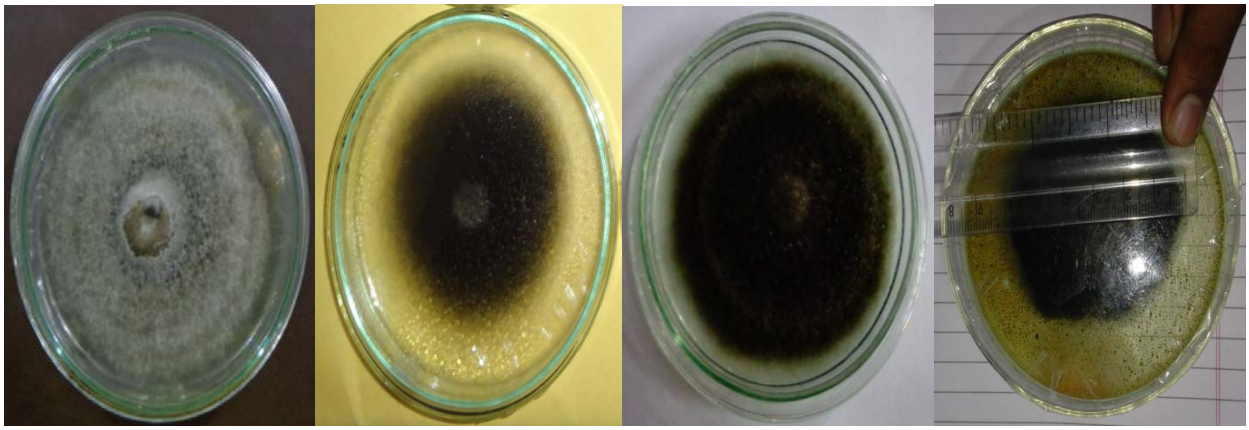
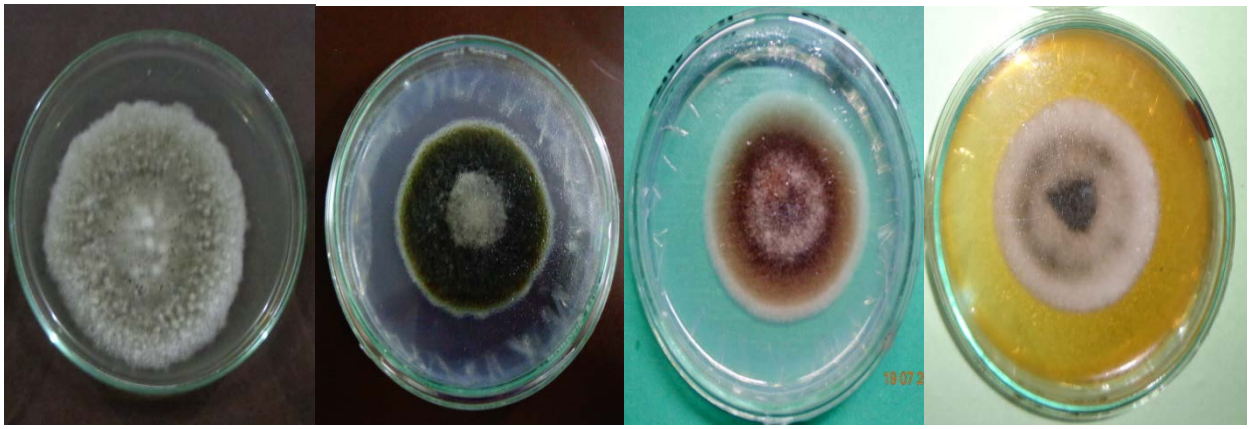


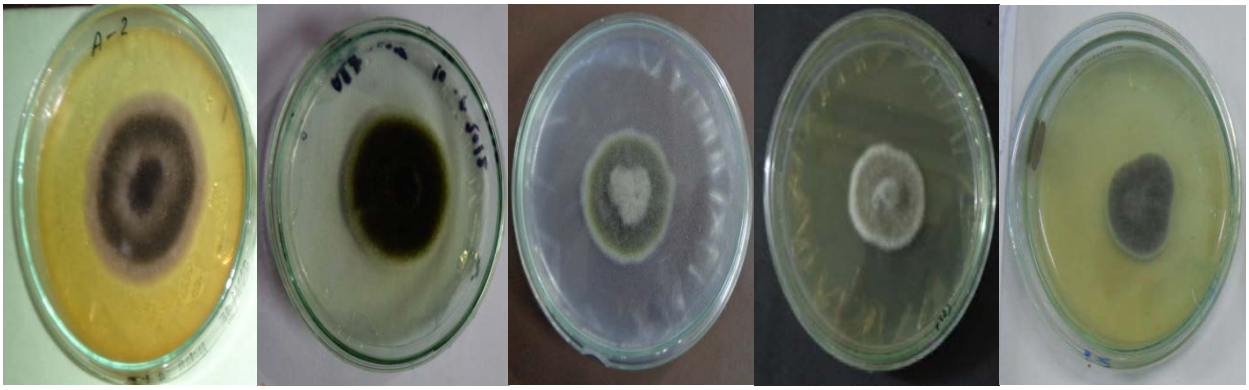
Figure : 4.4b. *In vitro* efficacy of botanical extracts at 25 % concentration on inhibition % over control of *Alternaria brassicae* at different hrs of interval.



T 13 **T 2** **T 11** **T 5**



T 4 **T 9** **T 1** **T 6**



T 10 **T 8** **T 3** **T 7** **T 12**

Plate: 26. *In vitro* efficacy of botanical extracts at 25 % concentration on mycelial growth of *Alternaria brassicae* at 120 hrs after incubation.

4.5 *In vitro* efficacy of botanical extracts at 50 % concentration on mycelial growth of *Alternaria brassicae* :

Data are presented in the Table : 4.5 and depicted in Fig : 4.5ab, results shows that all the aqueous plant extracts at 50% concentration was significantly reduced the growth of *Alternaria brassicae* at 48, 72, 96 and 120 hrs after incubation.

After 48 hrs of incubation, the botanical was noticed significantly reduced the colony growth and maximum inhibition of mycelial growth of *Alternaria brassicae* over untreated control. The treatment T_7 –*Azadirachta indica* was significantly recorded least mean colony diameter (7.70 mm) as compared with other plant extracts excluding Bordeaux mixture. The highest per cent inhibition (56.67) was found in *Azadirachta indica* followed by T_3 –*Mentha peperata* (49.63), T_1 –*Ocimum sanctum* (46.26), T_8 –*Eucalyptus globules* (46.26), T_6 –*Datura stramonium* (38.82), T_{10} –*Mentha arvensis* (38.27), T_9 –*Zingiber officinale* (37.25), T_4 –*Allium sativum* (33.87), T_5 –*Lantana camara* (29.65), T_2 –*Calotropis procera* (29.26) and T_{11} –*Croton boplandianum* (26.56) including with T_0 - control (00.0). Whereas, the treatments T_7 and T_{12} were recorded non-significant among themselves but they are significant from rest all other treatments. However, treatments (T_3, T_1, T_8), (T_6, T_{10}, T_9), (T_{10}, T_9, T_4), (T_4, T_5) and (T_5, T_2, T_4) were observed non-significant among themselves but they are significant to ach other. While control petri plates were maximum mycelial growth of *A. brassicae*.

At 72 hrs, results shows that the minimum radial growth (10.45mm) and maximum per cent inhibition (77.71) was found in the treatment T_7- *A. indica* followed by T_8 - *Eucalyptus globules* (11.00), Bordeaux mixture and all the rest botanical treatments including with control. Whereas, the treatments T_7, T_8 and T_{12} were found non-significant among themselves but they are significant from T_{10}, T_3, T_1, T_6, T_2 and T_9. However, treatments (T_{10}, T_3, T_1, T_6) and (T_2, T_9) were found non-significant among themselves but they are showed significant to each other. While treatments T_4, T_5 and T_{11} were found significantly superior over rest of the botanical extracts.

At 96 hrs after incubation, the radial growth of *A. brassicae* was inhibited in the Bordeaux mixture (14.30 mm) and neem leaf extract (15.30mm) followed by rest of the botanical leaf extracts. Among botanical extracts, neem was showed minimum mycelium growth and

maximum inhibition as compared with all other botanical treatments. Whereas, treatment T_7, T_{10}, T_1 and T_3 were shows non-significant among themselves but they are significant from T_8, T_2, T_6, T_9, T_4, T_5 and T_{11}. However, treatments T_9, T_4, T_5 and T_{11} were found significantly differ from each other.

At 120 hrs after incubation of *A. brassicae* was reduced the mycelium growth in the chemical treatment (18.82 mm) and neem leaf extract (19.25mm) followed by all other botanical leaf extracts including with control. Among botanical extracts, neem was showed minimum mycelium growth and highest per cent inhibition as compared with all other botanical treatments. Whereas, treatments T_8, T_3, T_1 and T_{10} were shows no significant effect on radial growth but they are significant from T_2 and T_6. However, treatments T_9, T_4, T_5 and T_{11} were found significantly difference to each other.

Table : 4.5 *In vitro* efficacy of botanical extracts at 50 % concentration on mycelial growth of *Alternaria brassicae* at different hrs of interval

Treatments	Mycelial radial growth (mm)							
	48 hrs	% Inhibition over control	72 hrs	% Inhibition over control	96 hrs	% Inhibition over control	120 hrs	% Inhibition over control
T_1 (*Ocimum sanctum*)	9.55 f	46.26	12.40f	73.56	16.37gh	78.11	21.00g	76.67
T_2 (*Calotropis procera*)	12.57 b	29.26	14.82e	68.40	18.75f	74.92	23.25f	74.17
T_3 (*Mentha peperata*)	8.95f	49.63	12.22fg	73.94	16.37gh	78.10	20.87g	76.81
T_4 (*Allium sativum*)	11.75cd	33.87	16.62d	64.56	24.27d	67.54	29.32d	67.42
T_5 (*Lantana camara*)	12.50bc	29.65	19.22c	59.01	26.22c	64.93	31.17c	65.37
T_6 (*Datura stramonium*)	10.87e	38.82	12.60f	73.13	18.75f	74.92	23.32f	74.08
T_7 (*Azadirachta indica*)	7.70g	56.67	10.45h	77.71	15.30hi	79.54	19.25h	78.61
T_8 (*Eucalyptus globules*)	9.55f	46.26	11.00h	76.55	16.92g	77.37	20.60g	77.11
T_9 (*Zingiber officinale*)	11.15de	37.25	14.90e	68.23	22.85e	69.43	26.00e	71.11
T_{10} (*Mentha arvensis*)	10.97de	38.27	12.22fg	73.95	15.70h	79.00	21.12g	76.53
T_{11} (*Croton boplandianum*)	13.05b	26.56	21.02b	55.18	31.27b	58.18	38.35b	57.39
T_{12} (Bordeaux mixture)	8.05g	54.69	11.15gh	76.23	14.30i	80.87	18.82h	79.08
T_0 (Control)	17.77a	-	46.90a	-	74.77a	-	90.00a	-
F-test	S		S		S		S	
S. Ed (±)	0.39		0.55		0.53		0.42	
CD (P = 0.05)	0.78		1.10		1.07		0.85	

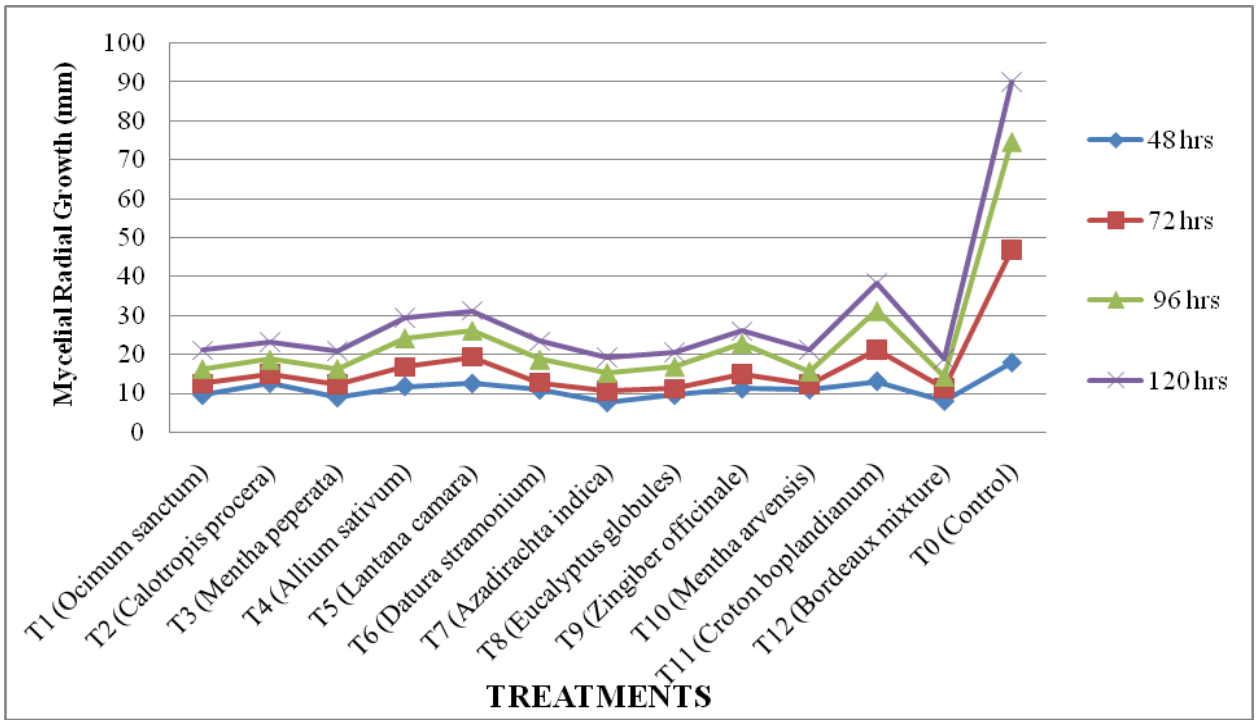


Figure : 4.5a. Effect of selected botanical extracts at 50 % concentration on mycelial radial growth of *Alternaria brassicae* at different hrs of interval.

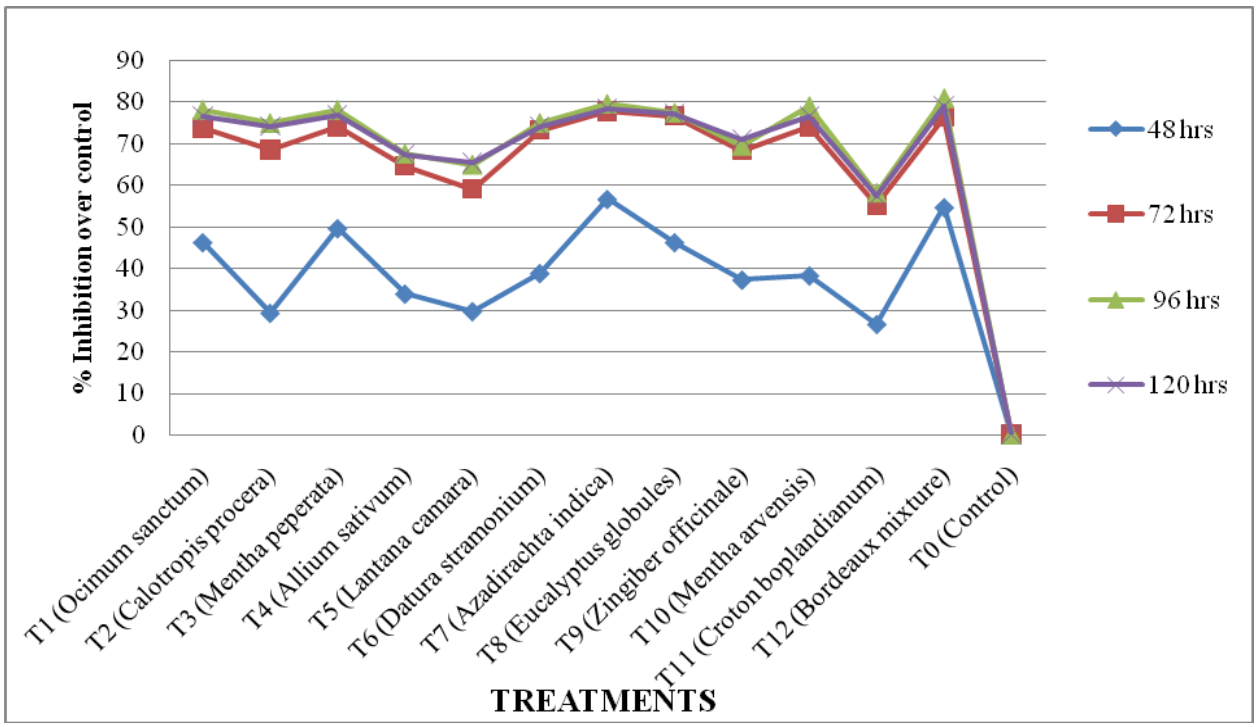
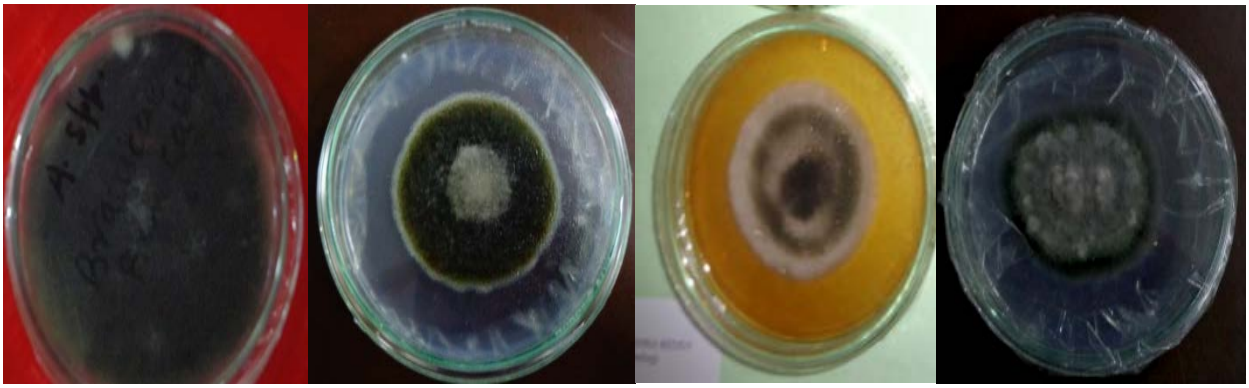
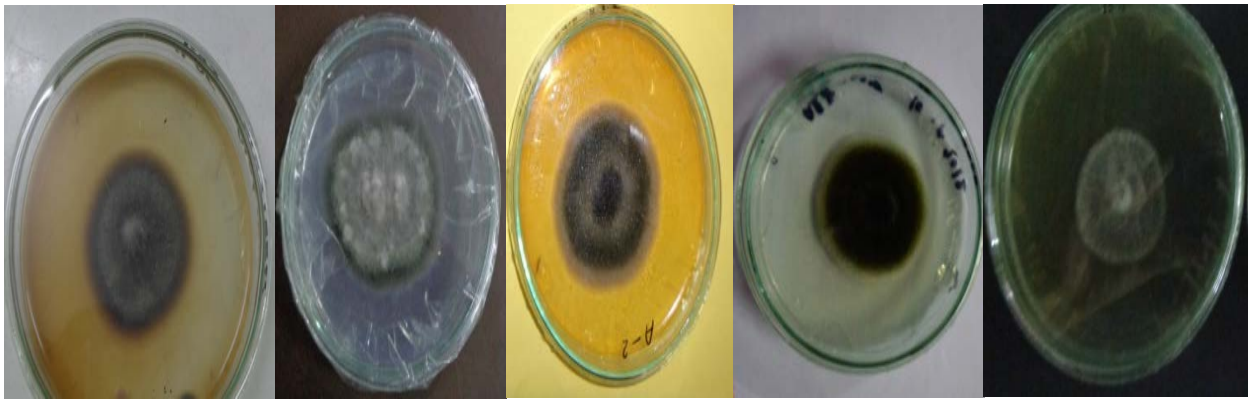


Figure : 4.5b. Effect of 50 % selected botanical extracts at 50 % concentration on inhibition % over control of *Alternaria brassicae* at different hrs of interval.



T $_{13}$ **T $_{11}$** **T $_{5}$** **T $_{4}$**



T $_{9}$ **T $_{6}$** **T $_{2}$** **T $_{10}$** **T $_{1}$**

T $_{3}$ **T $_{8}$** **T $_{7}$** **T $_{12}$**

Plate: 27. *In vitro* efficacy of botanical extracts at 50 % concentration on mycelial growth of *Alternaria brassicae* at 120 hrs after incubation.

4.6 Effect of botanical extracts at 75 % concentration on radial growth of *Alternaria brassicae* :

The results of Table and Fig : 4.6ab, revealed that all the aqueous plant extracts at 75% concentration was significantly reduced the mycelial radial growth of *A. brassicae* in the treatment of Bordeaux mixture at 48, 72, 96 and 120 hrs after incubation.

At 48 hrs after incubation, the radial growth (mm) of *Alternaria brassicae* was significantly reduced in the treatment T_7 –*Azadirachta indica* (8.30) as compared with rest botanical extracts including over untreated control. However, treatments T_8 –*Eucalyptus globules* (10.35), T_3 – *Mentha peperata* (11.70), T_{10} –*Mentha arvensis* (12.62), T_6 –*Datura stramonium* (13.47) were significantly superior from T_1 –*Ocimum sanctum* (14.27), T_9 –*Zingiber officinales* (14.77), T_4 – *Allium sativum* (15.32), T_2 – *Calotropis procera* (15.65), T_{11} –*Croton boplandianum* (15.77) and T_5 –*Lantana camara* (16.30) including with control (22.07). Whereas, all the botanical treatments were observed significantly maximum inhibition per cent excepting (T_1, T_9), (T_9, T_4) and (T_4, T_2, T_{11}). While, they are non-significant among themselves. Maximum growth of *A. brassicae* was observed in the un-treated petriplates.

After 72 hrs, the minimum colony growth was significantly shows in the leaf extracts of neem (10.57mm) and eucalyptus (12.35) followed by all the botanical extracts including with control. However, treatments T_3 and T_{10} were found non significant among themselves but they are significant from T_6, T_1, T_9, T_4, T_{11}, T_5, T_2 and T_0. Whereas, treatment T_9 was found significant from T_4, T_{11}, T_5 and T_2. While, the treatments T_4, T_{11}, T_5 and T_2 were not significant among themselves.

At 96 hrs after incubation, the mycelium growth (mm) of *A. brassicae* was significantly reduced in the treatment T_7 –*Azadirachta indica* (11.37) and T_8 –*Eucalyptus globules* (10.35) followed by all other botanical extracts including over untreated control (71.15). However, the treatments T_{10} - *Mentha arvensis* (15.10) and T_3 - *Mentha peperata* (15.30) were also recorded least colony growth as comparison from T_1, T_6, T_9, T_4, T_2, T_5 and T_{11}. Whereas, treatments (T_1, T_6), (T_6, T_9), (T_9, T_4) and (T_4, T_2, T_5, T_{11}) were found not significant among themselves.

At 120 hrs after incubation of *A. brassicae*, the least mean colony growth (mm) was recorded in the neem leaf extract (13.32) as compared with rest botanical leaf extracts excluding with Bordeaux mixture. Neem leaf extract and Bordeaux mixture were found non-significant in the mycelial growth of *A. brassicae*. It was observed highest per cent inhibition of *A. brassicae* as compared with all other aqueous leaf extracts including control. Whereas, treatment T8 was showed significant from T_{10}, T_3, T_1, T_9, T_6, T_4, T_2, T_5 and T_{11}. While, the treatments (T_{10}, T_3), (T_1, T_9, T_6), (T_2, T_5, T_{11}) and (T_5, T_{11}) were found no significant in results but those are significant to each other.

One of the most effective measures to control the disease caused by *Alternaria* is the effective application of fungicides. The arbitrary usage of the common fungicides has made the man and wild life susceptible to a wide array of diseases. The use of various herbal extracts and natural products is being encouraged because these cause no health hazard or pollution. The natural plant products are bio-degradable and thus eco-friendly, are catching the concentration of the scientists worldwide.

Our results showed that leaf extracts of *Azadirachta indica* and *Eucalyptus globules* are found effective in the management of leaf spot disease. Similar finding results have reported by **Sharma *et al.* (2007).** That is why, because plants contain various kinds of phytochemicals like, Saponins, Alkaloids, Flavanoids *etc.,* (commonly called secondary metabolites) are bringing the antimicrobial effects. In the current study it is well proved that all most plants used in the study have at least little effect in controlling the fungus. Yet, it is clearly shown that except neem and eucalyptus no other extracts have the similar effect of chemical fungicide (positive controls).

This finding is also supported by **Hassanein *et al.* (2008)** reported that the neem leaf extract showed maximum inhibition percentages were 17.88%, 23.66, 52.77 % and 70.55% for *A. solani* at 5%, 10%, 15% and 20 % concentrations, while those for *F. oxysporum* were 14.77 %, 23.88%, 31.22 % and 100%, respectively.

Sheikh and Agnihotri (1972) also reported that the extracts of *Canna indica*, *Convolvulus arvensis, Ipomoea palmata*, *Cenchrus catharticus*, *Mentha piperita*, *Prosopsis spicigera*, *Allium cepa*, *A. sativum*, *Lawsonia inermis*, *Argemone mexicana*, *Datura stramonium* and *Clerodendron inerme* completely inhibited the spore germination of *A. brassicae* isolated from leaves of cauliflower.

The diameters of inhibition zones increased with the increase of concentration levels of plant extracts. Similar antifungal properties of *Mentha piperita* L., *Allium cepa* L., *A. sativum* L. and *Datura stramonium* have been reported by **Sheikh and Agnihotri (1972),** these extracts completely inhibited the spore germination of *A. brassicae* isolated from leaves of cauliflower. **Yasmeen & Saxena (1990)** also observed that the extracts prepared from the leaves of *Mentha piperita* had fungitoxic activities against *A. brassicae* isolated from cauliflower leaves.

Table : 4.6 *In vitro* efficacy of botanical extracts at 75 % concentration on mycelial growth of *Alternaria brassicae* at different hrs of interval

Treatments	Mycelial radial growth (mm)							
	48 hrs	**% Inhibition over control**	**72 hrs**	**% Inhibition over control**	**96 hrs**	**% Inhibition over control**	**120 hrs**	**% Inhibition over control**
T_1 (*Ocimum sanctum*)	14.27e	35.34	16.20 d	65.42	16.70e	76.52	17.47e	80.58
T_2 (*Calotropis procera*)	15.65bc	29.08	18.65b	60.19	19.40b	72.73	20.20c	77.56
T_3 (*Mentha peperata*)	11.70h	46.98	13.80e	70.54	15.30f	78.49	16.45f	81.72
T_4 (*Allium sativum*)	15.32cd	30.58	18.22b	61.10	19.12bc	73.12	19.10d	78.77
T_5 (*Lantana camara*)	16.30b	26.14	18.52b	60.47	19.62b	72.42	20.97bc	76.70
T_6 (*Datura stramonium*)	13.47f	38.72	16.10d	65.64	17.02de	76.07	18.10e	79.88
T_7 (*Azadirachta indica*)	8.30j	62.39	10.57g	77.44	11.37h	84.02	13.32h	85.20
T_8 (*Eucalyptus globules*)	10.35i	53.10	12.35f	73.64	13.32g	81.27	15.12g	83.20
T_9 (*Zingiber officinale*)	14.77de	33.07	17.02c	63.67	18.02cd	74.67	18.02e	79.97
T_{10} (*Mentha arvensis*)	12.62g	42.81	14.10e	69.90	15.10f	78.77	16.35f	81.83
T_{11} (*Croton boplandianum*)	15.77bc	28.54	18.45b	60.61	19.70b	72.31	21.70b	75.89
T_{12} (Bordeaux mixture)	7.20k	67.37	8.92h	80.72	9.92i	86.06	12.42h	86.20
T_0 (Control)	22.07a	-	46.85a	-	71.15a	-	90.00a	-
F-test	S		S		S		S	
S. Ed (±)	0.39		0.34		0.58		0.48	
CD (P = 0.05)	0.76		0.68		1.16		0.72	

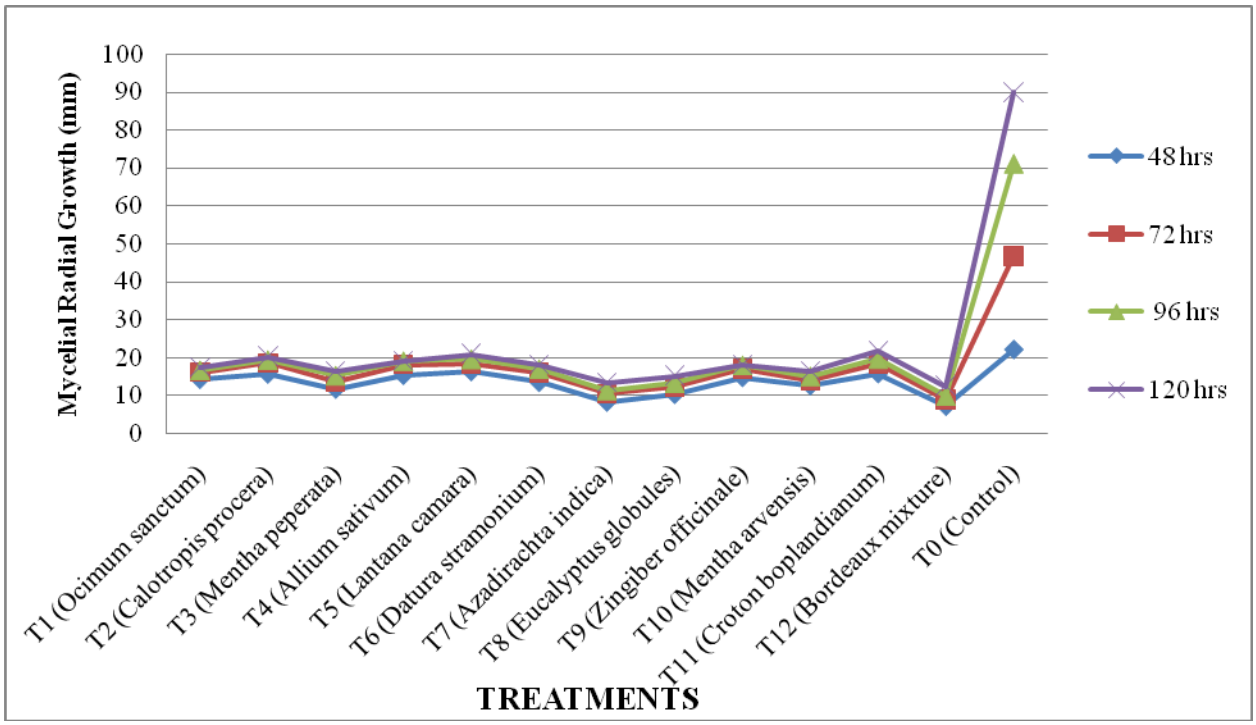


Figure : 4.6a Effect of selected botanical extracts at 75 % concentration on mycelial radial growth of *Alternaria brassicae* at different hrs of interval.

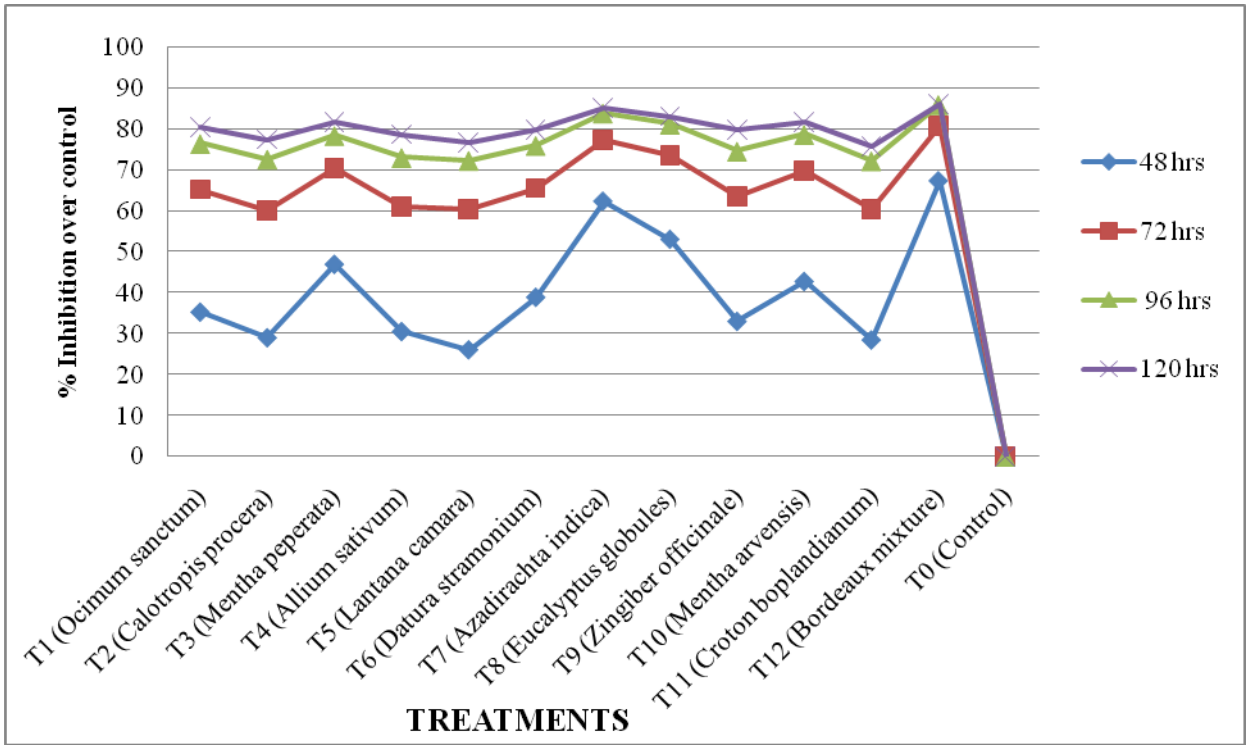
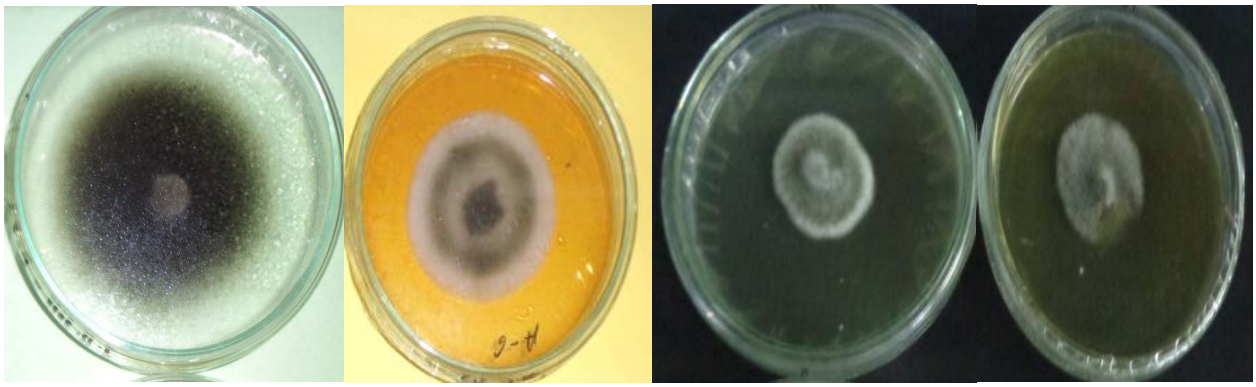
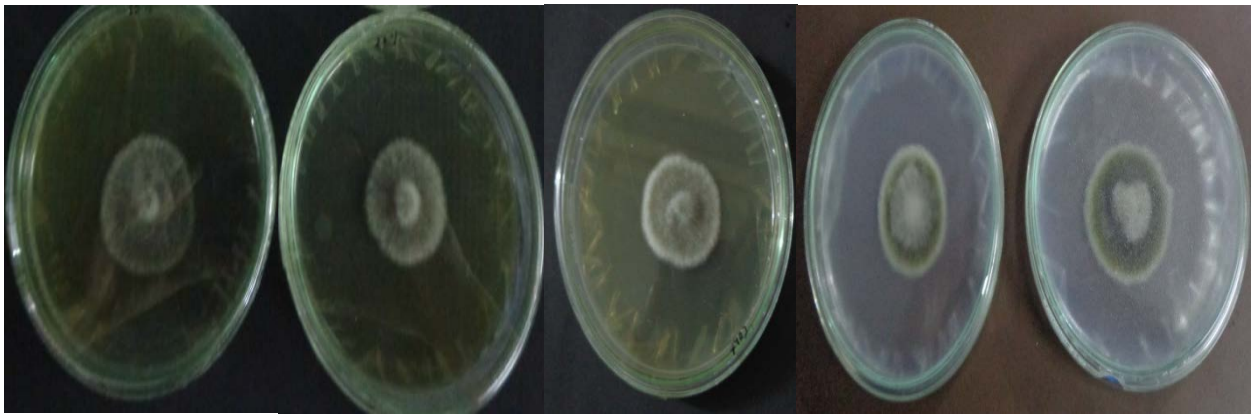


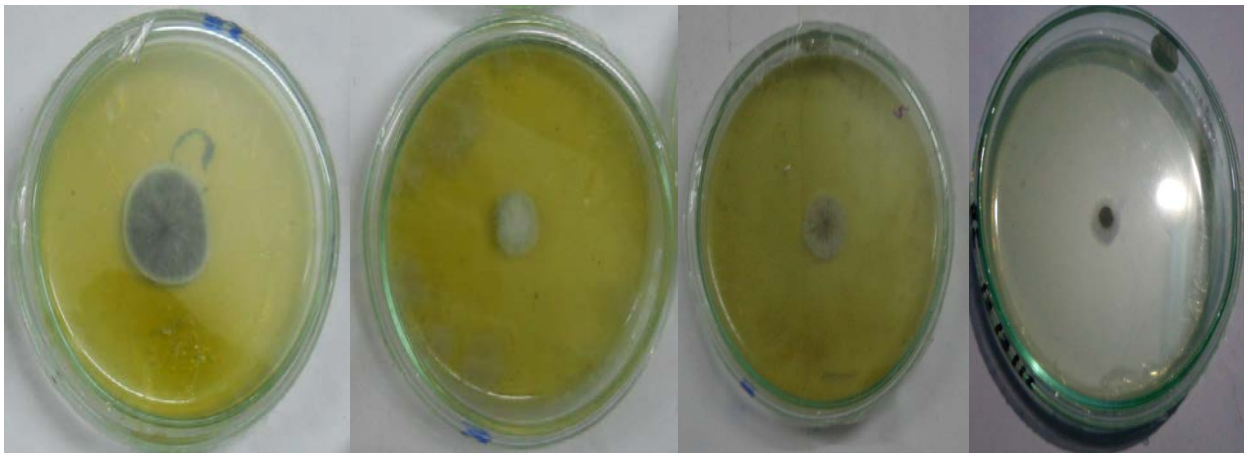
Figure : 4.6b. Effect of selected botanical extracts at 75 % concentration on % inhibition over control of *Alternaria brassicae* at different hrs of interval.



T 13 **T 11** **T 5** **T 2**



T 4 **T 6** **T 9** **T 1** **T 3**



T 10 **T 8** **T 7** **T 12**

Plate: 28. ***In vitro*** **efficacy of botanical extracts at 75 % concentration on mycelial growth of** ***Alternaria brassicae*** **at 120 hrs after incubation.**

4.7 *In vitro* efficacy of essential oils at 2% concentration on the radial growth and inhibition % of *Alternaria brassicae* :

The results Table and Fig : 4.7ab shows that *Azadirachta indica* treatment was significantly reduced the radial growth of *A. brassicae* as comparison with Bordeaux mixture at 48 hrs after incubation. Whereas, at 72 and 96 hrs after incubation the growth of *A. brassicae* was significantly reduced in Bordeaux mixture as compared with *Azadirachta indica.* At 120 hrs after incubation the growth of *A. brassicae* reduced in *Azadirachta indica* and Bordeaux mixture were found non significant among each other.

At 48 hrs, among the essential treatments significantly reduced the colony growth (6.25 mm) and inhibition % (64.78) was found in neem oil followed by T_{10} – *Mentha peperata* (8.0mm, 54.8%), T_1 – *Eucalyptus globules* (8.9mm, 49.5%), T_{11} –*Mentha arvensis* (10.5mm, 40.8%), T_4 – *Buchnera hispida* (11.6mm, 34.6%), T_3 –*Lantana camera* (12.0mm, 32.3%), T_{12} –*Ocimum sanctum* (12.8mm, 27.4%), T_5 –*Syzygium aromaticum* (14.6mm, 17.6%), T_7 – *Allium sativum* (15.0mm, 15.4%), T_6 –*Carica papaya* (16.5mm, 6.6%), T_8 –*Zingiber officinale* (16.6mm, 6.3%) and T_9 – *Psidium guajava* (17.0mm, 4.1%) including with control. Whereas, the treatments (T_4, T_3), (T_5, T_7) and (T_6, T_8, T_9) are not significant from each other.

At 72 hrs of incubation *A. brassicae* in essential oil it was observed that T_7 – *Allium sativum* (37.47mm) and T_6 –*Carica papaya* (38.30mm) are not significantly differ from each other, rest of the treatments are significantly differ from one another. Whereas, the least reduction of the *A. brassicae* growth was found in T_9 – *Psidium guajava* (41.22) and T_9 – *Psidium guajava* (41.22), but they are significantly reduced from control (47.15).

At 96 and 120 hrs of incubation the treatments T_{11} –*Mentha arvensis* (19.90mm), T_1 – *Eucalyptus globules* (20.27mm), T_{12} –*Ocimum sanctum* (25.15mm) and T_4 –*Buchnera hispida* (25.85) were found insignificant from each other at 96 hrs after incubated but 120 hrs after, the growth of *A. brassicae* was non significantly reduced in the treatment T_3 –*Lantana camera* (28.45mm) and T_{12} –*Ocimum sanctum* (28.75mm). Further, the remaining treatments are significant from one another. While, the least reduction of *A. brassicae* was also found in T_8 and T_9. The percentage inhibition of *A. brassicae* was increased in T_{13}- Bordeaux mixture (81) and T_2 – *Azadirachta indica* (80) as the incubation period from 48 hrs to 120 hrs as compared with other essential oil treatments.

Table : 4.7 *In vitro* efficacy of essential oils at 2 % concentration on the radial growth and per cent inhibition of *Alternaria brassicae* at different hrs of interval

Treatments	Mycelial radial growth (mm)							
	48 hrs	**% Inhibition**	**72 hrs**	**% Inhibition**	**96 hrs**	**% Inhibition**	**120 hrs**	**% Inhibition**
T_1 *(Eucalyptus globules)*	8.95g	49.58	18.67i	60.40	20.27i	71.72	21.77j	75.81
T_2 (*Azadirachta indica*)	6.25i	64.78	13.12l	72.17	15.27k	78.87	18.00 l	80.00
T_3 (*Lantana camera*)	12.00e	32.39	26.67f	43.44	27.72g	61.66	28.45h	68.39
T_4 (*Buchnera hispida*)	11.60e	34.65	24.17g	48.74	25.85h	64.25	30.15g	66.50
T_5 (*Syzygium aromaticum*)	14.62c	17.63	34.60e	26.61	35.70f	50.62	36.10f	59.89
T_6 (*Carica papaya*)	16.57b	6.65	38.30d	18.77	42.07d	41.81	42.92d	52.31
T_7 (*Allium sativum*)	15.00c	15.49	37.47d	20.53	39.12e	45.89	39.15e	56.50
T_8 (*Zingiber officinale*)	16.62b	6.37	42.22b	10.46	45.17c	37.52	48.02c	46.64
T_9 (*Psidium guajava*)	17.02b	4.11	41.22c	12.57	48.82b	32.48	50.80b	43.56
T_{10} (*Mentha peperata*)	8.02h	54.82	15.20k	67.76	17.30j	76.07	19.00k	78.89
T_{11} (*Mentha arvensis*)	10.50f	40.85	16.17j	65.70	19.90i	72.48	23.35i	74.06
T_{12} (*Ocimum sanctum*)	12.87d	27.49	21.62h	54.15	25.15h	65.21	28.75h	68.06
T_{13} (Bordeaux mixture)	6.42i	63.83	11.72m	75.14	13.57 l	81.23	17.10 l	81.00
T_0 (Control)	17.75a	-	47.15a	-	72.30 a	-	90.00a	-
F-test	S		S		S		S	
S. Ed (±)	0.26		0.48		0.51		0.48	
CD (P = 0.05)	0.50		0.95		1.00		0.94	

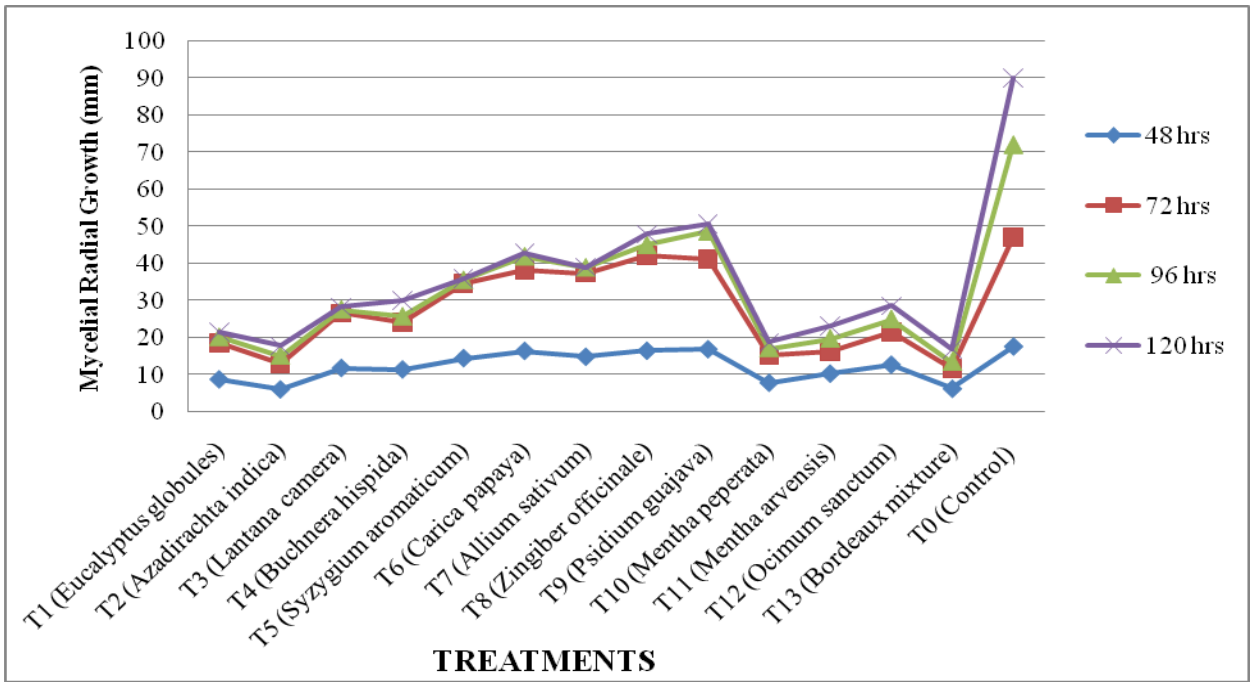


Figure : 4.7a Effect of essential oils at 2 % concentration on mycelial radial growth of *Alternaria brassicae* at different hrs of interval.

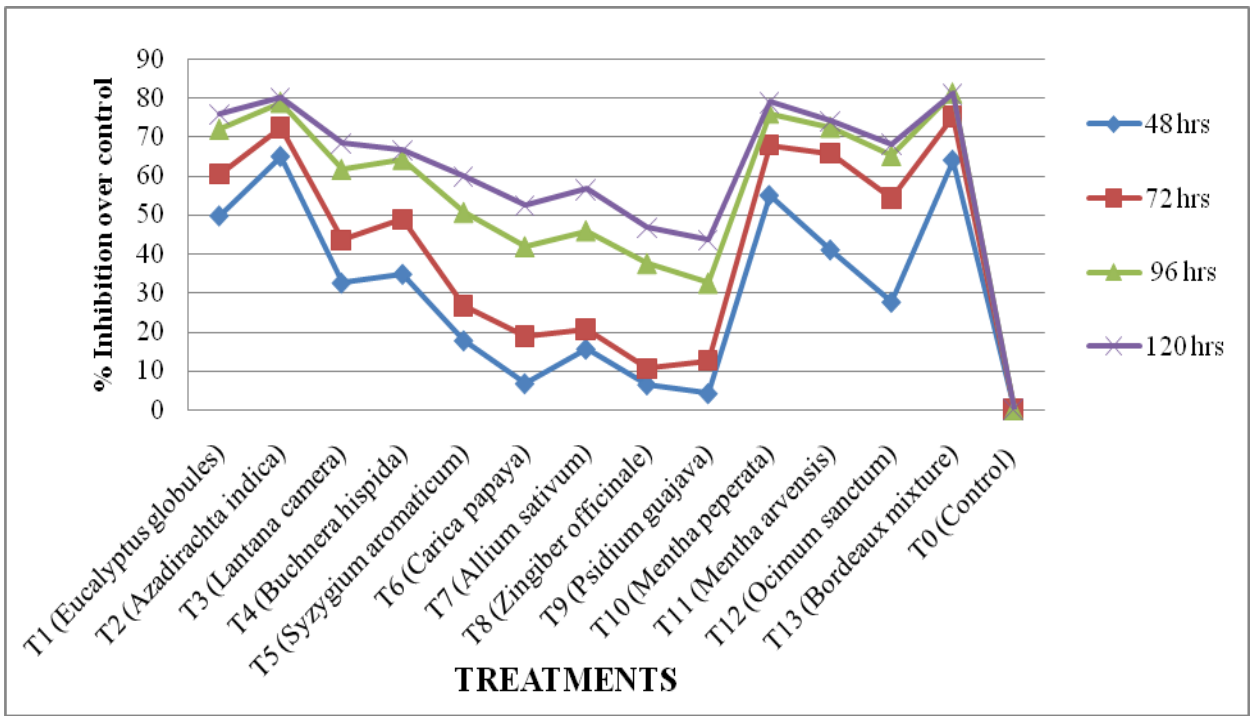


Figure : 4.7b Effect of essential oils at 2 % concentration on % inhibition over control of *Alternaria brassicae* at different hrs of interval.

4.8 *In vitro* efficacy of essential oils at 4% concentration on the radial growth and inhibition % of *A. brassicae* :

Data recorded in Table : 4.8 and Fig : 4.8ab, showed the antifungal effect of essential oils at 4% concentration on the mycelial growth (mm) of *A. brassicae* was non significantly reduced in the treatments of T_{13} – Bordeaux mixture (6.52, 8.42, 10.47, 12.02) and T_2 - *Azadirachta indica* (7.22, 8.67, 10.57, 12.70) as compared with rest essential oils including with control at 48, 72, 96 and 120 hrs after incubation, respectively. All the essential oil treated plats significantly reduced the radial growth of *A. brassicae* over control.

At 4 % concentration of essential oils, it was recorded that after 48 hrs of incubation the maximum reduction of mycelial growth was found in *Azadirachta indica* (7.22) followed by T_{10} –*Mentha peperata* (7.87), T_1 – *Eucalyptus globules* (8.40), T_{11} – *Mentha arvensis* (8.52), T_{12} – *Ocimum sanctum* (8.55), T_3 – *Lantana camara* (9.95), T_4 – *Buchnera hispida* (10.37), T_5 – *Syzygium aromaticum* (11.12), T_7 – *Allium sativum* (13.75), T_8 – *Zingiber officinale* (15.60), T_6 – *Carica papaya* (15.75) and T_9-*Psidium guajava* (16.35) including with control (18.75). However, the treatments (T_{10}, T_1, T_{11}, T_{12}) are non significant among each other but they are significant from rest of essential oils. The treatments (T_3, T_4), (T_4, T_5) and (T_8, T_6) were found no significant differences among themselves but they are differ to each other.

At 72 hrs after incubation of *A. brassicae*, it was recorded non significantly reduced the radial growth (mm) in the treatment T_2 - *Azadirachta indica* (8.67) and T_1 – *Eucalyptus globules* (9.72) as compared rest of essential oils excluding Bordeaux mixture. However, treatments T_1 – *Eucalyptus globules* (8.40) and T_{10} –*Mentha peperata* (7.87) were insignificant difference to each other but are significant from T_{11} (11.12), T_{12} (12.25), T_3 (12.75), T_4 (13.60), T_5 (14.02), T_7 (15.17), T_6 (19.15), T_8 (19.92) and T_9 (22.80) including with control (47.15). Among the essential oil treatments (T_{12}, T_3), (T_3, T_4), (T_4, T_5) and (T_6, T_8) were found no significant differences between among themselves but they are difference from T_9 and T_0.

At 96 hrs incubation of *A. brassicae*, the least radial growth (mm) was insignificantly reduced in the treatments T_1 – *Eucalyptus globules* (12.50) and T_{10} –*Mentha peperata* (12.80) as comparison with rest of essential oils excluding Bordeaux mixture and neem oil. However, the treatments (T_{12}, T_{11}, T_3) and (T_3, T_4) were found no significant differences between among

themselves but are significant from T_5, T_7, T_6, T_8 and T_9 including with control. Whereas, treatments T_6 – *Carica papaya* (24.12) and T_8 – *Zingiber officinale* (24.97) were not significantly differ.

After 120 hrs incubation of *A. brassicae*, it was observed that not significantly reduced the radial growth in T_{10} –*Mentha peperata* (14.50) and T_1 – *Eucalyptus globules* (15.10) followed by T_{11} (15.97), T_3 (16.00), T_{12} (16.70), T_4 (17.42), T_5 (19.07), T_7 (23.60), T_6 (26.85), T_8 (29.02) and T_9 (30.85) including with control (90.00). However, treatments (T_1, T_{11}, T_3), (T_3, T_{12}) and (T_{12}, T_4) were found no significantly difference among each other but they are significant from rest essential oils including with control. Rest of the treatments are shows significant among each other.

The percentage inhibition of *A. brassicae* was increased in T_{13}- Bordeaux mixture (86.64) and T_2 – *Azadirachta indica* (85.88) on the incubation period from 48 hrs to 120 hrs as compared with other essential oil treatments. However, *Mentha peperata* (83.89) *Eucalyptus globules* (83.22) were also recorded maximum per cent inhibition of *A. brassicae* over control.

Table : 4.8 *In vitro* efficacy of essential oils at 4 % concentration on mycelial radial growth of *Alternaria brassicae* at different hrs of interval

Treatments	Mycelial radial growth (mm)							
	48 hrs	**% Inhibition over control**	**72 hrs**	**% Inhibition over control**	**96 hrs**	**% Inhibition over control**	**120 hrs**	**% Inhibition over control**
T_1 (*Eucalyptus globules)*	8.40f	55.20	9.72 ij	79.38	12.50h	82.68	15.10ij	83.22
T_2 (*Azadirachta indica*)	7.22gh	61.49	8.67 jk	81.61	10.57i	85.35	12.70k	85.88
T_3 (*Lantana camera*)	9.95e	46.93	12.75fg	72.95	14.47fg	79.95	16.00hi	82.22
T_4 (*Buchnera hispida*)	10.37de	44.69	13.60ef	71.16	15.27f	78.84	17.42g	80.64
T_5 (*Syzygium aromaticum*)	11.12d	40.69	14.02e	70.27	17.20e	76.17	19.07f	78.81
T_6 (*Carica papaya*)	15.75b	16.00	19.15c	59.38	24.12c	66.58	26.85d	70.16
T_7 (*Allium sativum*)	13.75c	26.67	15.17d	67.83	21.42d	70.32	23.60e	73.78
T_8 (*Zingiber officinale*)	15.60b	16.80	19.92c	57.75	24.97c	65.40	29.02c	67.75
T_9 (*Psidium guajava*)	16.35a	12.80	22.80b	51.64	26.57b	63.18	30.85b	65.72
T_{10} (*Mentha peperata*)	7.87fg	58.03	9.77i	79.28	12.80h	82.26	14.50j	83.89
T_{11} (*Mentha arvensis*)	8.52f	54.56	11.12h	76.42	14.22g	80.29	15.97hi	82.26
T_{12} (*Ocimum sanctum*)	8.55f	54.40	12.25g	74.02	14.02g	80.57	16.70gh	81.44
T_{13} (Bordeaux mixture)	6.52h	65.23	8.42k	82.14	10.47i	85.49	12.02k	86.64
T_0 (Control)	18.75a	-	47.15a	-	72.17a	-	90.00a	-
F-test	S		S		S		S	
S. Ed (±)	0.46		0.54		0.53		0.57	
CD (P = 0.05)	0.91		1.06		1.04		1.11	

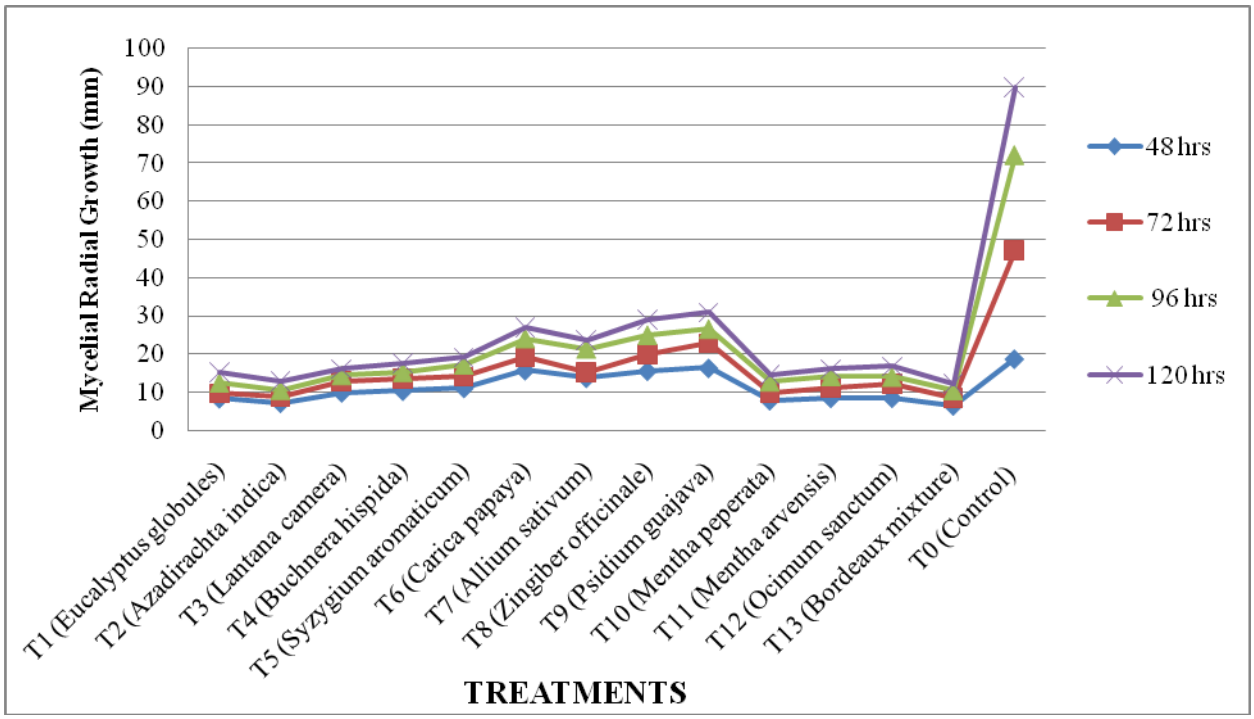


Figure : 4.8a Effect of essential oils at 4 % concentration on radial growth of *Alternaria brassicae* at different hrs of interval.

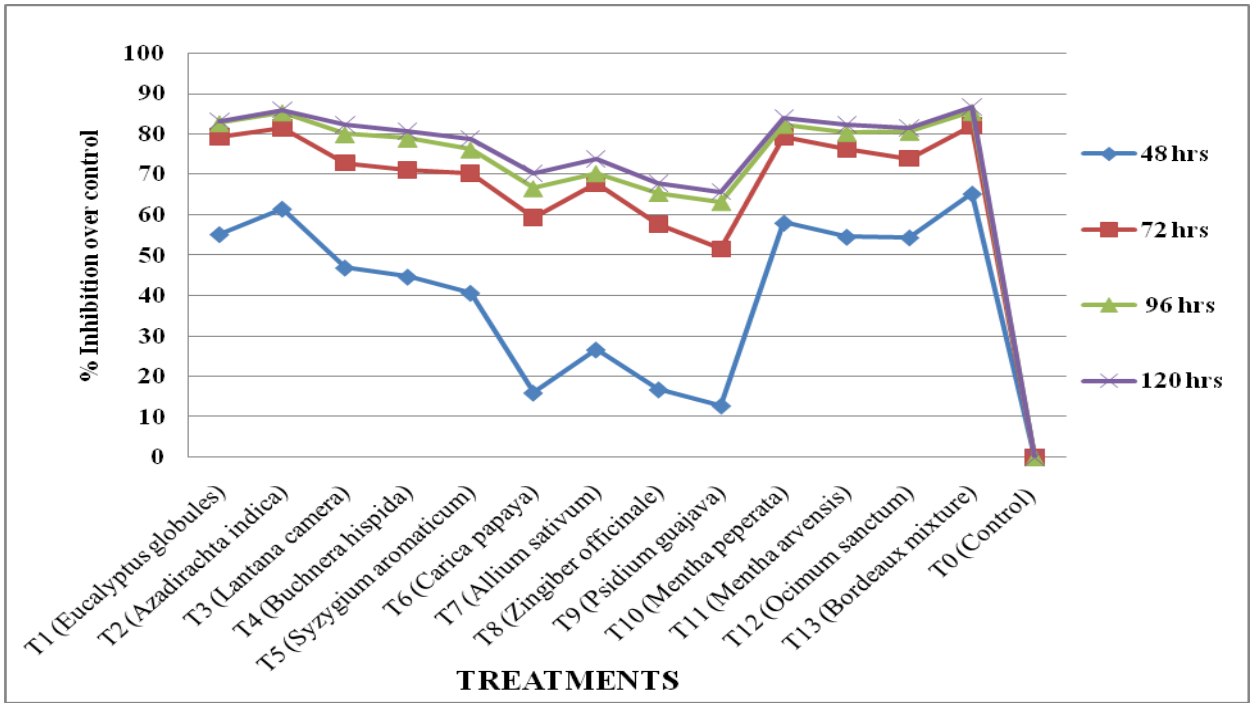


Figure : 4.8b Effect of essential oils at 4 % concentration on % inhibition over control of *Alternaria brassicae* at different hrs of interval.

4.9 *In vitro* efficacy of essential oils at 6% concentration on the radial growth (mm) and inhibition % of *A. brassicae* :

The data presented in Table and depicted in Fig : 4.9ab, revealed that the antifungal effect of essential oils at 6% concentration on the colony growth (mm) of *A. brassicae* was significantly reduced in T_{13}- Bordeaux mixture (6.25, 7.97, 9.75 14.75) as compared with all the essential oils at 48, 72, 96 and 120 hrs after incubation.

After 48 hrs incubation of *A. brassicae*, it was observed that T_2 – *A. indica* (6.75), T_{10} - *Mentha peperata* (7.35), T_{11} – *Mentha arvensis* (7.72) and T_1 –*Eucalyptus globules* (7.95) are not significantly differ from each other. But they are significant from T_{12} – *Ocimum sanctum* (8.70), T_4 –*Buchnera hispida* (8.75), T_3 – *Lantana camara* (8.85), T_5 –*Syzygium aromaticum* (9.75), T_7 –*Allium sativum* (11.55), T_8 –*Zingiber officinale* (13.25), T_6 – *Carica papaya* (14.57) and T_9- *Psidium guajava* (16.45) including with control (23.32). However, the treatments (T_{11}, T_{12}, T_4, T_3) and (T_{12}, T_4, T_3, T_5) were found no significant differences among each other but they are significant from rest of essential oils. While the treatments T_7, T_8, T_6 and T_9 were showed significant differ over control.

At 72 hrs after incubation, maximum reduction of colony growth (mm) of *A. brassicae* was recorded in the treatments T_2 - *A. indica* (13.05), T_{10} - *Mentha peperata* (13.70), T_4 –*Buchnera hispida* (13.92), T_1– *Eucalyptus globules* (13.95) and T_{11}– *Mentha arvensis* (14.77) are not significantly differ from each other, but are significant from T_3 (15.35), T_5 (15.60), T_{12} (16.17), T_6 (19.62), T_7(20.92), T_8 (24.75) and T9 (26.52) including with control (36.45). However, rest of treatments were found non significant among each other.

At 96 hrs incubation of *A. brassicae*, the minimum radial growth was recorded in the treatments T_2 - *A. indica* (14.60), T_{10} - *Mentha peperata* (15.55) and T_1– *Eucalyptus globules* (15.57) are not significantly differ among each other, but they are also shows significantly reduction as compared rest of essential oils including with control. However, the essential oil treatments (T_3, T_{11}, T_4), (T_4, T_{12}) and (T_{12}, T_5) were found no significant differences between among each other but they are difference from $T_{6,}$ T_7, $T_{8,}$ T_9 and T_0. While the treatments T_6, T_7, T_8, T_9 were showed significant differences each other.

After 120 hrs, maximum reduction of colony growth (mm) was non significantly recorded in T_2 - *A. indica* (16.05) and T_1– *Eucalyptus globules* (16.75) which are significantly reduced the growth from T_3 (17.75), T_{10} (17.80), T_4 (18.32), T_{11} (18.90), T_5 (19.30), T_{12} (20.57), T_7 (23.45), T_6 (23.90), T8 (26.70), T9 (28.92) and T_0 (90.00). The treatments (T_1, T_3, T_{10}), (T_3, T_{10}, T_4, T_{11}) and (T_4, T_{11}, T_5) were found non-significant among each other but they are significant from each remaining essential oils. However, the treatment T_{12} was found significant from T_7, T_6, T_8 and T_9. Whereas, treatments (T_7, T_6) and (T_8, T_9) are not significant differ among each other.

The highest percentage inhibition of *A. brassicae* was obtained in T_{13}- Bordeaux mixture (83.61) on the incubation period from 48 hrs to 120 hrs as compared with all the essential oil treatments. Among the essential oils, neem oil (82.17) was showed maximum per cent inhibition of *A. brassicae* as compared rest of essential oils. However, *Eucalyptus globules* (81.39) and *Mentha peperata* (80.22) were also recorded maximum per cent inhibition of *A. brassicae* over control.

Neem (*A. indica*) is widely used and well known plant from which seed extracts and oils are commonly used to control pathogens. A high content of azadirachtin, its active ingredient can be found both in oil and in the extract. The results are in agreement with those of **Chattopadhyay *et al.* (2004); Bhalodia *et al*. (2011),** who reported that number of plant derived natural products and its oils have to be antifungal and antibacterial in nature. Green plants and their products have proved their fruitfulness in providing less phytotoxic, more systemic, easily biodegradable and host metabolism stimulatory fungicides.

Essential oils have been used by many workers for controlling fungi, bacteria, viruses and insect pests **(Singh *et al*., 2001)**. The antimicrobial properties of essential oils invariably depend on the chemical nature of the constituents present in them **(Nidiry, 1998).** Essential oils, being lipophilic in nature can easily penetrate deeper through living tissue unbarred by the selective permeability of the cell membrane, hence they are of interest in the management of fungal and bacterial diseases.

Table : 4.9 *In vitro* efficacy of essential oils at 6 % concentration on radial growth of *Alternaria brassicae* at different hrs of interval

Treatments	Mycelial radial growth (mm)							
	48 hrs	% Inhibition over control	72 hrs	% Inhibition over control	96 hrs	% Inhibition over control	120 hrs	% Inhibition over control
T_1 *(Eucalyptus globules)*	7.95 gh	65.91	13.95ef	61.73	15.57ij	78.56	16.75hi	81.39
T_2 (*Azadirachta indica*)	6.75hi	71.05	13.05f	64.19	14.60j	79.89	16.05i	82.17
T_3 (*Lantana camera*)	8.85fg	62.05	15.35de	57.89	16.25hi	77.62	17.75gh	80.28
T_4 (*Buchnera hispida*)	8.75fg	62.48	13.92ef	61.81	16.82gh	76.84	18.32fg	79.64
T_5 (*Syzygium aromaticum*)	9.75f	58.19	15.60de	57.20	18.05f	75.14	19.30f	78.56
T_6 (*Carica papaya*)	14.57c	37.52	19.62c	46.17	21.92e	69.82	23.92d	73.42
T_7 (*Allium sativum*)	11.55e	50.47	20.92c	42.60	23.20d	68.05	23.45d	73.94
T_8 (*Zingiber officinale*)	13.25d	43.18	24.75b	32.09	25.70c	64.61	26.70c	70.33
T_9 (*Psidium guajava*)	16.45b	29.45	26.52b	27.24	27.67b	61.89	28.92b	67.87
T_{10} (*Mentha peperata*)	7.35hi	68.48	13.70ef	62.41	15.55ij	78.59	17.80gh	80.22
T_{11} (*Mentha arvensis*)	7.72gh	66.89	14.77def	59.47	16.40hi	77.42	18.90fg	79.00
T_{12} (*Ocimum sanctum*)	8.70fg	62.69	16.17d	55.64	17.82fg	75.46	20.57e	77.14
T_{13} (Bordeaux mixture)	6.25i	73.19	7.97g	78.13	9.75k	86.57	14.75j	83.61
T_0 (Control)	23.32a	-	36.45a	-	72.62a	-	90.00a	-
F-test	S		S		S		S	
S. Ed (±)	0.63		1.09		0.41		0.59	
CD (P = 0.05)	1.24		2.14		1.21		1.16	

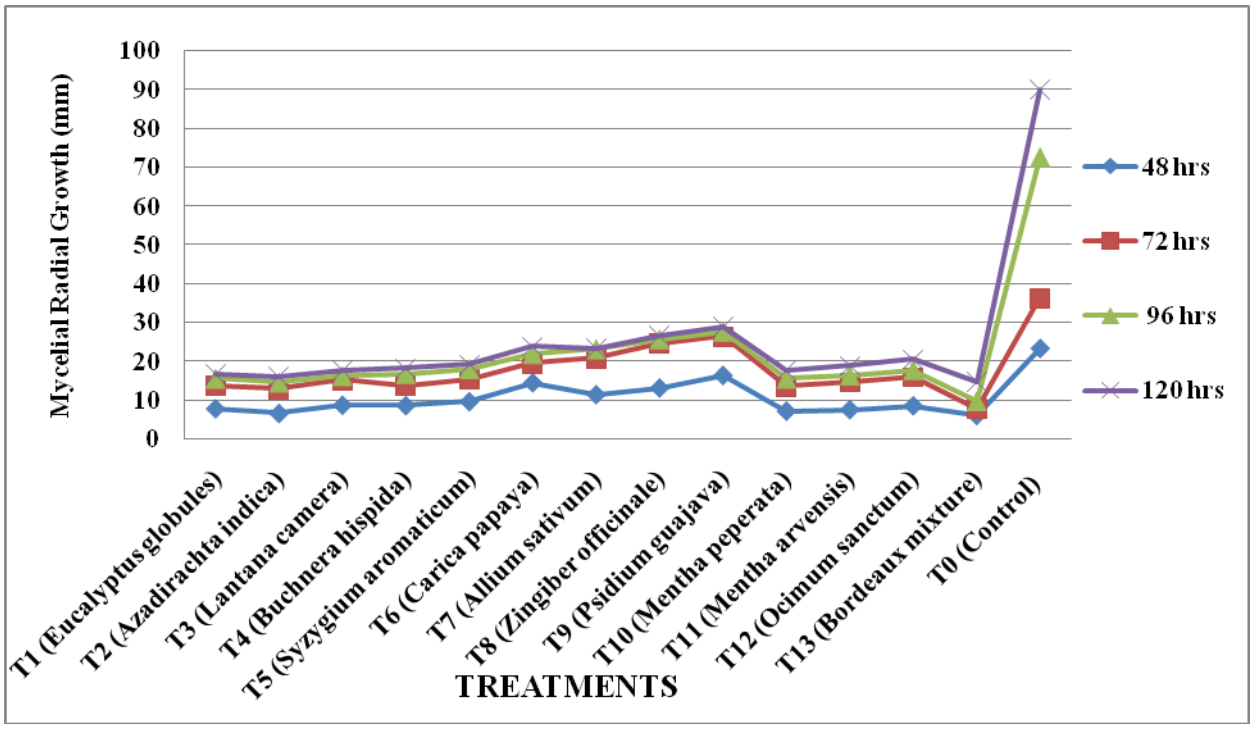


Figure : 4.9a Effect of essential oils at 6 % concentration on mycelial radial growth of *Alternaria brassicae* at different hrs of interval.

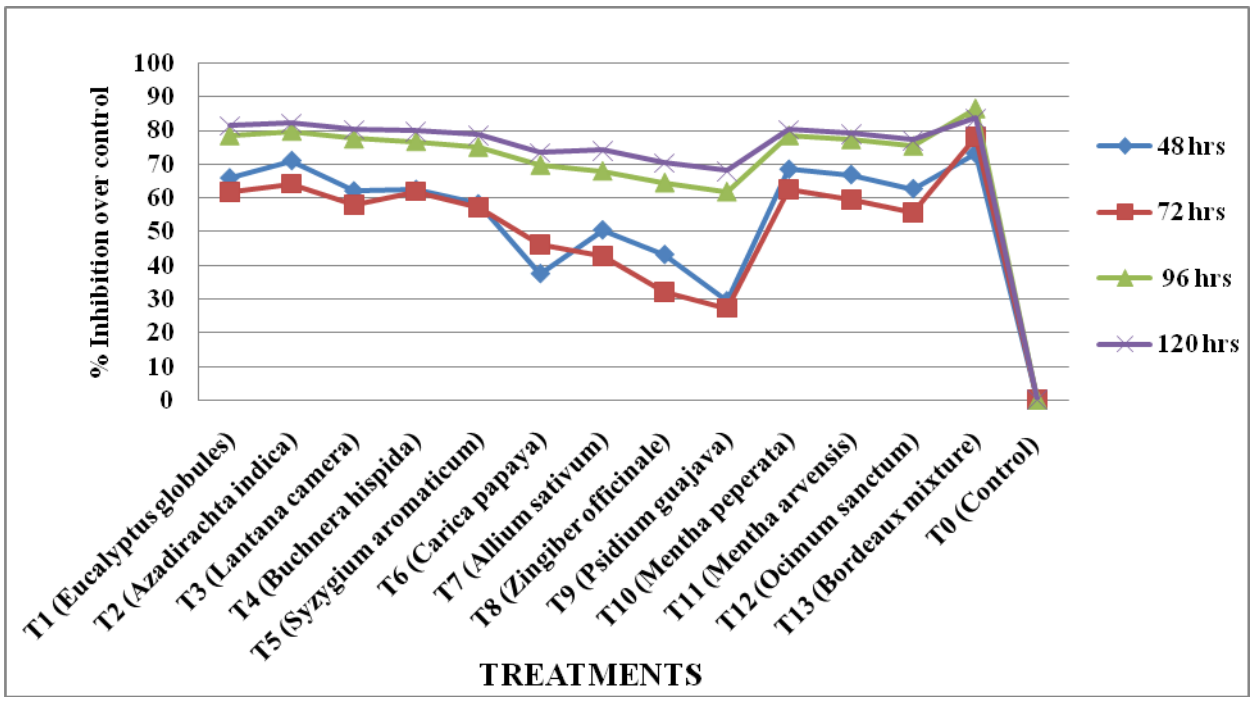


Figure : 4.9b Effect of essential oils at 6 % concentration on % over control of *Alternaria brassicae* at different hrs of interval.

4.10 *In vitro* efficacy of selected botanical extracts at 25 % on population of *Xanthomonas campestris* pv. *campestris* :

A perusal data documented in Table : 4.10 and Fig : 4.10ab, elucidate that the antibacterial effect of selected botanical extracts at 25% concentration on the no. of colonies of *X. campestris* pv. *campestris* was found minimum in the treatment T_7 - *Azadirachta indica* (8.66) as compared with other botanical extracts including bordeaux mixture and control at 48 hrs after incubation.

Among the botanical extracts the treatments T_2 (*Calotropis procera*), T_{11} (*Croton boplandianu*), T_9 (*Zingiber officinale*), T_{10} (*Mentha arvensis*) are found maximum no. of colonies ranging from 22.6 to 29.3. The treatments T_{12} - Bordeaux mixture (21.00), T_4 - *Allium sativum* (20.33) and T_8 - *Eucalyptus globules* (19.33) are not significantly differ among each other. Whereas, all the treatments significantly reduced the no. of colonies from control (without treatment).

At 72 hrs after incubation of *X. campestris* pv. *campestris*, it was noticed that among the different treatments significantly reduced the no. of colonies in the treatment T_7 –*Azadirachta indica* (23.33) and T_1 – *Ocimum sanctum* (24.00) as compared with rest of botanical leaf extracts including with Bordeaux mixture and control. Among the treatments, the maximum no. of colonies was seen in T_2 (*Calotropis procera*) followed by T_{12} (Bordeaux mixture), T_{11} (*Croton boplandianu*), T_9 (*Zingiber officinale*), T_{10} (*Mentha arvensis*) and T_4 (*Allium sativum*), which are not significantly differ the total no. of colonies among themsevles. Whereas, all the treatments significantly reduced the no. of colonies from control (without treatment).

The highest percentage inhibition of *X. campestris* pv. *campestris* was obtained in T_7 – *Azadirachta indica* (87.59) on the incubation period from 48 hrs to 72 hrs as compared with all the botanical extracts. However, the treatments T_1 – *Ocimum sanctum* (87.23), T_5 – *Lantana camara* (85.81), T_6 – *Datura stramonium* (84.93) and T_3 –*Mentha peperata* (84.04) were also recorded highest per cent inhibition as compared remaining leaf extracts.

Table: 4.10 *In vitro* efficacy of selected botanical extracts at 25 % concentration on number of colonies of *X. c.* pv. *campestris* at different hrs of interval

Treatments	CFU ml^{-1} of 10^{-7} dilution factor			
	48 hrs	% Inhibition over control	72 hrs	% Inhibition over control
T_1 (*Ocimum sanctum*)	10.33hi	90.80	24.00g	87.23
T_2 (*Calotropis procera*)	29.33b	73.88	43.00b	77.12
T_3 (*Mentha peperata*)	15.66fg	86.05	30.00defg	84.04
T_4 (*Allium sativum*)	20.33de	81.90	35.33bcde	81.20
T_5 (*Lantana camara*)	11.66hi	89.62	26.66fg	85.81
T_6 (*Datura stramonium*)	13.33gh	88.13	28.33efg	84.93
T_7 (*Azadirachta indica*)	8.66i	92.29	23.33g	87.59
T_8 (*Eucalyptus globules*)	19.33ef	82.79	34.33cdef	81.73
T_9 (*Zingiber officinale*)	23.66cd	78.93	39.66bc	78.90
T_{10} (*Mentha arvensis*)	22.66cde	79.82	37.33bcd	80.14
T_{11} (*Croton boplandianum*)	26.00bc	76.85	41.00bc	78.19
T_{12} (Bordeaux mixture)	21.00de	81.30	42.66bc	77.30
T_0 (Control)	112.33a	-	188.00a	-
F-test	S		S	
S. Ed (±)	1.98		4.19	

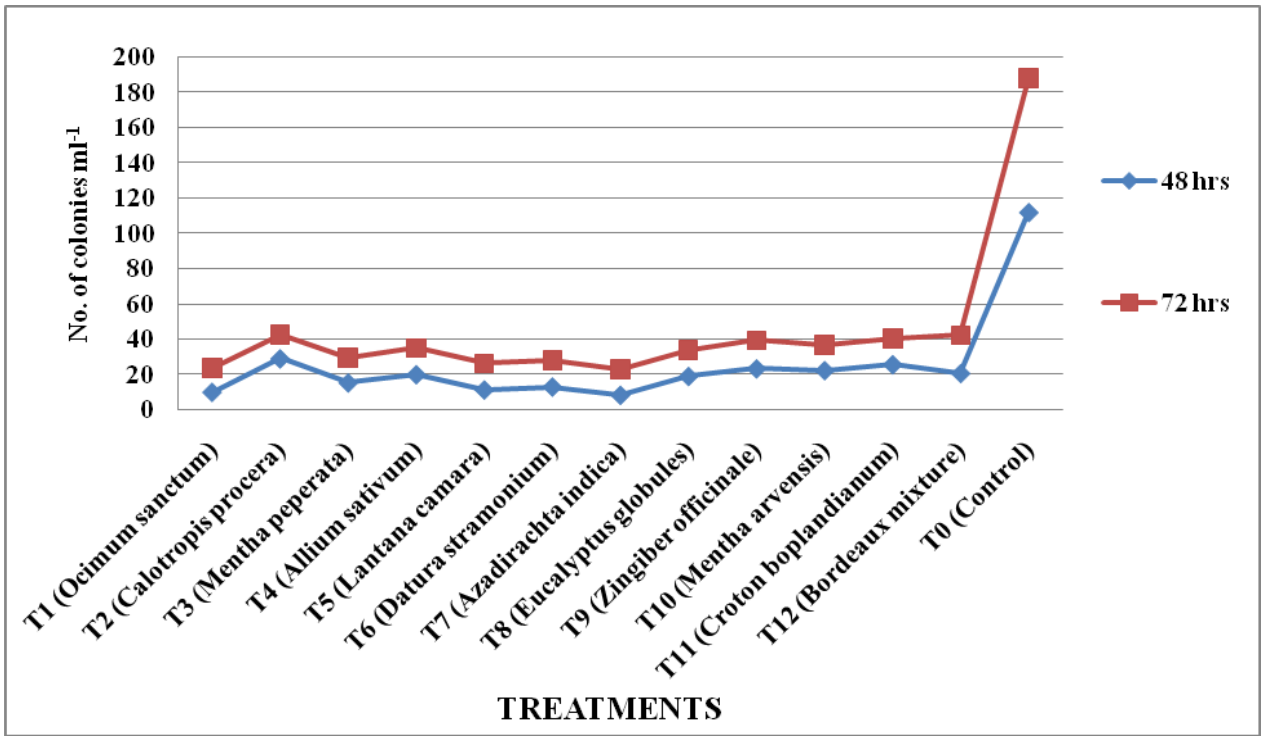


Figure : 4.10a Effect of selected botanical extracts at 25 % concentration on no. of colonies of *Xanthomonas campestris* pv. *campestris* at different hrs of interval.

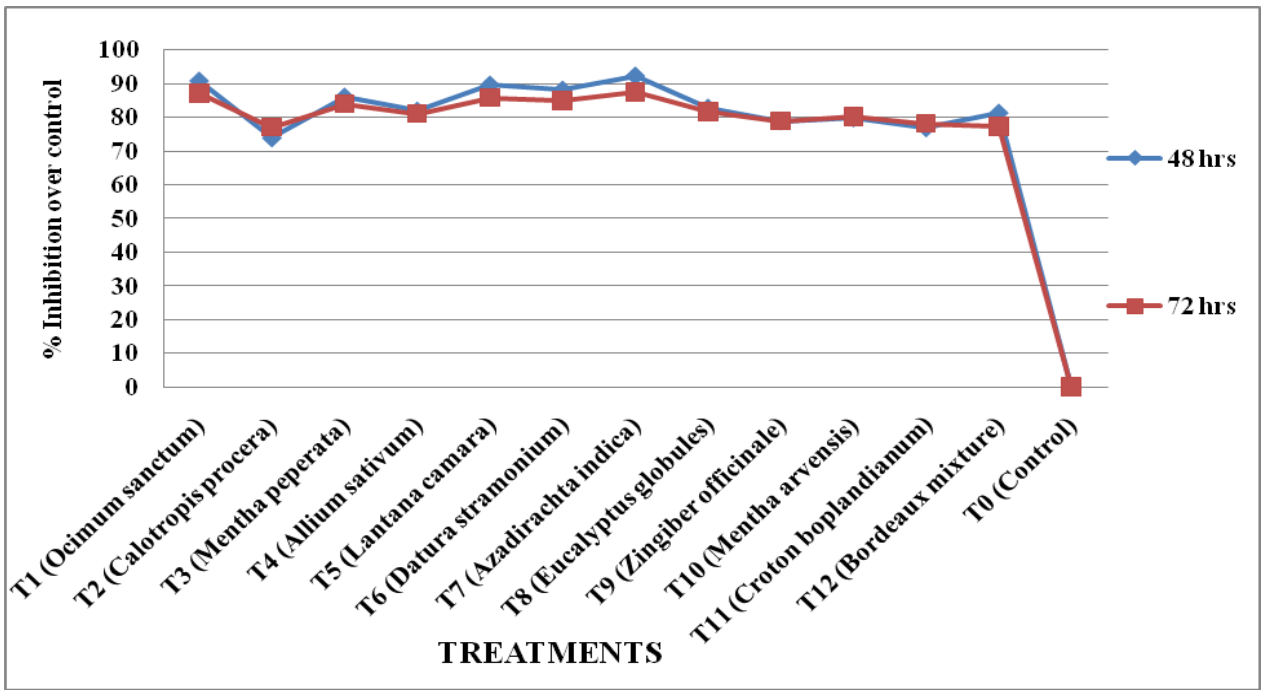


Figure : 4.10b Effect of selected botanical extracts at 25 % concentration on % reduction over control of *Xanthomonas campestris* pv. *campestris* at different hrs of interval.

4.11 *In vitro* efficacy of selected botanical extracts at 50 % concentration on no. of colonies of *Xanthomonas campestris* pv. *campestris* :

The results of Table :4.11 and Fig : 4.11ab, reveal that after 48 hrs of incubation the least no. of colonies and maximum inhibition percentage was obtained in T_7- *Azadirachta indica* (6.00, 94.8%) followed by T_5 –*Lantana camara* (6.66, 94.3%), T_1-*Ocimum sanctum* (6.66, 94.3%), T_3 –*Mentha peperata* (9.00, 92.3%) and T_8 –*Eucalyptus globules* (9.00, 92.3%), respectively. However, the treatments (T_7, T_5, T_1, T_3, T_8, T_6) are non significantly differ among each other but they are significant from T_4, T_{10}, T_9, T_{11}, T_2 and T_{12} in reduction the no. of *X. campestris* pv. *campestris* colonies. The treatments T_{12} - Bordeaux mixture (20.0) and T_2 - *Calotropis procera* (18.6) are found non significantly differ to each other. Whereas, all the treatments significantly reduced the no. of colonies from control.

At 72 hrs after incubation of *X. campestris* pv. *campestris* in leaf extracts, it was observed that non significantly reduced the no. of colonies and highest percentage inhibition in T_7- *Azadirachta indica* (10.0, 94.69%), T_1-*Ocimum sanctum* (13.3, 92.9%), T_5 –*Lantana camara* (13.3, 92.9%), T_6 - *Datura stramonium* (14, 92.5%) and T_3 –*Mentha peperata* (16, 91.5%) as compared with T_8 - *Eucalyptus globules* (19.3), T_4- *Allium sativum* (20.3), T_{10} - *Mentha arvensis* (23.0), T_9 - *Zingiber officinale* (24.0), T_{11} - *Croton boplandianum* (25.6), T_2 - *Calotropis procera* (27.6) and T_{12} - Bordeaux mixture (32.3) including with control (188.3). Among the botanical leaf extracts the treatments T_{12}, T_2, T_{11}, T_9 are found maximum no. of *X. campestris* pv. *campestris* colonies. However, all the treatments were found non significantly differ among each other.

Table : 4.11 *In vitro* efficacy of selected botanical extracts at 50 % concentration on no. of colonies of *Xanthomonas campestris* pv. *campestris* at different hrs of interval

Treatments	CFU ml^{-1} of 10^{-7} dilution factor			
	48 hrs	% Inhibition over control	72 hrs	% Inhibition over control
T_1 (*Ocimum sanctum*)	6.66 fg	94.30	13.33fg	92.92
T_2 (*Calotropis procera*)	18.66b	84.05	27.66bc	85.31
T_3 (*Mentha peperata*)	9.00efg	92.30	16.00efg	91.50
T_4 (*Allium sativum*)	10.33cde	91.17	20.33cdef	89.20
T_5 (*Lantana camara*)	6.66fg	94.30	13.33fg	92.92
T_6 (*Datura stramonium*)	9.33def	92.03	14.00fg	92.56
T_7 (*Azadirachta indica*)	6.00g	94.87	10.00g	94.69
T_8 (*Eucalyptus globules*)	9.00efg	92.30	19.33def	89.73
T_9 (*Zingiber officinale*)	12.33cd	89.46	24.00cd	87.26
T_{10} (*Mentha arvensis*)	10.66cde	90.89	23.00cde	87.78
T_{11} (*Croton boplandianum*)	13.33c	88.61	25.66bcd	86.38
T_{12} (Bordeaux mixture)	20.00b	82.91	32.33b	82.83
T_0 (Control)	117.00a	-	188.33a	-
F-test	S		S	
S. Ed (±)	1.63		3.87	
CD (P = 0.05)	3.28		7.78	

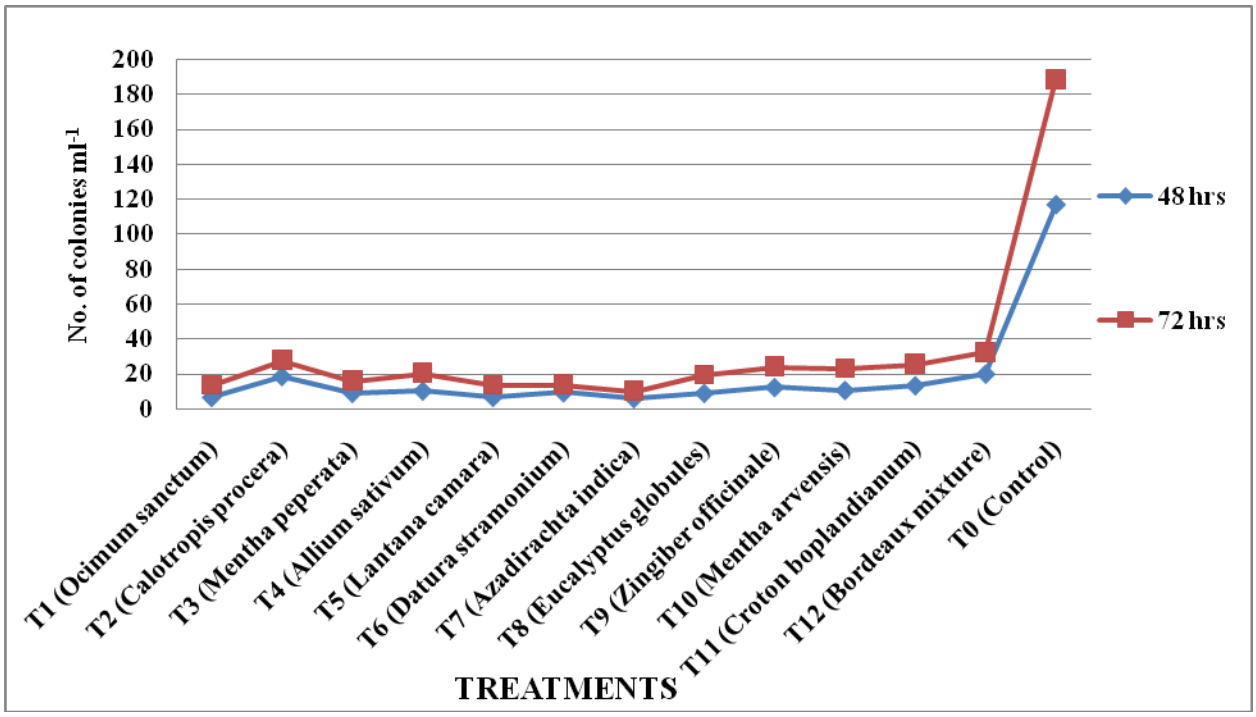


Figure : 4.11a Effect of selected botanical extracts at 50 % concentration on no. of colonies of *Xanthomonas campestris* pv. *campestris* at different hrs of interval

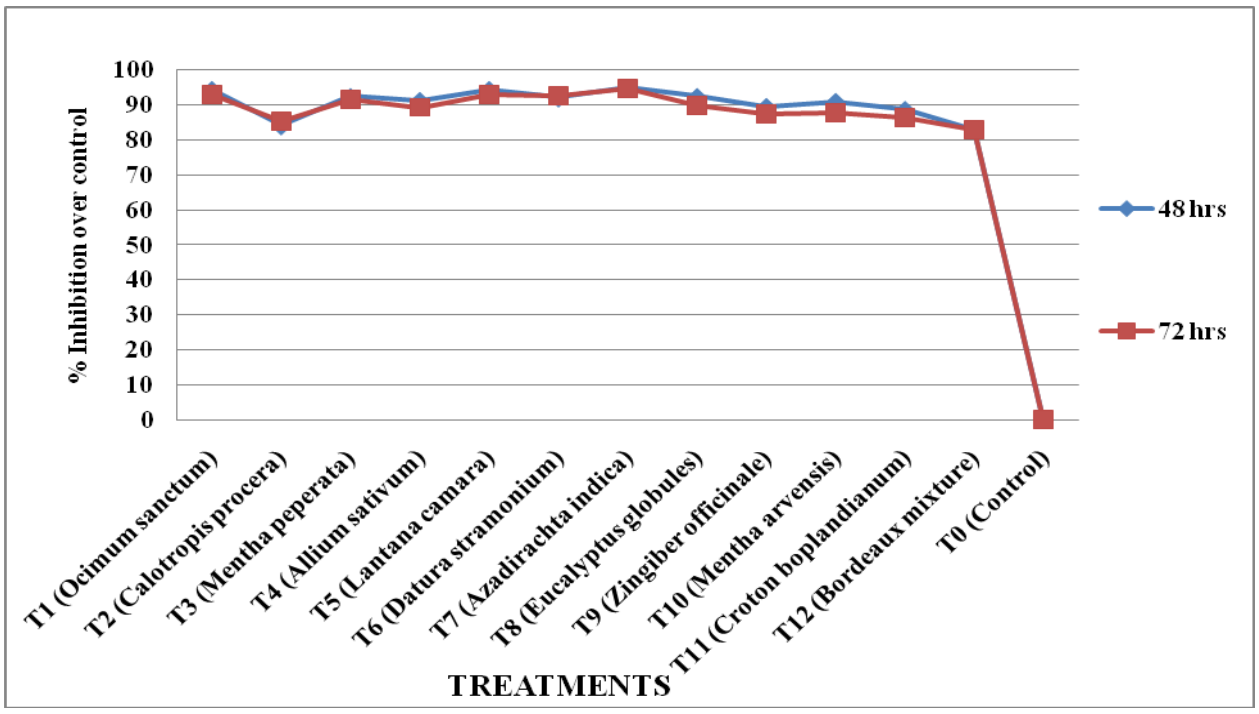


Figure : 4.11b Effect of selected botanical extracts at 50 % concentration on reduction population over control of *X. campestris* pv. *campestris* at different hrs of interval.

4.12 *In vitro* efficacy of selected botanical extracts at 75 % concentration on no. of colonies of *Xanthomonas campestris* pv. *campestris* :

The data documented in Table : 4.12 and Fig : 4.12ab, elucidate that the least no. of colonies and maximum per cent inhibition was obtained from 75 % concentration of the treatment T_7 - *Azadirachta indica* (1.66, 98.2%) as compared with T_1 –*Ocimum sanctum* (3.00, 96.7%), T_5- *Lantana camara* (3.33, 92.6%), T_3 –*Mentha peperata* (6.0, 93.5%), T_8 –*Eucalyptus globules* (7.0, 92.4%), T_6- *Datura stramonium* (7.0, 92.4%), T_{10}-*Mentha arvensis* (9.0, 90.2%), T_9- *Zingiber officinales* (9.6, 89.5%) and T_4-*Allium sativum* (10.3, 88.8%) including over control (92.6), respectively. Among the botanical extracts the treatments T_{12} (Bordeaux mixture), T_{11} (*Croton boplandianum*) and T_2 (*Calotropis procera*) are seen maximum no. of *X. campestris* pv. *campestris* colonies ranging from 12.3 to 21.6. However, the treatments (T_5, T_1, T_7), (T_4, T_9, T_{10}, T_6, T_8, T_3) and (T_{12}, T_{11}) are not significantly differ among each other. While, without treated petriplates were showed maximum no. of colonies.

After 72 hrs incubation of *X. campestris* pv. *campestris*, it was observed that the least no. of colonies were recorded in the treatment T_7 –*Azadirachta indica* (6.67) followed by T_5- *Lantana camara* (7.33) and T_1 –*Ocimum sanctum* (7.6) including rest of botanical leaf extracts with Bordeaux mixture and control. Among the treatments, the maximum no. of colonies was seen in T_{12} (Bordeaux mixture) followed by T_2 (*Calotropis procera*), T_{11} (*Croton boplandianum*), T_9 (*Zingiber officinales*) and T_{10} (*Mentha arvensis*), which are not significantly differ the total no. of colonies among themselves. All the botanicals were found non-significant difference among themselves but they are significant from control.

Leaf extract of *A. indica* was observed to show effective reduction of the population of *X. campestris*, which might be due various antimicrobial substances present in the plant sample, some phytochemicals such as coumarins, flavonoids and alcoholic soluble phenols. The reduction of the bacterial population was increased with the increase of concentration levels of plant extracts. Similar antibacterial properties of these plants have been reported in many studies, but most studied the antibacterial activity of the extracts against human pathogens.

The various crude extracts of *A. indica, Ocimum canum, Plumbago zeylanica* at concentrations of 1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm, and 5000 ppm showed significant activity

against five plant pathogenic bacteria and one bacterial isolate as well as positive bacteria **(Perumal *et al.*, 1999; Okunade, 2002, Karuppusamy *et al.*, 2001; Prakash *et al.*, 2006a, 2006b).** Our results are supported by **Atar *et al.* (2014),** who reported that neem oil was found best effective for control of *X. axonopodis* pv. *punicae* at all concentration by forming 14.47 per cent inhibition followed by Garlic leaf extract, Tulsi leaf extract, Ginger extract, Guava leaf extract and Aloe vera extract with 12.86%, 12.28%, 11.70%, 11.13%, 9.36% and 7.34 per cent average inhibition, respectively.

Our finding is supported by **Thirumalesh *et al.* (2012)** who reported that antibacterial activity of crude extracts of eight plants including A. indica, by using solvents like petroleum ether, chloroform, methanol and water against *X. campestris* pv. *mangiferaeindicae*. **Maragathavalli *et al.* (2012)** evaluated antimicrobial activity of the chemical compounds obtained from Neem leaves.

Table : 4.12 *In vitro* efficacy of selected botanical extracts at 75 % concentration on no. of colonies of *Xanthomonas campestris* pv. *campestris* at different hrs of interval

Treatments	CFU ml^{-1} of 10^{-7} dilution factor			
	48 hrs	% Inhibition over control	72 hrs	% Inhibition over control
T_1 (*Ocimum sanctum*)	3.00de	96.76	7.66 e	93.33
T_2 (*Calotropis procera*)	12.33bcd	86.69	23.00bc	80.00
T_3 (*Mentha peperata*)	6.00cde	93.52	10.33de	91.01
T_4 (*Allium sativum*)	10.33cde	88.85	11.66de	89.86
T_5 (*Lantana camara*)	3.33de	92.60	7.33e	93.62
T_6 (*Datura stramonium*)	7.00cde	92.44	12.66de	88.99
T_7 (*Azadirachta indica*)	1.66e	98.20	6.66e	94.20
T_8 (*Eucalyptus globules*)	7.00cde	92.44	12.33de	89.27
T_9 (*Zingiber officinale*)	9.66cde	89.57	14.33cde	87.53
T_{10} (*Mentha arvensis*)	9.00cde	90.28	14.33cde	87.53
T_{11} (*Croton boplandianum*)	13.33bc	85.61	18.00bcd	84.34
T_{12} (Bordeaux mixture)	21.66b	76.62	27.66b	75.94
T_0 (Control)	92.66a	-	115.00a	-
F-test	S		S	
S. Ed (±)	4.82		4.90	
CD (P = 0.05)	9.69		9.85	

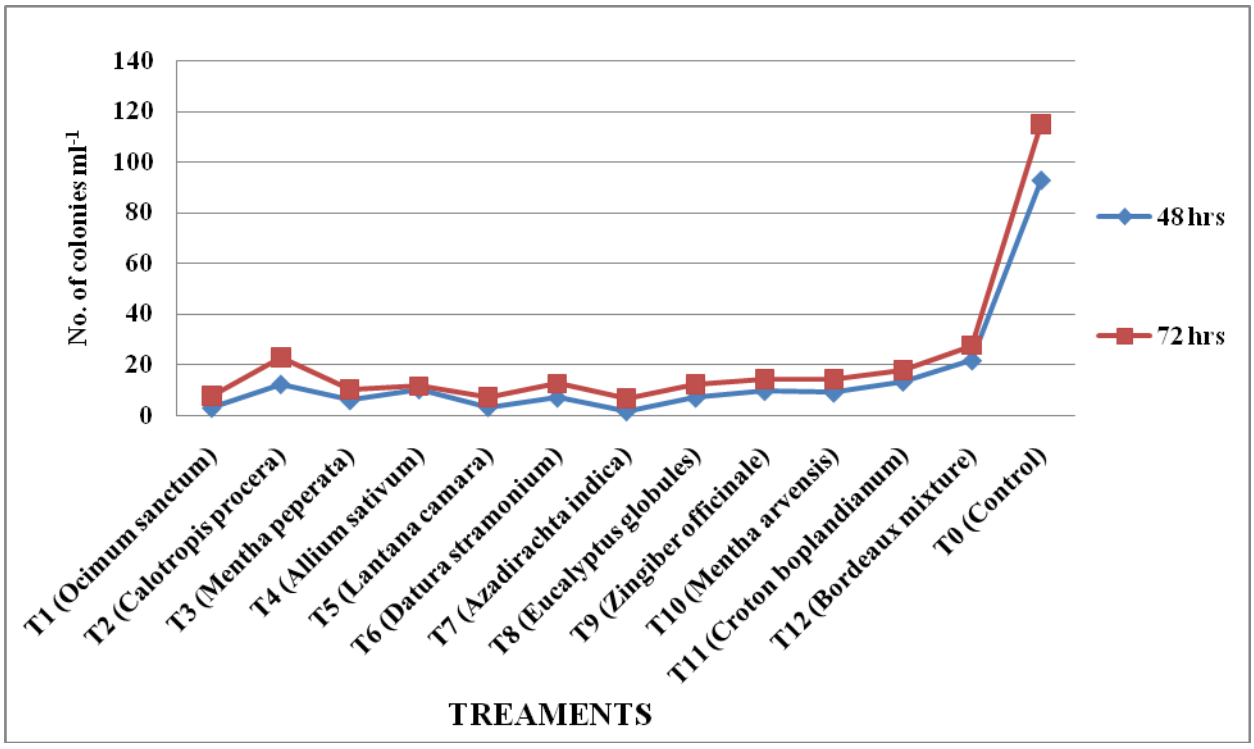


Figure : 4.12a Effect of selected botanical extracts at 75 % concentration on no. of colonies of *Xanthomonas campestris* pv. *campestris* at different hrs of interval.

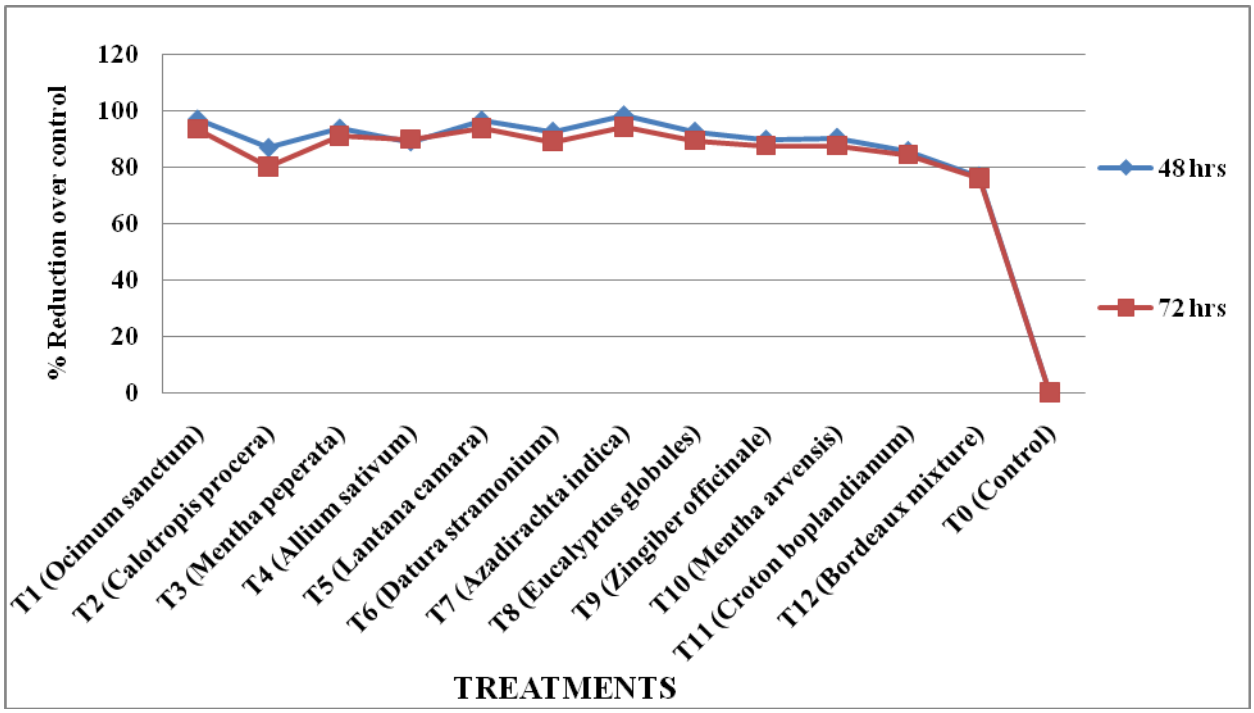


Figure :4.12b Effect of selected botanical extracts at 75 % concentration on % reduction population over control of *Xanthomonas campestris* pv. *campestris* at different intervals.

4.13 *In vitro* efficacy of selected essential oils at 2 % concentration on no. of colonies of *Xanthomonas campestris* pv. *campestris* :

The results of Table and Fig : 4.13ab, reveled that the antibacterial effect of essential oils at 2% concentration on 48 hrs after incubation , the no. of colonies of *X. campestris* pv. *campestris* was found minimum in the treatment T_2 - *Azadirachta indica* (6.0) followed by T_{10} - *Mentha peperata* (7.0), T_{11} - *Mentha arvensis* (9.0), T_{12} - *Ocimum sanctum* (10.3), T_1 - *Eucalyptus globules* (10.6), T_8 - *Zingiber officinale* (11.6) and T_7 - *Allium sativum* (11.6), are non significantly differ among each other.

Among the essential oils, the maximum colonies was recorded in T_9 (*Psidium guajava*) followed T_{13} (Bordeaux mixture), T_6 (*Carica papaya*) and T_5 (*Syzygium aromaticum*) which are not significantly differ in the no. of colonies among themselves. However, the treatments T_3 and T_4 were also shows non significant to each other but they are significant from T_5, T_6 and T_{13} including with T_0.

At 72 hrs after incubation, results revealed that T_2 - *Azadirachta indica* oil was recorded least no. of bacterial colonies (15.0) and highest per cent inhibition (85.5%) as compared with T_{10} - *Mentha peperata* (16), T_{11} - *Mentha arvensis* (18), T_{12} - *Ocimum sanctum* (19.3), T_1 - *Eucalyptus globules* (19.3), T_8 - *Zingiber officinale* (20.6), T_7 - *Allium sativum* (20.6) and T_4 - *Buchnera hispida* (25.3) which are non significantly differ among themselves. Among the treatments T_9 (*Psidium guajava*) was obtained maximum no. of *X. campestris* pv. *campestris* colonies followed by T_6 (*Carica papaya*) and T_{13} (Bordeaux mixture). However, the treatments (T_2, T_{10}, T_{11}, T_{12}, T_1), (T_8, T_7) and (T_4, T_3, T_5, T_5, T_{13}, T_6) were found non significant among each other. Whereas, the treatments (T_{12}, T_1) and (T_8, T_7) are similar in the no. of colonies. Comparison the results with CD value, all the essential oil was found no significant difference among each other.

The highest percentage inhibition of *X. campestris* pv. *campestris* was obtained in T_2 (*Azadirachta indica*), T_{10} (*Mentha peperata*), T_{11} (*Mentha arvensis*), T_{12} (*Ocimum sanctum*), T_1 (*Eucalyptus globules*), T_8 (*Zingiber officinale*) and T_7 (*Allium sativum*), which are ranging from 85.5% to 80.1% on the incubation period of 72 hrs as compared with rest of essential oils.

Table : 4.13 *In vitro* efficacy of essential oils at 2 % concentration on no. of colonies of *Xanthomonas campestris* pv. *campestris* at different hrs of interval

Treatments	CFU ml^{-1} of 10^{-7} dilution factor			
	48 hrs	% Inhibition over control	72 hrs	% Inhibition over control
T_1 *(Eucalyptus globules)*	10.66ef	86.16	19.33ef	81.41
T_2 (*Azadirachta indica*)	6.00f	92.20	15.00f	85.57
T_3 (*Lantana camera*)	15.66de	79.66	25.66cde	75.32

T_4 (*Buchnera hispida*)	16.00de	79.22	25.33cdef	75.64
T_5 (*Syzygium aromaticum*)	17.00cde	77.92	26.00cde	75.00
T_6 (*Carica papaya*)	23.66bcd	69.27	32.66bc	68.59
T_7 (*Allium sativum*)	11.66ef	84.85	20.66def	80.13
T_8 (*Zingiber officinale*)	11.66ef	84.85	20.66def	80.13
T_9 (*Psidium guajava*)	31.66b	58.89	37.33b	64.10
T_{10} (*Mentha peperata*)	7.00f	90.91	16.00ef	84.61
T_{11} (*Mentha arvensis*)	9.00ef	88.31	18.00ef	82.69
T_{12} (*Ocimum sanctum*)	10.33ef	86.58	19.33ef	81.41
T_{13} (Bordeaux mixture)	25.00bc	67.53	31.00bcd	70.19
T_0 (Control)	77.00a	-	104.00a	-
F-test	S		S	
S. Ed (±)	4.35		5.27	
CD (P = 0.05)	8.53		10.33	

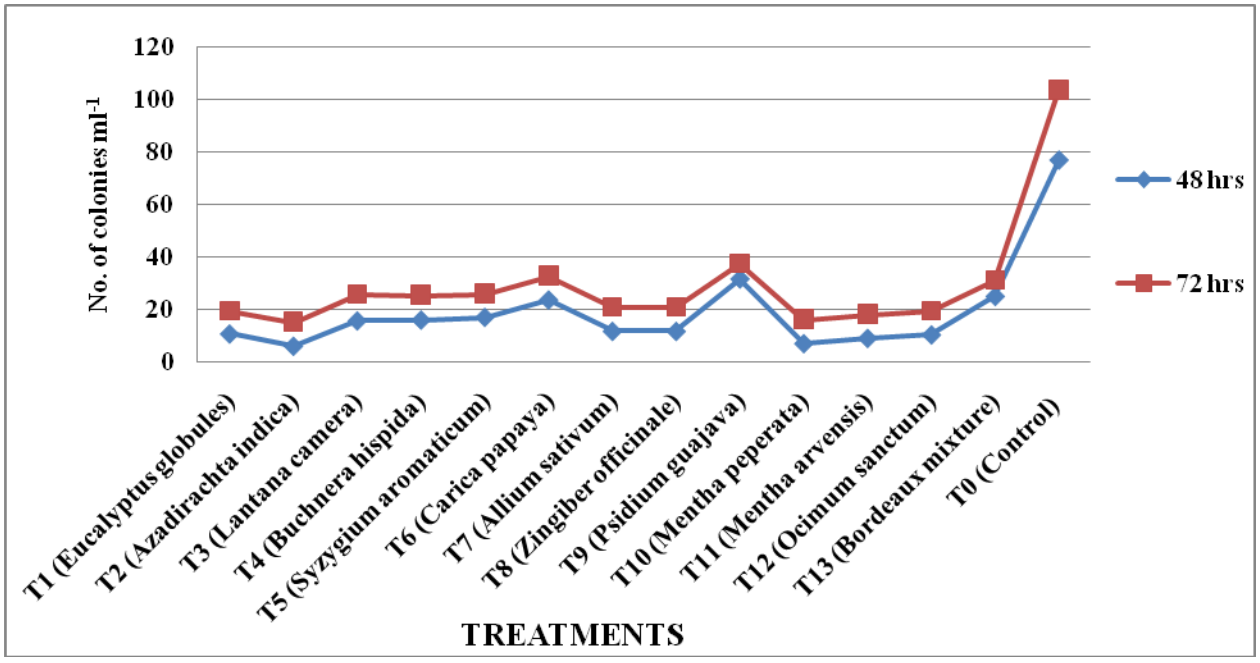


Figure : 4.13a Effect of selected essential oils at 2 % concentration on no. of colonies of *Xanthomonas campestris* pv. *campestris* at different intervals.

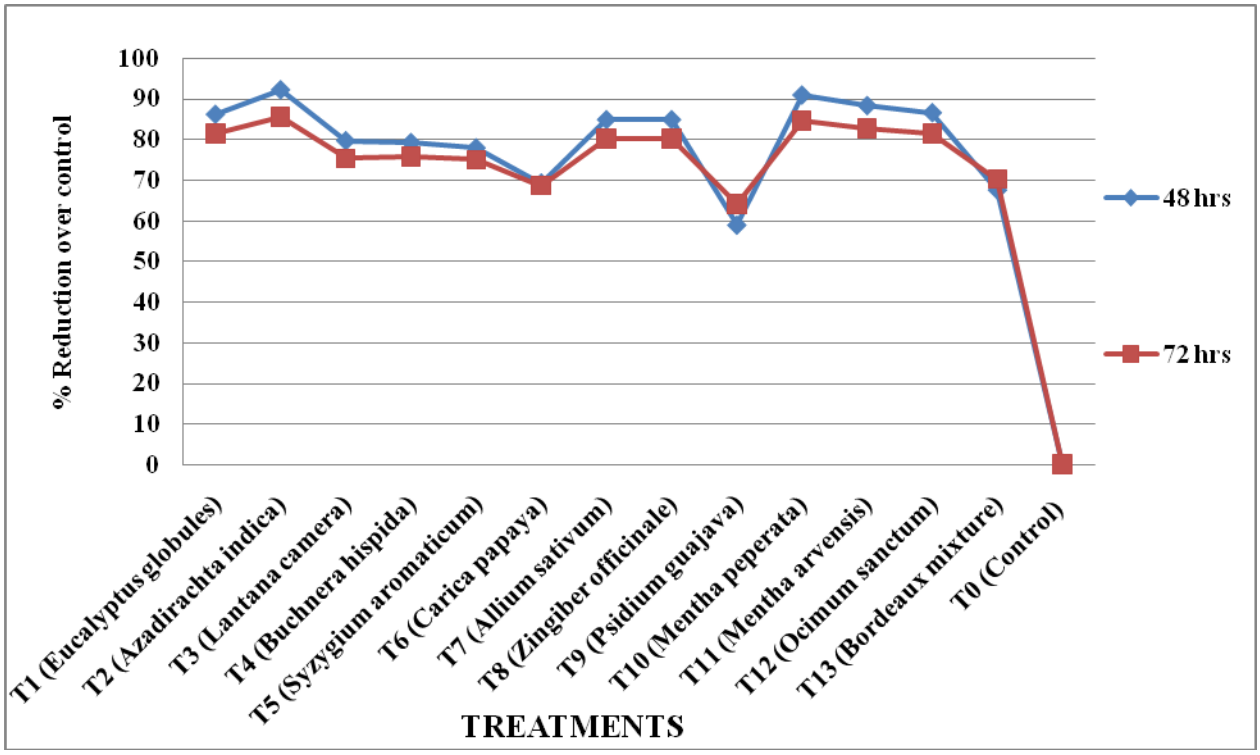


Figure : 4.13b Effect of selected essential oils at 2 % concentration on % reduction population over control of *Xanthomonas campestris* pv. *campestris* at different intervals.

4.14 *In vitro* efficacy of essential oils at 4 % concentration on no. of colonies of *Xanthomonas campestris* pv. *campestris* :

The results of Table and Fig : 4.14ab, reveled that the antibacterial effect of essential oils at 4 % concentration on 48 hrs after incubation , the no. of colonies of *X. campestris* pv. *campestris* was found minimum in the treatment T_2 - *Azadirachta indica* (5.6) and T_{10} - *Mentha peperata* (6.3) followed by T_{11} - *Mentha arvensis* (7.3), T_{12} - *Ocimum sanctum* (7.6), T_7 - *Allium sativum* (8.3), T_8 - *Zingiber officinale* (9.6,) and T_1 - *Eucalyptus globules* (10.0) including with rest essential oils and Bordeaux mixture.

Among the essential oils, the maximum colonies was recorded in T_9 (*Psidium guajava*) and T_{13} (Bordeaux mixture) followed by T_6 (*Carica papaya*) and T_5 (*Syzygium aromaticum*) which are not significantly differ in the no. of colonies among themselves. The treatments T_2, T_{10}, T_{11}, T_{12}, T_7, T_8 and T_1 were found non significant to each other. However, the treatments T_3 and T_4 were

shows similarly non significant among themselves but they are significant from T_5, T_6, T_9 and T_{13} including with T_0.

	CFU ml^{-1} of 10^{-7} dilution factor

Results shows on 72 hrs after incubation of *X. campestris* pv. *campestris* in 4% essential oils, it was observed that the minimum no. of colonies and maximum % inhibition in the treatment T_2 - *Azadirachta indica* (9.6, 90.1%) as compared with T_{10} - *Mentha peperata* (10.3, 89.4%), T_{12} - *Ocimum sanctum* (11.6, 88.1%), T_{11} - *Mentha arvensis* (11.6, 88.1%), T_7 - *Allium sativum* (13.0, 86.7%), T_8 - *Zingiber officinale* (14.0, 85.7%) and T_1 - *Eucalyptus globules* (14.0, 85.7%), which are not significantly differ among each other but they are significant from rest of essential oil treatments including Bordeaux mixture and control also. However, the treatments, T_3 (*Lantana camera*, 18.3) and T_4 (*Buchnera hispida*, 18.3) are found similarly non significant effect among themselves but they are significant from T_5, T_6, T_{13} and T_9 including with T_0. Whereas, treatments T_9 and T_{13} were seen maximum no. of colonies followed by T_6 and T_5.

Table : 4.14 *In vitro* efficacy of selected essential oils at 4 % concentration on no. of colonies of *Xanthomonas campestris* pv. *campestris* at different hrs of interval

	48 hrs	**% Inhibition over control**	**72 hrs**	**% Inhibition over control**
T_1 *(Eucalyptus globules)*	10.00def	87.23	14.00def	85.76
T_2 (*Azadirachta indica*)	5.66f	92.77	9.66f	90.18
T_3 (*Lantana camera*)	14.33cde	81.71	18.33cde	81.35
T_4 (*Buchnera hispida*)	14.33cde	81.71	18.33cde	81.35
T_5 (*Syzygium aromaticum*)	15.00cd	80.85	19.00cd	80.67
T_6 (*Carica papaya*)	19.33bc	75.32	24.00bc	75.59
T_7 (*Allium sativum*)	8.33def	89.37	13.00def	86.77
T_8 (*Zingiber officinale*)	9.66def	87.67	14.00def	85.76
T_9 (*Psidium guajava*)	26.33b	66.38	30.66b	68.82
T_{10} (*Mentha peperata*)	6.33f	91.92	10.33ef	89.49
T_{11} (*Mentha arvensis*)	7.33ef	90.64	11.66def	88.14
T_{12} (*Ocimum sanctum*)	7.66ef	90.22	11.66def	88.14
T_{13} (Bordeaux mixture)	23.33b	70.21	27.66b	71.87
T_0 (Control)	78.33a	-	98.33a	-
F-test	S		S	
S. Ed (±)	3.70		4.21	
CD (P = 0.05)	7.26		8.26	

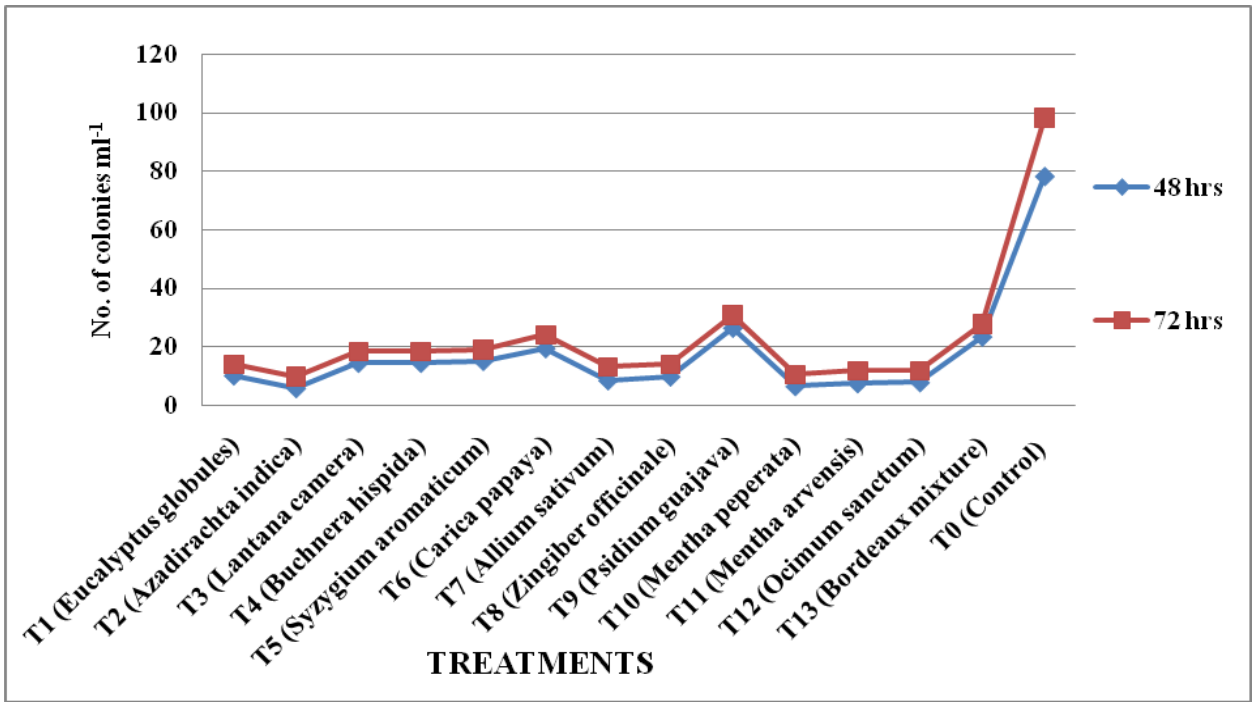


Figure : 4.14a Effect of essential oils at 4 % concentration on no. of colonies of *Xanthomonas campestris* pv. *campestris* at different hrs of interval.

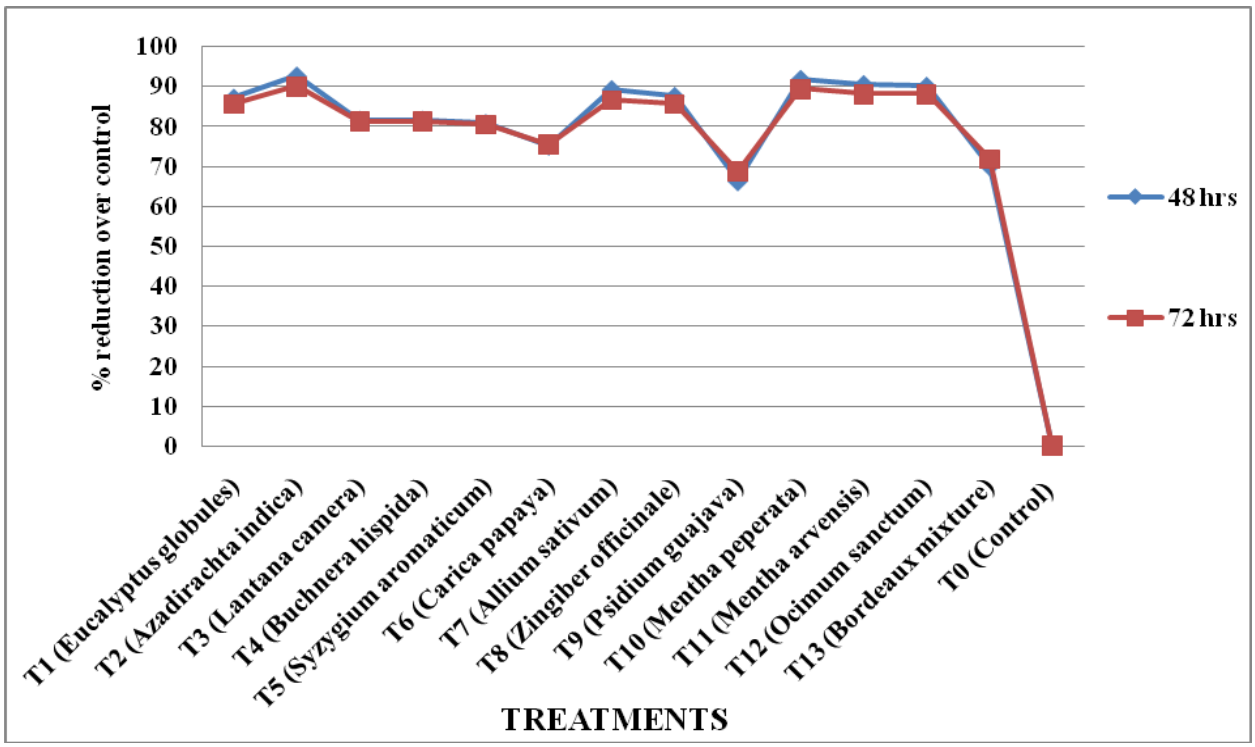


Figure : 4.14b Effect of essential oils at 4 % concentration on % reduction population over control of *Xanthomonas campestris* pv. *campestris* at different hrs of interval.

4.15 *In vitro* efficacy of essential oils at 6 % concentration on no. of colonies of *Xanthomonas campestris* pv. *campestris* :

A perusal of data documented in Table and Fig : 4.15ab, elucidate that the antibacterial effect of selected oils after 48 hrs at 6% concentration on the no. of colonies of *X. campestris* pv. *campestris* was least in the treatment T_2 - *Azadirachta indica* (2.66) followed by T_1 - *Eucalyptus globules* (4.33), T_3 - *Lantana camera* (5.66), T_{10} - *Mentha peperata* (6.66), T_{11} - *Mentha arvensis* (7.00), T_4 - *Buchnera hispida* (7.33) and T_{12} - *Ocimum sanctum* (8.33) including control (84.00). However, the treatments $T_{2,}$ $T_{1,}$ $T_{3,}$ T_{10} , $T_{11,}$ T_4 and T_{12} were found non significant effect in no. of bacterial colonies but they are significant from $T_{5,}$ $T_{7,}$ T_8 , $T_{6,}$ T_9 and $T_{13.}$ Among the treatments, the maximum no. of colonies was seen in T_{13} (Bordeaux mixture) followed by T_9 (*Psidium guajava*) and T_6 (*Carica papaya*) which are non significant among each other.

At 72 hrs after incubation of *X. campestris* pv. *campestris,* it was observed that minimum no. of colonies in T_2 - *Azadirachta indica* (8.66) as compared with T_{12} - *Ocimum sanctum* (10.0), T_1 - *Eucalyptus globules* (11.0), T_3 - *Lantana camera* (11.6), T_{10} - *Mentha peperata* (12.6), T_{11} - *Mentha arvensis* (13.0), T_4 - *Buchnera hispida* (13.6) including bordeaux mixture and control (84.00). Among the essential oil treatments, the maximum no. of colonies was significantly noticed in T_{13} – Bordeaux mixture (28.3) followed byT_9 (*Psidium guajava*), T_8 (*Zingiber officinale*) and T_6 (*Carica papaya*), which are not significant among each other. However, the treatments ($T_{2,}$ $T_{12,}$ $T_{1,}$ T_3 , $T_{10,}$ $T_{11,}$ T_4) and ($T_{5,}$ $T_{7,}$ $T_{6,}$ T_8 , T_9) were found non significant effect in no. of bacterial colonies but they are significant from T_{13} and $T_{0.}$

The highest percentage inhibition of *X. campestris* pv. *campestris* was obtained in T_2 (*Azadirachta indica*), T_{12} (*Ocimum sanctum*), T_1 (*Eucalyptus globules*), T_3 (*Lantana camera*), T_{10} (*Mentha peperata*), T_{11} (*Mentha arvensis*) and T_4 (*Buchnera hispida*), which are ranging from 91.8% to 87.1% on the incubation period of 72 hrs as compared with rest of essential oils.

Among all essential oils, neem oil was found maximum inhibition per cent at increasing with increasing concentration. Our results are agreement with **Atar *et al.* (2014)** who found neem oil was best effective for control *of X. axonopodis* pv. *punicae* at all concentration by forming 14.47

per cent inhibition followed by leaf extracts of garlic, Tulasi, Ginger, Guava and Aloe vera with 12.86%, 12.28%, 11.70%, 11.13%, 9.36% and 7.34 per cent average inhibition respectively.

Neem (*A. indica*) is widely used and well known plant from which seed extracts and oils are commonly used to control pathogens. It is probably reason of a high content of azadirachtin, its active ingredient, can be found both in oil and in the extract. Essential oils, being lipophilic in nature can easily penetrate deeper through living tissue unbarred by the selective permeability of the cell membrane, hence they are of interest in the management of fungal and bacterial diseases. The results are similar with those of **Chattopadhyay *et al.* (2004); Bhalodia *et al.* (2011),** who reported that number of plant derived natural products and its oils have to be antifungal and antibacterial in nature. Green plants and their products have proved their fruitfulness in providing less phytotoxic, more systemic, easily biodegradable and host metabolism stimulatory fungicides.

Essential oils have been used by many workers for controlling fungi, bacteria, viruses and insect pests **(Singh *et al*., 2001).** The antimicrobial properties of essential oils invariably depend on the chemical nature of the constituents present in them **(Nidiry, 1998).**

Treatments	CFU ml^{-1} of 10^{-7} dilution factor			
	48 hrs	% Inhibition over control	72 hrs	% Inhibition over control

Table : 4.15 *In vitro* efficacy of selected essential oils at 6 % concentration on no. of colonies of *Xanthomonas campestris* pv. *campestris* at different hrs of interval

T_1 *(Eucalyptus globules)*	4.33fg	94.84	11.00efg	89.62
T_2 (*Azadirachta indica*)	2.66g	72.83	8.66g	91.83
T_3 (*Lantana camera*)	5.66fg	93.26	11.66efg	89.00
T_4 (*Buchnera hispida*)	7.33defg	91.27	13.66defg	87.11
T_5 (*Syzygium aromaticum*)	9.33cdef	88.89	15.33def	85.53
T_6 (*Carica papaya*)	13.66cd	83.73	19.00cd	82.08
T_7 (*Allium sativum*)	10.66cdef	87.31	16.66cde	84.28
T_8 (*Zingiber officinale*)	13.00cde	84.52	19.00cd	82.08
T_9 (*Psidium guajava*)	15.66bc	81.36	21.66c	79.57
T_{10} (*Mentha peperata*)	6.66efg	92.07	12.66efg	88.06
T_{11} (*Mentha arvensis*)	7.00efg	91.67	13.00defg	87.74
T_{12} (*Ocimum sanctum*)	8.33defg	90.08	10.00fg	90.57
T_{13} (Bordeaux mixture)	21.33b	74.60	28.33b	73.27
T_0 (Control)	84.00a	-	106.00a	-
F-test	S		S	
S. Ed (±)	3.28		3.16	
CD (P = 0.05)	6.42		6.20	

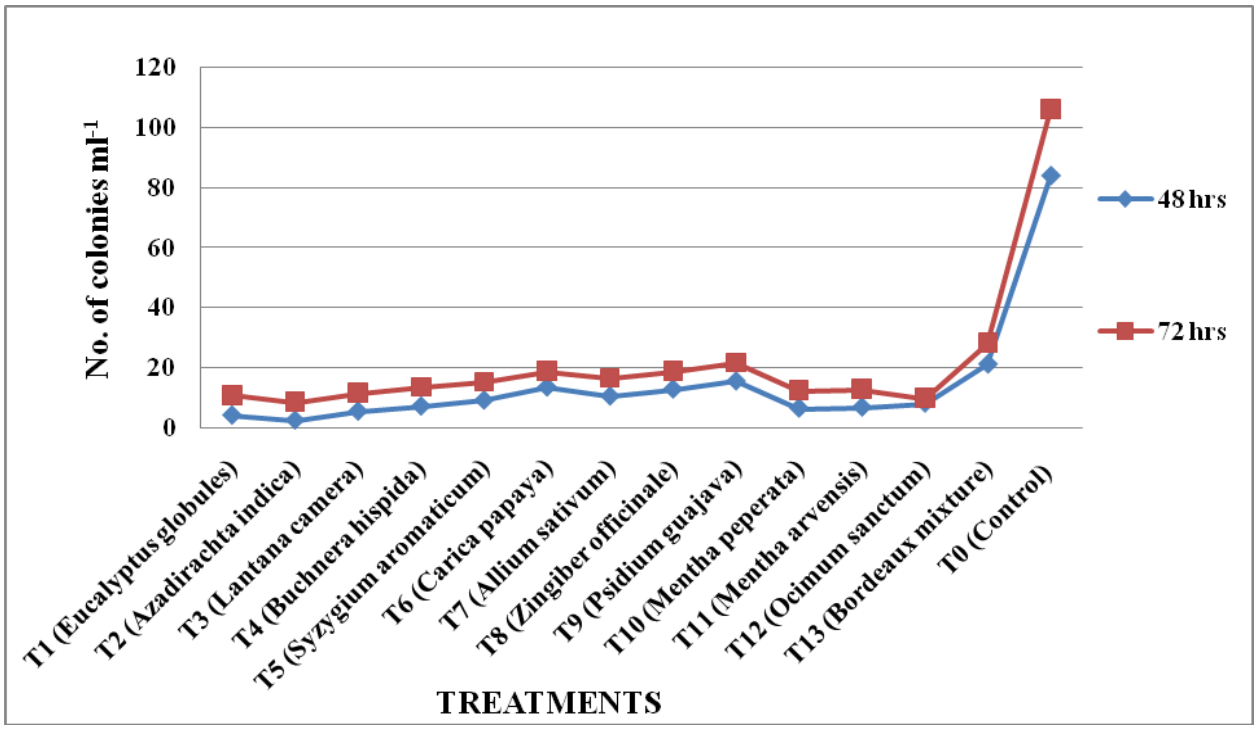


Figure : 4.15a Effect of essential oils at 6 % concentration on no. of colonies of *Xanthomonas campestris* pv. *campestris* at different hrs of interval.

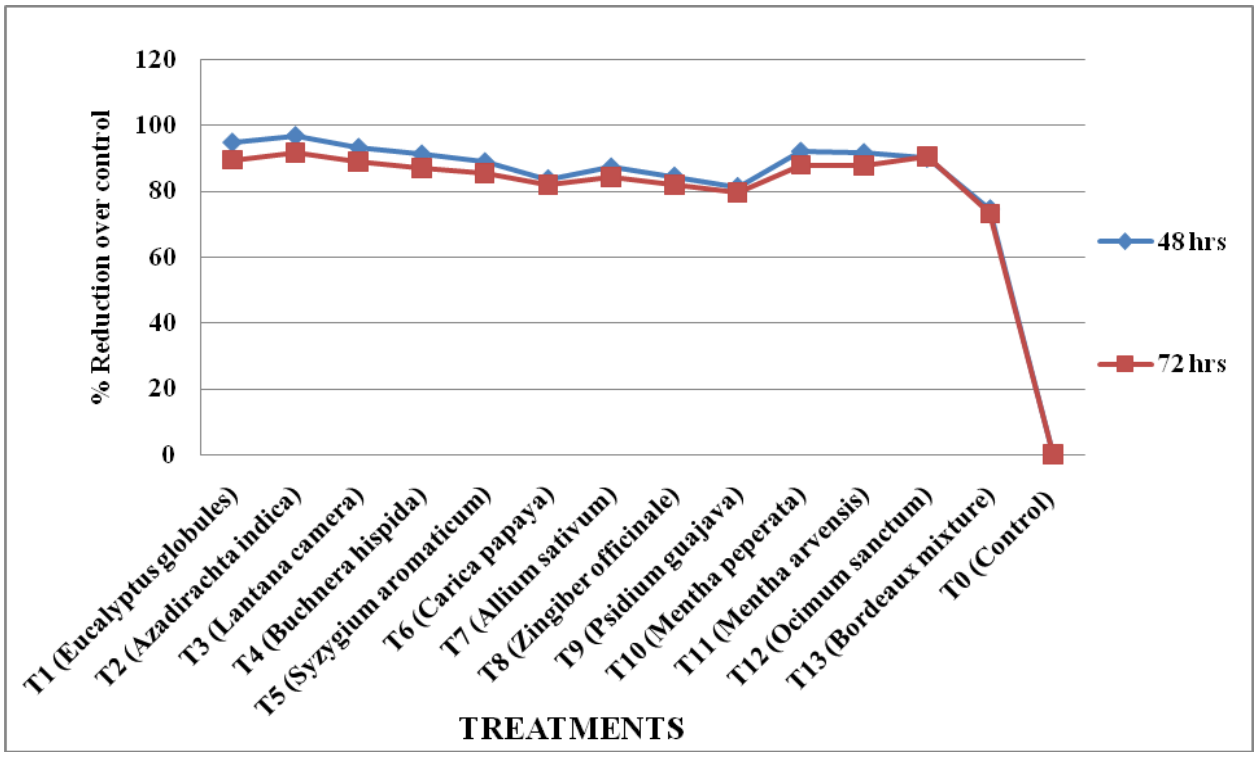


Figure : 4.15b Effect of essential oils at 6 % concentration on % reduction population over control of *Xanthomonas campestris* pv. *campestris* at different hrs of interval.

4.16 Field efficacy of selected botanical extracts at 75% concentration on disease intensity of black spot (*Alternaria brassicae*) of cabbage :

A perusal of data documented in Table and Fig : 4.16, elucidate that the antifungal effect of selected botanical extracts at 75% concentration on the disease intensity of black spot was reduced in the treatment T_{12} – Bordeaux mixture (15.2, 16.3, 19.9 and 21.9) at 45, 60, 75 and 90 DAS as compared with all the botanical extracts and also from control.

At 45 days after sowing, the disease intensity was reduced in T_7 – *Azadirachta indica* (15.53) followed by T_{12} (Bordeaux mixture), both are not significant from each other, but they are significantly reduced from rest of the botanical extracts including control. However, the treatments (T_8, T_2, T_9), (T_{11}, T_5, T_1, T_4) and (T_{10}, T_3, T_6) were showed non-significant effect among each other. Among the botanical extracts, the maximum per cent diseases intensity was recorded in the treatment T_6 (*Datura stramonium*) followed byT_3 (*Menthe peperata*), T_{10} (*Mentha arvensis*) and T_4 (*Allium sativum*).

At 60 DAS, results revealed that among the plant extracts the per cent disease intensity was significantly reduced in the treated plots with *Azadirachta indica* leaf extract (17.16) followed by Eucalyptus (19.03), Calotropis (19.76), Garlic (22.16), Peppermint (27.16), Tulsi (23.86), Lantana (25.20), Datura (25.66), Mint (27.16), Ginger (28.60) and Croton (30.86) including control (34.10). Whereas, all the botanicals were shows significantly reduction of the black leaf spot disease remaining T_5 and T_6. While treatment T_5 and T_6 were found non-significant to each other but they are significantly reduced from T_{10}, T_9, T_{11} and T_0.

At 75 days after sowing, the disease intensity was observed in the treated plots with leaf extracts of neem (20.2) and eucalyptus (21.3), which are insignificantly differ among them selves but it was significant from rest of botanical extracts including control. However, the treatments (T_2, T_4) and (T_9, T_{10}) were found non-significant effect among each other. Whereas, rest of botanical extracts are seen significant effect from T_0.

Final data was observed that maximum reduction of leaf spot disease of cabbage was in the treatment T_{12} – Bordeaux mixture (21.93) as compared with all the aqueous leaf extracts. Among leaf extracts, neem and eucalyptus were observed not significantly reduction of the disease but

they are significantly reduced the disease intensity from all other plant extracts. Whereas, treatments T_2, T_4, T_3, T_1, T_5 were found significant in results. While, treatment (T_{10}, T_9) and (T_9, T_{11}) were showed non significant among each other.

A. indica seed kernel, leaves, bark and roots possess antimicrobial, antiseptic and insecticidal properties. Different plant parts of *A. indica* contain many chemicals inhibitory to microbes. Most of these plant parts are bitter in taste due to presence of an array of complex compounds known as limonoids. The fruits containing nimbiol and azadirachtin. Azadirachtin and limonoids are considered as most bioactive in gradients having microbial property **(Prajapati *et al.*, 2003).** Several leaf extracts like, *Eucalyptus, Citriodora* **(Ramezani, 2006*),*** *Lantana camara* **(Deena and Thoppil, 2000),** *Ocimum basilium* **(Boyraz and Ozcan, 2005)** and *Azadirachta indica* **(Bhonde *et al.*, 1999)** can be efficient in controlling agents against *Alternaria brassicae*.

The results are agreement with those of **Patni and Kolte (2006)** who revealed that neem and eucalyptus spray gave significantly lesser number of mustard black leaf spots/leaf (2.05), minimum size of spot (1.28 mm), minimum sporulation intensity (1.22X105) and minimum disease index (13.72) followed by Calotropis, Ocimum and Polyanthai extracts spray, which might be due to neem leaves contain nimbin, nimbinene, 6-desacetylnimbinene, nimbandiol, nimbolide and quercetin.

Hassanein *et al.* (2008) also reported tomato plants spraying with 20% aqueous neem leaf extracts lowered the disease incidence to 42.54% while spray and irrigation reduced disease incidence to 39.49%. Spray and irrigation with 20% aqueous extracts from neem leaves seemed to work in a synergism in controlling the disease.

Srivastava *et al.* (1997) showed the properties of aqueous leaf extracts of *A. indica* against *A. alternata* from pear fruits with 85 % control of fruit rot *in vivo*.

Results were in conformity with those by **Chattopadhyay (1999)** who found that foliar spray of *A. indica* leaf extract and azadirachtin reduced mycelia growth of *A. alternata* (causing loss of sunflower and tomato), decreased disease severity and increased yield over control.

Table : 4.16 Field efficacy of selected botanical extracts at 75% concentration on disease intensity of black spot (*Alternaria brassicae*) of cabbage at different days of interval

Treatments	% Disease Intensity Mean			
	45 DAS	60 DAS	75 DAS	90 DAS
T_1 (*Ocimum sanctum*)	17.13bcd	23.86 f	27.86 f	34.13f
T_2 (*Calotropis procera*)	16.46cde	19.76i	23.70h	26.16i
T_3 (*Mentha peperata*)	17.40bc	23.06g	26.33g	30.63g
T_4 (*Allium sativum*)	17.26bcd	22.16h	24.53h	27.80h
T_5 (*Lantana camara*)	17.13bcd	25.20e	29.93e	35.90e
T_6 (*Datura stramonium*)	17.93b	25.66e	32.10d	37.63d
T_7 (*Azadirachta indica*)	15.53ef	17.16k	20.20i	23.60j
T_8 (*Eucalyptus globules*)	16.30de	19.03j	21.03i	24.30j
T_9 (*Zingiber officinale*)	16.80cd	28.60c	34.26c	41.83bc
T_{10} (*Mentha arvensis*)	17.33bc	27.16d	34.46c	40.46c
T_{11} (*Croton boplandianum*)	17.06bcd	30.86b	36.26b	42.90b
T_{12} (Bordeaux mixture)	15.23f	16.33 l	19.93i	21.93k
T_0 (Control)	20.46a	34.10a	41.72a	51.56a
F-test	S	S	S	S
S. Ed (±)	0.47	0.29	0.60	0.66
CD (P = 0.05)	1.02	0.65	1.31	1.44

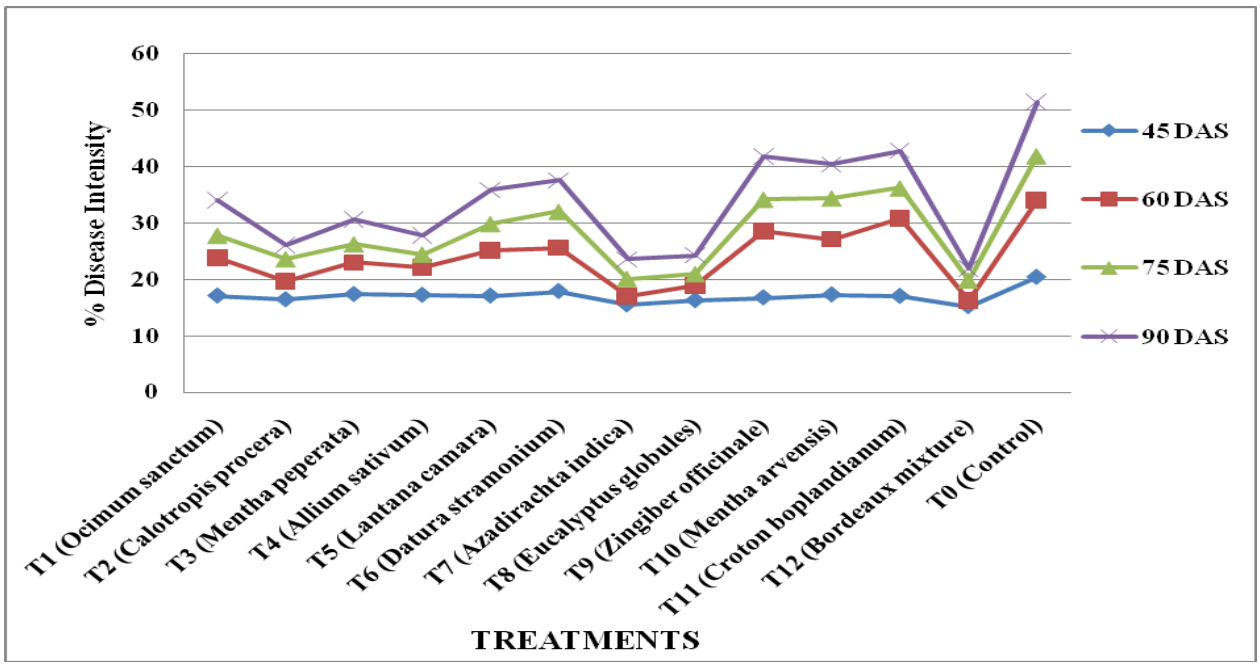


Figure : 4.16 Effect of selected botanical extracts on disease intensity of black spot (*Alternaria brassicae*) of cabbage at different days interval.

4.17 Field efficacy of selected botanical extracts at 75 % concentration on disease intensity of black rot (*Xanthomonas campestris* pv. *campestris*) :

The data are presented in Table and Fig : 4.17, elucidate that the antibacterial effect of selected botanical extracts at 75% concentration on the disease intensity of black rot was significantly reduced in the treated plots with T_7 –*Azadirachta indica* (5.8, 12.1, 14.5, 17.2%) followed by rest of botanical leaf extracts at 45, 60, 75 and 90 days after sowing, respectively.

At 45 days after sowing, the disease intensity of black rot was significantly reduced in the treated plots with T_7 –*Azadirachta indica* (5.86) followed by T_8 –*Eucalyptus globules* (6.70), T_2 – *Calotropis procera* (7.40), T_4 –*Allium sativum* (7.93), T_3 –*Mentha peperata* (8.30), T_1 –*Ocimum sanctum* (9.16), T_5 –*Lantana camara* (9.93), T_6 –*Datura stramonium* (10.63), T_{12} –*Bordeaux mixture* (10.66), T_{10} –*Mentha arvensis* (11.36), T_{11} – *Croton boplandianum* (12.53) and T_0 (13.76). However, the treatments (T_8, T_2), (T_2, T_4) and (T_4, T_3) were shows no significant difference among each other but they are found significant from rest of botanical extracts. Whereas, rest of botanical treatments are seen non significant effect to each other excluding the treatment T_1.

At 60 DAS, results revealed that the disease intensity (%) of black rot of cabbage, it was observed that minimum disease intensity in the treatment T_7 - neem (12.10) and T_8 – Eucalyptus (13.36), which are shows non significant among themselves but significant from leaf extract of calotropis (15.00), garlic (15.70), peppermint (17.30), tulsi (17.73), lantana (18.76), datura (19.43), mint (20.93), ginger (21.16), croton (21.60) including Bordeaux mixture (22.1) and control (25.13). However, the treatments (T_2, T_4), (T_3, T_1), (T_1, T_5), (T_5, T_6) and (T_{10}, T_9, T_{11}, T_{12}) were shows non-significant reduction of the disease but they are significant from control.

At 75 DAS, disease intensity (%) of black rot was found significantly reduced in the treated plots with neem extract (14.50) as compared from all other plant extracts including Bordeaux mixture treated plants. However, sprayed plots with eucalyptus, calotropis, garlic and mint were found significantly least in disease intensity. Whereas, treatments (T_{12}, T_1, T_5), (T_{10}, T_{11}) are shown non significant among each other. All the plant extracts found significantly reduced the diseases intensity of black rot over contrl.

At 90 days after sowing, the results revealed that the reduction of black rot disease intensity in the plot that was sprayed with leaf extracts of neem (17.20) and eucalyptus (19.16) followed by other leaf extracts and Bordeaux mixture also. Whereas, the treatments (T_8, T_2), (T_2, T_4), (T_4, T_3) and (T_3, T_{12}, T_1) were showed no significant difference between among themselves which are significant from T_6, T_{10}, T_{11} and T_9 including with control.

Neem extracts which are cheap and environmentally safe, exhibited considerable control of disease development and may be considered promising for protection of cabbage plants against leaf spot and black rot pathogens with possible improvement of this economic crop.

Per cent disease intensity of black rot of cabbage was reduced in the spray plot with neem leaf extract followed by eucalyptus and all other botanical extracts at 75% concentration. This might be due to presence of various secondary metabolites such as alkaloids, quaternary alkaloids, coumarins, flavanoids, steroids/ terpenoids, phenols etc. have been reported in the various plants extracts **(Aswal *et al*., 1984; Chopra *et al*., 1992)** which may be responsible for the antibacterial properties of the plants studied.

This result is supported by **Didwania *et al.* (2013)** reported that *Azadirachta indica*, *Vernonia anthelmentica*, *Tagetes erecta* and *Plumbago zelanicum* showed very good inhibition zone of black rot pathogen 23.75, 22.83, 20.46 and 20.42 mm, at its 50% concentration, respectively.

Table : 4.17 Field efficacy of selected botanical extracts at 75 % concentration on disease intensity of black rot (*X. campestris* pv. *campestris*) of cabbage at different days interval

Treatments	% Disease Intensity Mean			
	45 DAS	60 DAS	75 DAS	90 DAS
T_1 (*Ocimum sanctum*)	9.16f	17.73 de	23.36 d	25.20de
T_2 (*Calotropis procera*)	7.40hi	15.00f	19.03g	20.56gh
T_3 (*Mentha peperata*)	8.30g	17.30e	21.93e	23.33ef
T_4 (*Allium sativum*)	7.93gh	15.70f	20.53f	22.33fg
T_5 (*Lantana camara*)	9.93e	18.76cd	24.26d	26.76d
T_6 (*Datura stramonium*)	10.63de	19.43c	25.70c	28.80c
T_7 (*Azadirachta indica*)	5.86j	12.10g	14.50i	17.20i
T_8 (*Eucalyptus globules*)	6.70i	13.36g	16.50h	19.16hi
T_9 (*Zingiber officinale*)	12.10bc	21.16b	27.53b	32.26b
T_{10} (*Mentha arvensis*)	11.36cd	20.93b	26.70bc	30.46bc
T_{11} (*Croton boplandianum*)	12.53b	21.60b	26.93bc	30.46bc
T_{12} (Bordeaux mixture)	10.66de	22.16b	23.30d	24.83de
T_0 (Control)	13.76a	25.13a	29.20a	36.13a
F-test	S	S	S	S
S. Ed (±)	0.34	0.62	0.57	0.92
CD (P = 0.05)	0.74	1.36	1.24	2.01

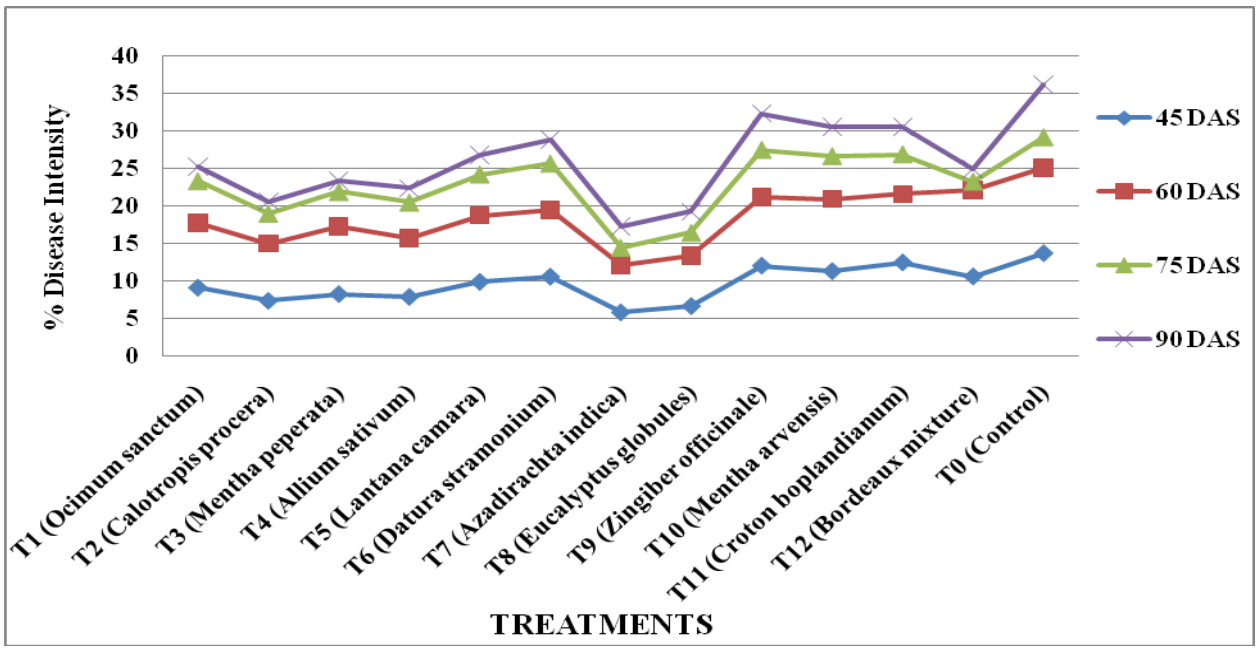


Figure : 4.17 Effect of selected botanical extracts at 75 % concentration on disease intensity of black rot (*X. campestris* pv. *campestris*) of cabbage at different days interval.

4.18 Field efficacy of selected botanical extracts at 75 % concentration on head diameter (cm) of cabbage :

A perusal of data documented in Table and Fig : 4.18, elucidate that all the selected botanical extracts at 75% concentration on head diameter (cm) of cabbage was increased in the treated plots with T_{12} - bordeaux mixture (17.0, 25.1) and leaf extracts of T_7- *Azadirachta indica* (16.0, 24.8) as compared with other botanical extracts at 90 and 120 days after sowing, respectively.

At 90 days after sowing, among the botanical leaf extracts the data shown that the head diameter (cm) of cabbage was increased in the treatment T_7- *Azadirachta indica* (16.00) followed by T_4- *Allium sativum* (15.56) and T_5- *lantana camara* (14.30), which are non significant among each other but they are significant from T_2 –*Calotropis procera* (11.76), T_6-*Datura stramonium* (11.46), T_3 –*Mentha peperata* (10.60), T_9- *Zingiber officinale* (9.86), T_8 – *Eucalyptus globules* (9.73), T_{11} – *Croton boplandianum* (9.33), T_{10} –*Mentha arvensis* (9.20) and T_1 –*Ocimum sanctum* (8.66) including with control (7.90). Among the leaf extracts, the minimum head diameter of cabbage was noticed in the treatment T_1 (*Ocimum sanctum*), T_{10} (*Mentha arvensis*), T_{11} (*Croton boplandianum*), T_8 (*Eucalyptus globules*), T_9 (*Zingiber officinale*) and T_3 (*Mentha peperata*) are non significant to each other.

At 120 DAS, the head diameter was increased with increasing of date and highest diameter (cm) was observed in the treated plots with neem leaf (24.8) extract followed by all other botanical treatments excluding Bordeaux mixture. However, the treatments T_5 (*Lantana camara*), T_4 (*Allium sativum*) and T_1 (*Ocimum sanctum*) were found insignificantly increased the head diameter as compared with rest of botanical extracts. Whereas, treatment T_2 (*Calotropis procera*) was recorded significantly increased the diameter of cabbage head as comparison from other leaf extracts treated plots.

All the sprayed botanical leaf extracts showed increasing the head diameter of cabbage as compared with control but neem was superior amongst the botanicals. The results are agreement with **Tuan *et al.* (2014)** who revealed that Garlic and chili combination solution was the effectively reduced cabbage insect pests. On other hand, the spray solution not only reduced the number of days required for the cabbage growth but also greatly enhanced the leaf number, head diameter, head weight and quality of cabbage. Garlic and chili combination solution have positive effects on pests reduction and improve growth, yield and quality of cabbage vegetable.

Table : 4.18 Field efficacy of selected botanical extracts at 75 % concentration on head diameter of cabbage at different days after sowing

Treatments	Head diameter (cm)	
	90 DAS	120 DAS
T_1 (*Ocimum sanctum*)	8.66 cd	21.66c
T_2 (*Calotropis procera*)	11.76bc	19.16d
T_3 (*Mentha peperata*)	10.60cd	17.53e
T_4 (*Allium sativum*)	15.56a	22.53bc
T_5 (*Lantana camara*)	14.30ab	23.46b
T_6 (*Datura stramonium*)	11.46bc	17.86e
T_7 (*Azadirachta indica*)	16.00a	24.86a
T_8 (*Eucalyptus globules*)	9.73cd	15.66fg
T_9 (*Zingiber officinale*)	9.86cd	14.80g
T_{10} (*Mentha arvensis*)	9.20cd	16.86ef
T_{11} (*Croton boplandianum*)	9.33cd	14.70g
T_{12} (Bordeaux mixture)	17.06a	25.13a
T_0 (Control)	7.90d	13.23h
F-test	S	S
S. Ed (±)	1.46	0.59
CD (P = 0.05)	3.19	1.29

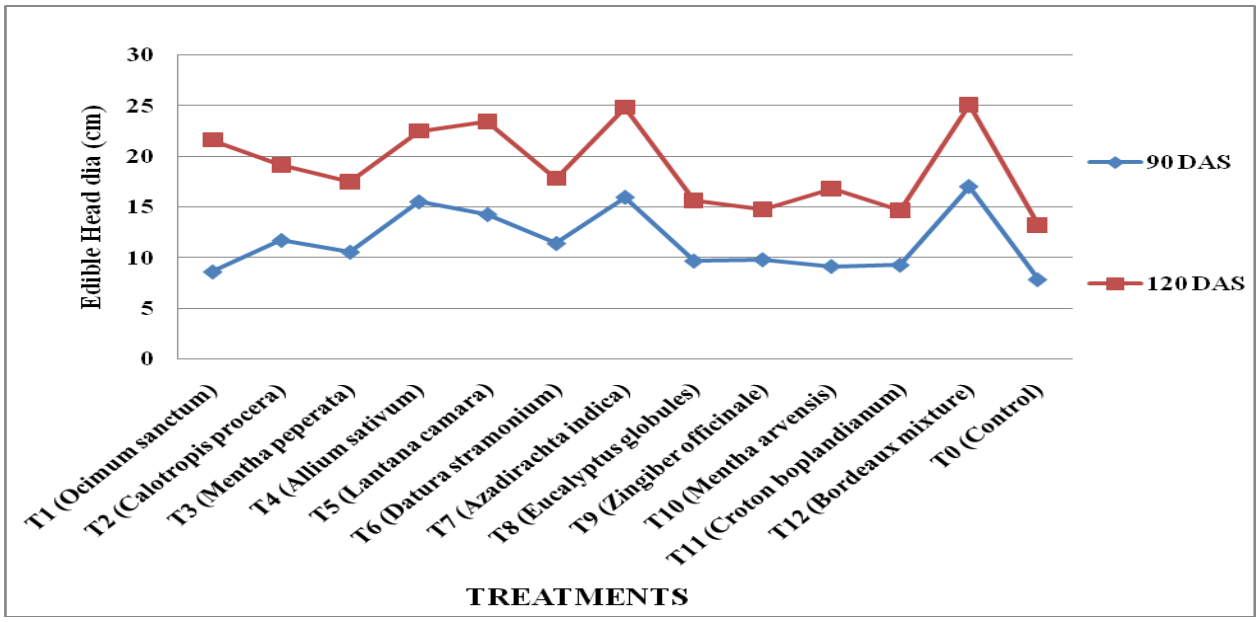


Figure : 4.18 Effect of selected botanical extracts at 75 % concentration on head diameter of cabbage at different days interval.

4.19 Field efficacy of selected botanical extracts at 75% concentration on edible head weight (gm) of cabbage :

The results of leaf extracts on yield (g) are presented in table and fig : 4.19, indicated that the Bordeaux mixture (1504.72g) was significantly increased the yield of edible head of cabbage followed by all extracts of leaf including with control. Among the leaf extracts treatments, T_7 - *Azadirachta indica* (1429.86) and T_4 - *Allium sativum* (1417.56) were shown not significant, but they are significant from T_5 –*Lantana camara* (1373.73), T_8 –*Eucalyptus globules* (1346.33), T_1 –*Ocimum sanctum* (1336.83), T_2 –*Calotropis procera* (1316.80), T_6 –*Datura stramonium* (1234.83), T_3 – *Mentha peperata* (1139.80), T_9 –*Zingiber officinale* (1015.56), T_{10} –*Mentha arvensis* (930.83) and T_{11} –*Croton boplandianum* (799.13) including with control (580.60). Whereas, the treatments (T_5, T_8, T_1) and (T_8, T_1, T_2) were found non-significant among themselves while these are significant from rest of botanical treatments. However treatments T_6, T_3, T_9, T_{10} and T_{11} were performed significant increased yield of cabbage from T_0.

The results of this study corroborated those of earlier works in reducing foliar diseases and subsequent increase in plant yield by neem and other botanical extracts. **Javaida *et al.* (2000)** had recorded the neem treated cabbage plant was significantly higher yield of marketable heads of cabbage and significantly better control of pest. Similar results have also been reported by **Patil *et al*. (2001)** showed that neem leaf extract was effective in reducing early blight incidence with increased yield of tomato infected by *A. solani*.

Table : 4.19 Field efficacy of selected botanical extracts at 75% concentration on edible head weight (gm) of cabbage at 120 DAS

Treatments	Cabbage weight (gm)			Mean
	R_1	R_2	R_3	
T_1 (*Ocimum sanctum*)	1326.2	1335.9	1348.4	1336.83cd
T_2 (*Calotropis procera*)	1312.3	1316.6	1321.5	1316.80d
T_3 (*Mentha peperata*)	1142.6	1143.9	1132.9	1139.80f
T_4 (*Allium sativum*)	1411.2	1415.8	1425.7	1417.56b
T_5 (*Lantana camara*)	1380.3	1365.1	1375.8	1373.73c
T_6 (*Datura stramonium*)	1220.3	1236.8	1247.4	1234.83e
T_7 (*Azadirachta indica*)	1424.1	1455.2	1410.3	1429.86b
T_8 (*Eucalyptus globules*)	1345.3	1351.4	1342.3	1346.33cd
T_9 (*Zingiber officinale*)	1002.4	1023.5	1020.8	1015.56g
T_{10} (*Mentha arvensis*)	930.5	920.7	941.3	930.83h
T_{11} (*Croton boplandianum*)	821.6	790.4	785.4	799.13i
T_{12} (Bordeaux mixture)	1542.1	1497.5	1475.3	1504.72a
T_0 (Control)	550.2	651.3	540.3	580.60j
F-test				S
S. Ed (±)				17.58
CD (P = 0.05)				38.33

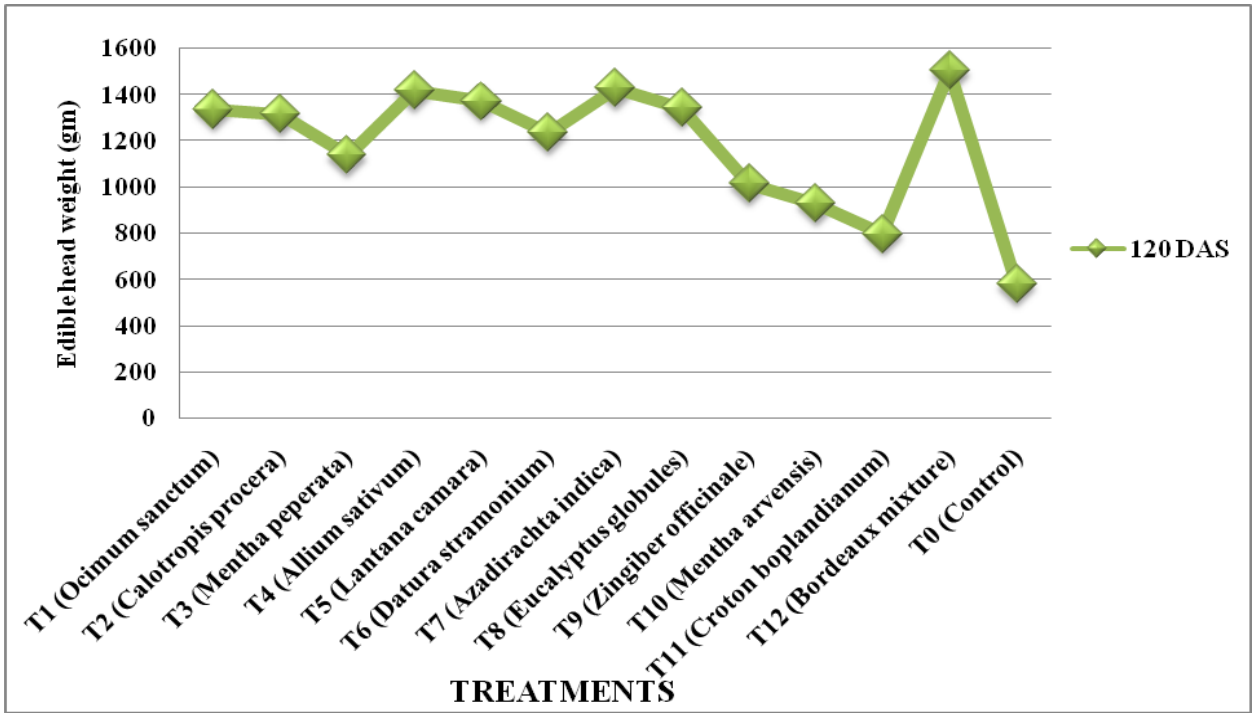


Figure : 4.19 Effect of selected botanical extracts at 75 % concentration on edible head weight (gm) of cabbage at 120 DAS.

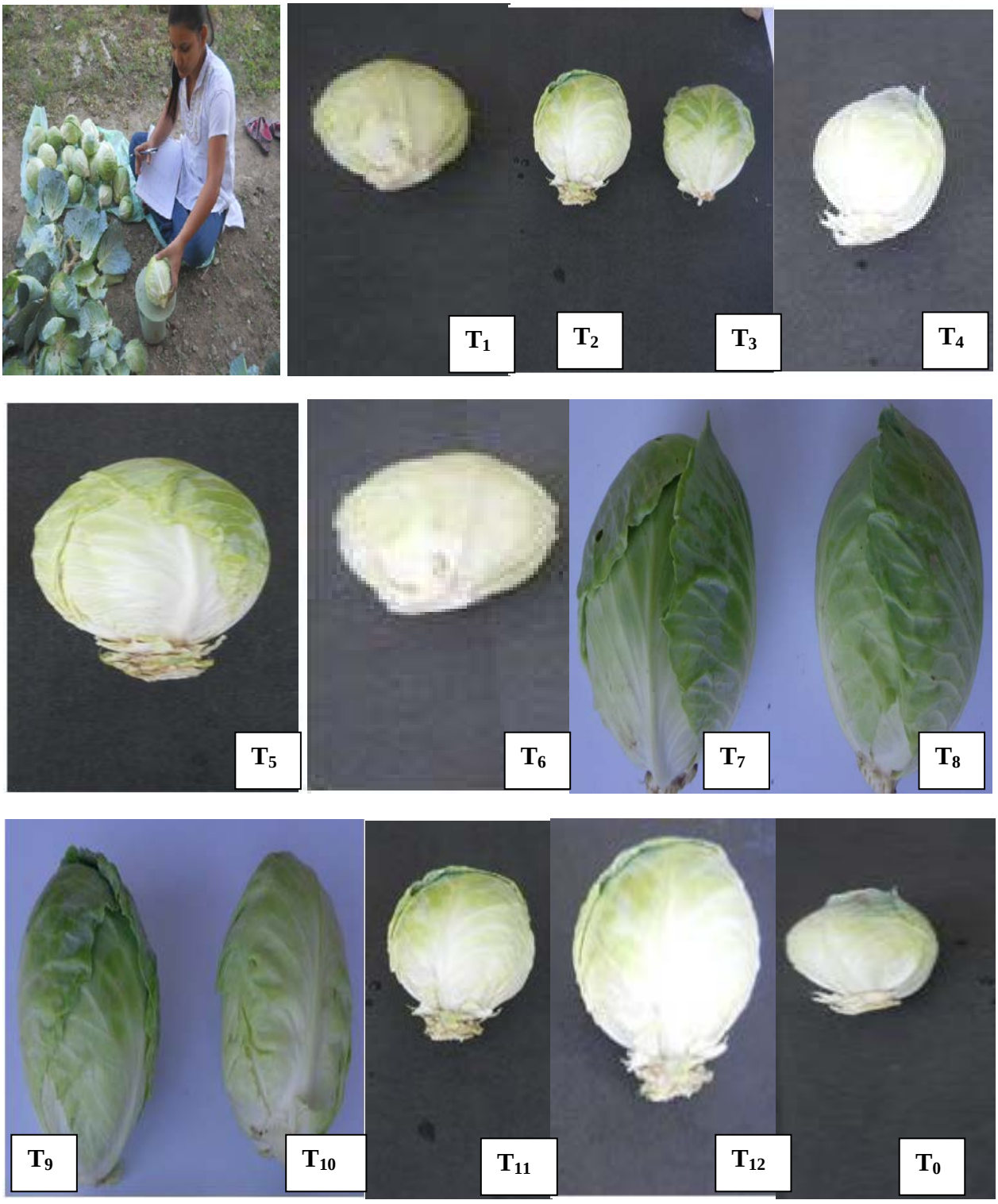


Plate: . Effect of selected botanical extracts at 75 % concentration on edible head weight (gm) of cabbage at 120 DAS.

4.20 Effect of essential oils at 6 % concentration on disease intensity of black spot (*Alernaria brassicae*) of cabbage :

The data documented in Table and Fig : 4.20, elucidate that the antifungal effect of selected essential oils at 6% concentration on disease intensity of black spot was reduced in the treated plots with T_2 –*Azadirachta indica* (7.96, 13.20, 18.20, 21.35%) followed by all other essential oils including Bordeaux mixture and control at 45, 60, 75 and 90 days after sowing, respectively.

At 45 DAS, results shows that the maximum reduction of disease intensity of black leaf spot was recorded in the treatment T_2 –*Azadirachta indica* (7.96) and T_{13} – *Bordeaux mixture* (8.03) followed by T_{10} – *Mentha peperata* (9.20), T_{11} – *Mentha arvensis* (9.50), T_7 – *Allium sativum* (9.63), T_1 – *Eucalyptus globules* (10.10), T_{12} – *Ocimum sanctum* (10.46), T_3 – *Lantana camara* (10.60), T_5 – *Syzygium aromaticum* (11.43), T_8 – *Zingiber officinale* (12.33), T_4 – *Buchnera hispida* (12.73), T_6 – *Carica papaya* (13.36) and T_9- *Psidium guajava* (13.56) including with control (17.26). Whereas, the treatments (T_{10}, T_{11}, T_7, T_1, T_{12}, T_3), (T_1, T_{12}, T_3, T_5), (T_5, T_8, T_4) and (T_8, T_4, T_6, T_9) were found non-significant among each other but they are significant from T_0.

At 60 days after sowing of cabbage seeds, the black leaf spot disease was noticed in the T_2 – *Azadirachta indica* (13.53) and T_{13} –Bordeaux mixture (13.20), which are non significant effect on leaf spot but they are significant from all the essential oil treatments including control. Among the oil treatments, T_{10} (*Mentha peperata*), T_7 *(Allium sativum*) and T_{11} (*Mentha arvensis*) were also recorded minimum disease intensity as compared with rest of essential oil treated plots. However, the treatments (T_1, T_3, T_{12}), (T_5, T_4, T_8) and (T_6, T_9) were shows no significant difference between to each other.

At 75 DAS, minimum % disease intensity was noticed in treated plots with T_2 –*Azadirachta indica* (18.2) followed by T_{13} - Bordeaux mixture (19.13), T_{11} – *Mentha arvensis* (20.1) and T_{10} – *Mentha peperata* (20.8) are not significant among each other but they are significant from rest of essential oils over control. Whereas, the treatments (T_{12}, T_7, T_1, T_5) and (T_3, T_4) were shows significant effect from T_8, T_6 and T_9 including with control.

At 90 DAS, among the essential oil, the treatment T_2 –*Azadirachta indica* (21.3) was observed maximum reduction of disease intensity (%) as compared with all other essential oil treatments including bordeaux mixture and control. However, the treatments T_{13} (*Bordeaux mixture*, 22.7), T_8 (*Zingiber officinale*, 23.3), T_{10} (*Mentha peperata*, 23.8), T_1 (*Eucalyptus globules*, 24.5), T_{11} (*Mentha arvensis*, 24.5), T_3 (*Lantana camara*, 24.8) and T_7 (*Allium sativum*, 24.8), are not significantly reduced the disease intensity among themselves but they are significant from T_{12}, T_5, T_4, T_6 and T_9, were shows non-significant in results.

Plant extracts and oils are known to possess toxic organic poison that is effective in reducing the intensity of plant diseases. This probably reason of a high content of azadiractin, its active ingredient, can be found both in the oil and in the neem extract. Moreover, several authors have shown the efficacy of different plant materials as biopesticides for the control of different pest **(Oparaeke *et al.*, 2000).** Similar result have been reported by **Babu *et al.*, (2000)** who mentioned that spraying with 3 % of neem oil in tomato pot cultures resulted in 53 % reduction in disease incidence over the control while **Patil *et al.*, (2001)** found that incidence of tomato early blight caused by *A. solani* was affected by a botanical like neem seed extract with increased fruit yield between 156.43 and 168.56q / ha. **Jackai and Oyediran (1991)** have also been reported that neem, black pepper, garlic bulb and nutmeg, Lippia adoensis Hoschst to be effective against some crop pests species.

Table : 4.20 Field efficacy of essential oils at 6% concentration on disease intensity of black spot (*Alternaria brassicae*) of cabbage at different days of interval

Treatments	% Disease Intensity Mean			
	45 DAS	60 DAS	75 DAS	90 DAS
T_1 *(Eucalyptus globules)*	10.10de	16.33de	22.06de	24.50 bc
T_2 (*Azadirachta indica*)	7.96f	13.2h	18.20h	21.30c
T_3 (*Lantana camera*)	10.60de	16.73de	22.43gh	24.86b
T_4 (*Buchnera hispida*)	12.73bc	17.56cd	22.43d	27.83b
T_5 (*Syzygium aromaticum*)	11.43cd	17.46cd	22.20de	27.16b
T_6 (*Carica papaya*)	13.36b	19.26b	25.20bc	28.70b
T_7 (*Allium sativum*)	9.63e	15.63ef	21.90de	24.86bc
T_8 (*Zingiber officinale*)	12.33bc	18.73bc	23.90c	23.30bc
T_9 (*Psidium guajava*)	13.56b	19.72b	26.50b	28.90b
T_{10} (*Mentha peperata*)	9.20ef	14.90fg	20.83ef	23.80bc
T_{11} (*Mentha arvensis*)	9.50ef	15.90ef	20.16fg	24.50bc
T_{12} (*Ocimum sanctum*)	10.46de	16.73de	21.60de	25.46b
T_{13} (Bordeaux mixture)	8.03f	13.53gh	19.13gh	22.70bc
T_0 (Control)	17.26a	27.56a	38.66a	50.16a
F-test	S	S	S	S
S. Ed (±)	0.74	0.67	0.66	3.05
CD (P = 0.05)	1.57	1.39	1.41	6.47

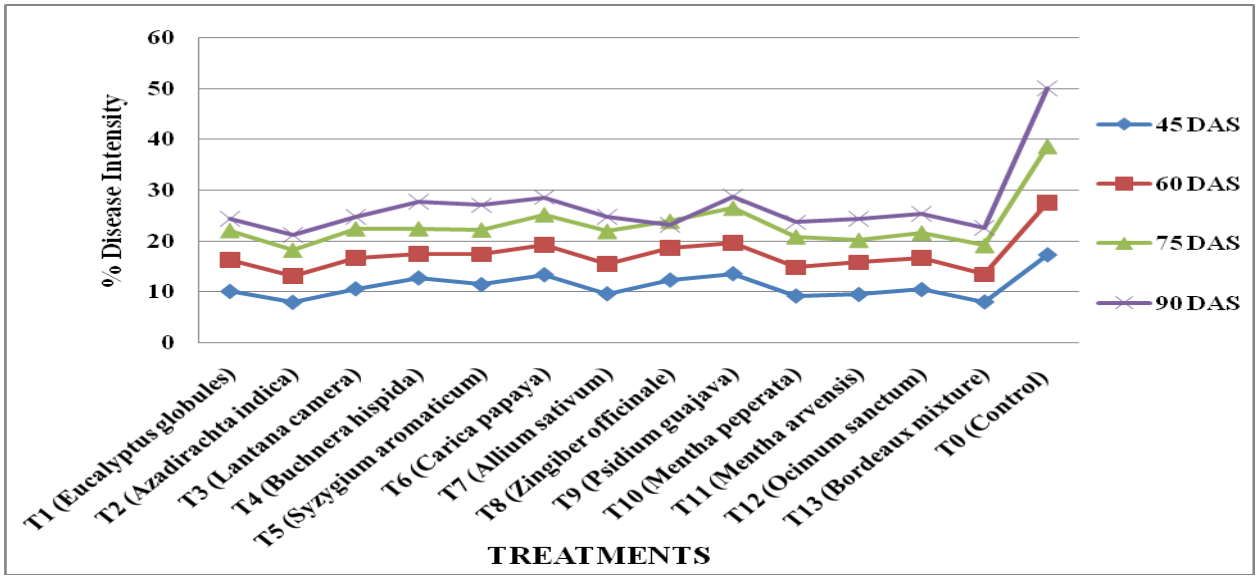


Figure : 4.20 Effect of selected essential oils at 6 % concentration on disease intensity of black spot (*Alernaria brassicae*) of cabbage at different days intervals.

4.21 Field efficacy of essential oils at 6 % concentration on disease intensity of black rot (*Xanthomonas campestris* pv. *campestris*) of cabbage :

The activity of the essential oils against % disease intensity of black rot of cabbage is presented in table and fig : 4.21. It was observed that essential oils of *Syzygium aromaticum* and *Eucalyptus globules* were significantly reduced in the disease intensity (3.23 and 5.33%, respectively) showed maximum reduction as compared with T_2 – *Azadirachta indica* (6.36), T_3 – *Lantana camera* (6.80), T_{10} – *Mentha peperata* (8.00), T_4 – *Buchnera hispida* (8.36), T_7 – *Allium sativum* (9.26), T_8 – *Zingiber officinale* (9.63), T_{11} – *Mentha arvensis* (10.56), T_{12} – *Ocimum sanctum* (10.60), T_{13}- Bordeaux mixture (13.53),T_6- *Carica papaya* (13.80) and T_9 – *Psidium guajava* (14.06) including with control (15.16). However, treatments (T_2, T_3), (T_{10}, T_4), (T_7, T_8), (T_{11}, T_{12}) and (T_{13}, T_6, T_9) were recorded non-significant differences among themselves but they are significant from control.

After 60 DAS, the disease intensity of black rot was significantly reduced in the oil of *Syzygium aromaticum* (8.8%) as compared with all the essential oils including Bordeaux mixture and control. However, the treatments T_1 and T_2 were showed non-significant and found least disease intensity but they are significant from T_3, T_4, T_7, T_8, T_{10}, T_{11}, T_{12}, T_{13}, T_9 and T_6. While, these treatments were found significant in results.

The result shows at 75 DAS, the intensity of black rot was significantly reduced in the treated plot with *Syzygium aromaticum* oil (11.73%) followed by T_1 (*Eucalyptus globules,* 13.76), T_2 – *Azadirachta indica* (15.26), T_3 – *Lantana camera* (16.66), T_4 – *Buchnera hispida* (1766), T_7 – *Allium sativum* (18.66), T_8 – *Zingiber officinale* (19.9), T_{10} – *Mentha peperata* (20.83), T_{11} – *Mentha arvensis* (21.53), T_{12} – *Ocimum sanctum* (22.23), T_6- *Carica papaya* (22.36), T_{13}- Bordeaux mixture (22.46) and T_9 – *Psidium guajava* (23.33) including with control (27.36). Whereas, treatments T_1, T_2, T_3, T_4, T_7 and T_8 were noticed significant in result. However, treatments (T_{10}, T_{11}), (T_{11}, T_{12}) and (T_{12}, T_6, T_{13}) were found non significant among each other but they are significant from T_9 and T_0.

At 90 DAS, showed the finally result was recorded significantly maximum reduction of disease intensity in the treatment T_5 - *Syzygium aromaticum* (14.73%) followed by *Eucalyptus globules*, *Azadirachta indica*, *Lantana camera*, *Buchnera hispida*, *Allium sativum*, *Mentha peperata*,

Zingiber officinale, *Mentha arvensis*, *Psidium guajava*, *Carica papaya*, *Ocimum sanctum* and Bordeaux mixture including with control. Whereas, treatments (T_1, T_2, T_3), (T_2, T_3, T_4), (T_4, T_7) and (T_7, T_{10}, T_8, T_{11}, T_9, T_6, T_{12}, T_{13}) were recorded not significant among themselves but they are significant from each other.

Table : 4.21 Field efficacy of essential oils at 6 % concentration on disease intensity of black rot (*Xanthomonas campestris* pv. *campestris*) of cabbage at different days interval

Treatments	% Disease Intensity Mean			
	45 DAS	**60 DAS**	**75 DAS**	**90 DAS**
T_1 *(Eucalyptus globules)*	5.33g	9.56 l	13.76 k	17.72 f
T_2 (*Azadirachta indica*)	6.36f	9.90 l	15.26j	19.23ef
T_3 (*Lantana camera*)	6.80f	10.73k	16.66i	20.26ef
T_4 (*Buchnera hispida*)	8.36e	12.16j	17.66h	21.36de
T_5 (*Syzygium aromaticum*)	3.23h	8.80m	11.73 l	14.73g
T_6 (*Carica papaya*)	13.80b	19.40b	22.36c	25.46b
T_7 (*Allium sativum*)	9.26d	13.10i	18.66g	23.13cd
T_8 (*Zingiber officinale*)	9.63d	13.83h	19.90f	24.10bc
T_9 (*Psidium guajava*)	14.06b	18.36c	23.33b	25.36bc
T_{10} (*Mentha peperata*)	8.00e	14.76g	20.83e	24.03bc
T_{11} (*Mentha arvensis*)	10.56c	16.30f	21.53de	25.10bc
T_{12} (*Ocimum sanctum*)	10.60c	16.86e	22.23cd	25.53b
T_{13} (Bordeaux mixture)	13.53f	17.70d	22.46c	25.93b
T_0 (Control)	15.16a	23.03a	27.36a	40.06a
F-test	S	S	S	S
S. Ed (±)	0.36	0.26	0.34	1.09
CD (P = 0.05)	0.77	0.56	0.73	2.32

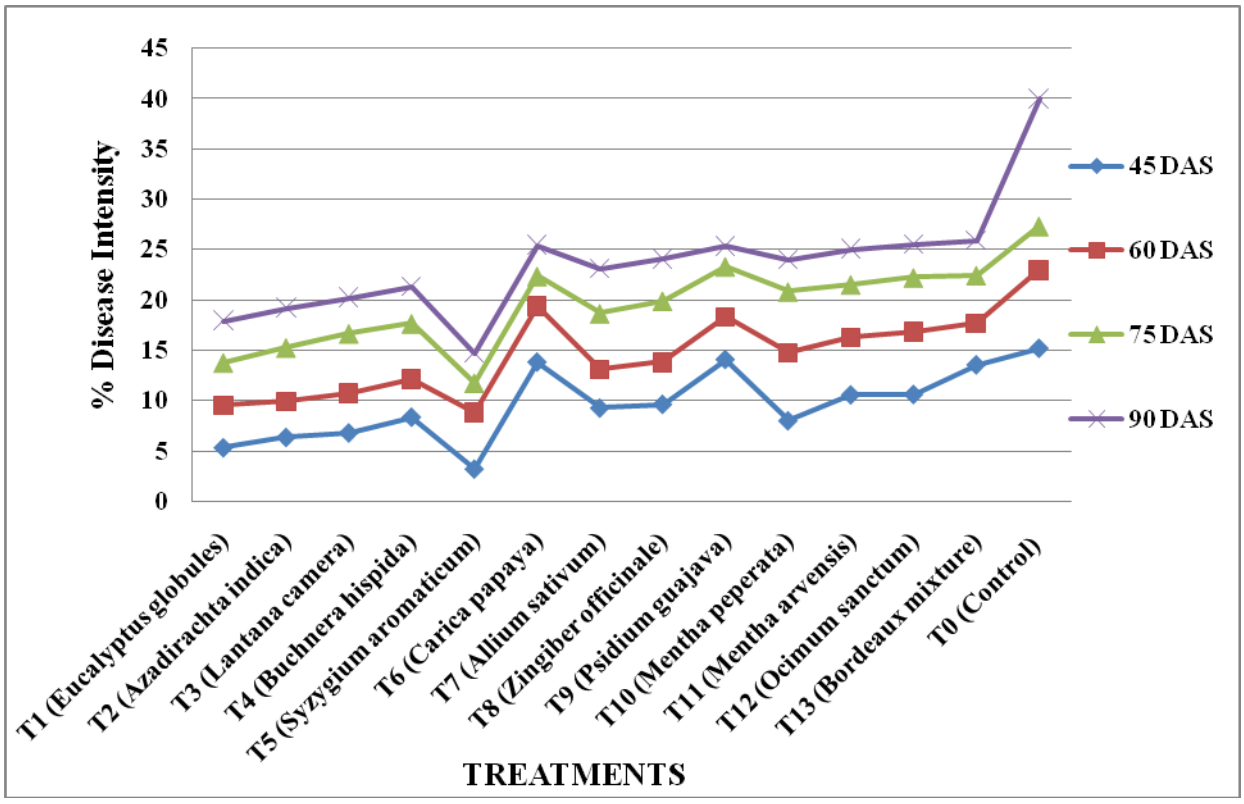


Figure : 4.21 Effect of essential oils at 6 % concentration on disease intensity of black rot (*Xanthomonas campestris* pv. *campestris*) of cabbage at different days intervals.

4.22 Field efficacy of essential oils at 6 % concentration on head diameter of cabbage :

The data documented in Table and Fig : 4.22, elucidate that the selected essential oils at 6% concentration on head diameter (cm) of cabbage was significantly increased in T_{13} - bordeaux mixture (15.5) at 90 DAS as compared with all the essential oils treatment including control.

At 90 days after sowing, the result shows that among the oil treatments the cabbage head diameter (cm) was significantly increased in the treated plot with T_2 - *Azadirachta indica* (14.10) followed T_1 - *Eucalyptus globules* (12.43) and rest of essential oils including with control. However, the treatments (T_1, T_{10}, T_3, T_{12}, T_7), (T_{11}, T_8) and (T_9, T_6, T_4) and (T_9, T_6, T_4) were found non-significant among each other but they are significant from control.

After harvest, the cabbage head diameter (cm) was increased in the treated plot with *Azadirachta indica* (25.20), bordeaux mixture (25.13), *Lantana camera* (24.50) and *Ocimum sanctum* (24.33), which are found non significant effect to each other but they are significant from *Eucalyptus globules* (23.30), *Allium sativum* (22.93), *Mentha peperata* (22.50), *Zingiber officinale* (21.90),

Mentha arvensis (21.86), *Syzygium aromaticum* (20.83), *Buchnera hispida* (20.06), *Carica papaya* (19.16) and *Psidium guajava* (16.16) including with control (14.66). However, the treatments (T_1, T_7, T_{10}), (T_{10}, T_8, T_{11}), (T_5, T_4) and (T_4, T_6) were showed no significant difference among themselves but they are significant from T_9 and T_0. While, control was found minimum cabbage head diameter (14.66 cm).

Similar result have been reported by **Javaida *et al.* (2000)** who recorded that neem treatments was significantly higher yield of marketable heads of cabbage and significantly better control of pest than the commonly used mixture of pyrethroids or the untreated control.

Table : 4.22 Field efficacy of essential oils at 6 % concentration on head diameter (cm) of cabbage at different days intervals

Treatments	Head diameter (cm)	
	90 DAS	120 DAS
T_1 *(Eucalyptus globules)*	12.43c	23.30b
T_2 (*Azadirachta indica*)	14.10b	25.20a
T_3 (*Lantana camera*)	11.63cd	24.50a
T_4 (*Buchnera hispida*)	10.06ef	20.06de
T_5 (*Syzygium aromaticum*)	9.46f	20.83d
T_6 (*Carica papaya*)	10.23ef	19.16e
T_7 (*Allium sativum*)	11.43cd	22.93b
T_8 (*Zingiber officinale*)	10.72de	21.90c
T_9 (*Psidium guajava*)	10.30ef	16.16f
T_{10} (*Mentha peperata*)	11.90cd	22.50bc
T_{11} (*Mentha arvensis*)	11.06de	21.86c
T_{12} (*Ocimum sanctum*)	11.46cd	24.33a
T_{13} (Bordeaux mixture)	15.50a	25.13a
T_0 (Control)	7.43g	14.66g
F-test	S	S
S. Ed (±)	0.45	0.46
CD (P = 0.05)	1.01	0.97

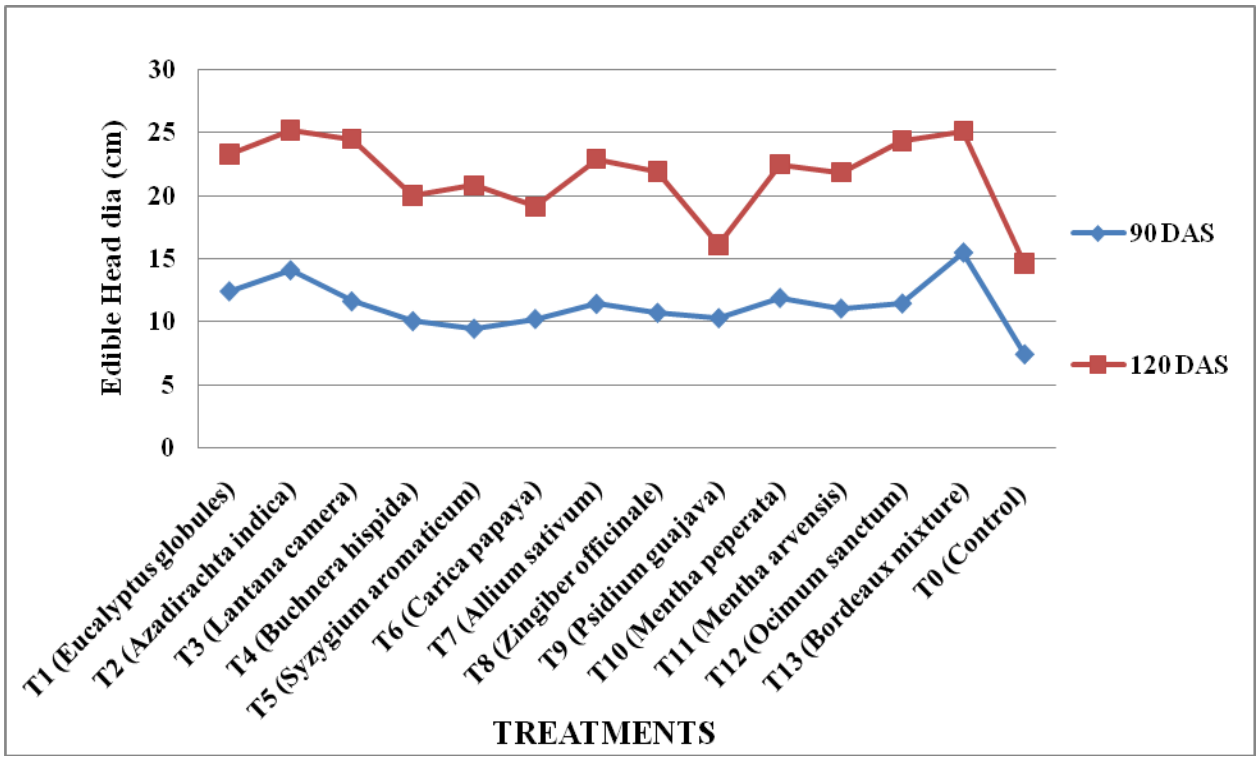


Figure : 4.22 Effect of essential oils at 6 % concentration on head diameter of cabbage at different days intervals.

4.23 Field efficacy of essential oils at 6 % concentration on edible head weight (gm) of cabbage :

The data are presented in the table and fig : 4.23, shows the maximum yield (g) in the treated plots with T_{13} – Bordeaux mixture (1782.06) and T_2 – *Azadirachta indica* (1740.46) followed by all the essential oils including with control. Whereas, treatment T_{13} and T_2 were shows no differences among themselves but they have maximum yield as compared with all other treatments. However, treatments (T_1, T_{10}), (T_{11}, T_3), (T_7, T_{12}), (T_8, T_5), (T_5, T_9) and (T_9, T_4) were found non-significant in yield of cabbage but they are significant from T_6 and T_0. Whereas, minimum yield was observed in the treatment T_6 (*Carica papaya*) followed by T_4 (*Buchnera hispida*) and T_9 (*Psidium guajava*).

These results are in good agreement with the findings of **Babu *et al.* (2000)** mentioned that spraying with 3 % of neem oil in tomato pot cultures resulted in 53 % reduction in disease

incidence over the control while **Patil *et al.* (2001)** found that incidence of tomato early blight caused by *A. solani* was affected by a botanical like neem seed extract with increased fruit yield between 156.43 and 168.56q / ha.

Table : 4.23 Field efficacy of essential oils at 6 % concentration on edible head weight (gm) of cabbage at 120 DAS

Treatments	Cabbage weight (gm)			Mean
	R_1	R_2	R_3	
T_1 *(Eucalyptus globules)*	1630.4	1632.4	1535.9	1599.56b
T_2 (*Azadirachta indica*)	1790.2	1710.8	1720.4	1740.46a
T_3 (*Lantana camera*)	1427.5	1465.3	1424.6	1439.13c
T_4 (*Buchnera hispida*)	791.2	786.4	776.3	784.63g
T_5 (*Syzygium aromaticum*)	890.1	885.4	888.9	888.13ef
T_6 (*Carica papaya*)	610.4	623.3	615.9	616.53h
T_7 (*Allium sativum*)	1098.1	1191.4	1185.2	1158.23d
T_8 (*Zingiber officinale*)	925.4	935.5	948.9	936.60e
T_9 (*Psidium guajava*)	810.9	821.4	833.5	821.93fg
T_{10} (*Mentha peperata*)	1535.2	1638.7	1536.3	1570.06b
T_{11} (*Mentha arvensis*)	1450.6	1536.4	968.3	1478.43c
T_{12} (*Ocimum sanctum*)	1115.1	1121.4	1110.9	1115.80d
T_{13} (Bordeaux mixture)	1810.3	1715.7	1820.2	1782.06a
T_0 (Control)	462.2	446.4	565.3	491.30i
F-test				S
S. Ed (±)				32.29
CD (P = 0.05)				68.47

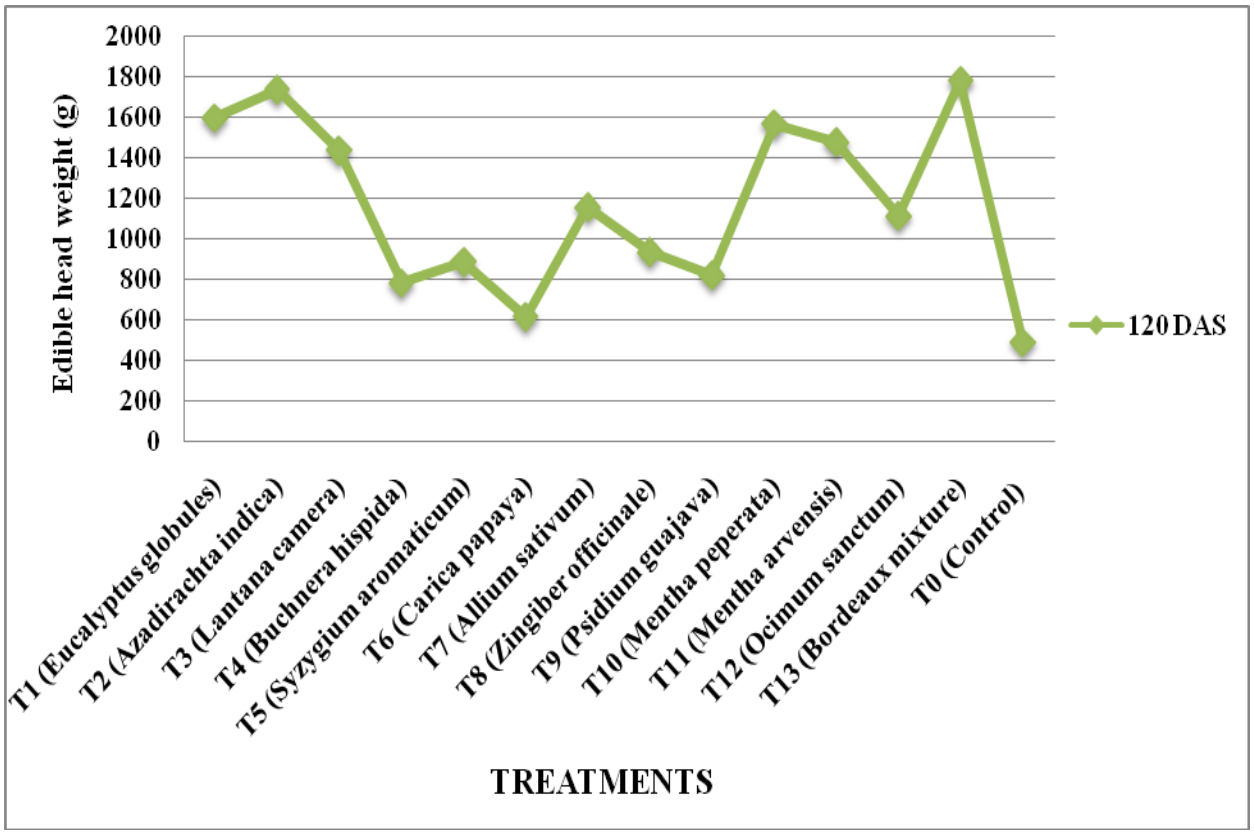


Figure : 4.23 Effect of essential oils at 6 % concentration on edible head weight (gm) of cabbage at 120 DAS.

CHAPTER - V

SUMMARY AND CONCLUSION

Cabbage (*Brassica oleracea* var. *capitata*) is leafy green vegetable crop, a popular cultivar of the species *oleraceae* under the family Brassicaceae. It's an introduced vegetable crop in India, but it has adapted itself well and is grown all over the country. It has excellent source of vitamin K, C, B_1, B_2, B_6, and also a very good source of manganese, dietary fiber, potassium, foliate, magnesium, calcium, selenium, iron, copper, chlorine, phosphorus, sodium, pantothenic acid, protein and niacin. Cabbage has anti-bacterial and anti-viral powers. It also contains numerous anticancer and antioxidant compounds especially those in the colorectal group. This is possibly due to the glucosinolates. The nations with the largest production were China, which produced 46 percent of the world followed by India produced 12 percent.

Cabbage crop is suffered from many diseases due to biotic and abiotic factors. Among biotic agents; fungi, bacteria and nematodes are responsible for the sever losses of the head quality and crop production. Black spot disease caused by *Alternaria brassicae* and *A. brassicicola* has been reported from all the continents of the world. Losses up to 30 - 47% were caused by *Alternaria brassicae.* Second most important disease, black rot caused by the bacterium *Xanthomonas campestris* pv. *campestris* is the most common and destructive disease of the cabbage family worldwide. A crop loss up to 50 per cent in cabbage and 50 to 70 per cent in cauliflower has been recorded. Botanical pesticides are considered as less toxic and environmentally safe. Green plants can be used as a source of a reservoir and can be effective chemotherapeutants which can provide valuable sources of natural pesticides because of environmental and economic considerations, plant scientists are involved to find the cheaper and more environmental friendly bio-compounds for the control of plant diseases using different forms of botanicals. Extracts from leaves have been reported to have antifungal, anti-proliferative, antibacterial, nematicidal, termicidal, anti-helmintic and anticancer activities. Beside this, the essential oil of the plants also possesses antifungal and antibacterial activities.

Plant extracts are least expensive and cause less health hazards. Several higher plants and their constituents have shown success in plant disease control and are proved to be harmless and non-phytotoxic unlike chemical fungicides.

Plant extracts are screened to detect secondary metabolites with biological activities, including antifungal activity. Keeping this in mind the present study entitled **"Botanical pesticides in the management of *Xanthomonas campestris* pv. *campestris* and *Alternaria brassicae* of cabbage"** was proposed with the following objectives –

1. To isolate and identify *Xanthomonas campestris* pv. *campestris* and *Alternaria brassicae* from infected leaves of cabbage.
2. To screen available botanicals for their antimicrobial activities against *Xanthomonas campestris* pv. *campestris* and *Alternaria brassicae*.
3. To detect secondary metabolites from the selected botanicals.
4. To evaluate the efficacy of selected botanical extracts and essential oils against *Xanthomonas campestris* pv. *campestris* and *Alternaria brassicae* of cabbage.
5. To study the effect of selected botanicals on edible head diameter (cm) and weight (g) of cabbage boll.

Results summarized that Bordeaux mixture @ 0.2% was recorded minimum mycelial radial growth (8.67, 12.07, 20.65 and 22.85 mm) and maximum % inhibition as compared with all the botanicals leaf extract at 48, 72, 96 and 120 hrs after incubation. While, neem leaf extract @ 25% concentration was found least radial growth and more % inhibition of *Alternaria brassicae*.

The least mycelium growth of *Alternaria* was found in the neem leaf extract @ 50 % concentration (7.70 and 10.45mm) at 48 and 72 hrs after incubation. But increasing of time, neem was less effective on 96 and 120 hrs after incubation, while Bordeaux mixture @ 0.3% are recorded best on 96 and 120 hrs after incubation.

Bordeaux mixture @ 0.4% was least colony diameter (7.20, 8.92, 9.92 and 11.37mm) at 48, 72, 96 and 120 hrs after incubation, respectively. While, neem extract @ 75% concentration was found reduced colony growth and maximum % inhibition among all the botanical treatments.

Minimum radial growth of *Alternaria brassicae* was recorded in the treated plates with Bordeaux mixture (11.72, 13.57 and 17.10mm) at 72, 96 and 120 hrs after incubation, respectively. However, essential oil of neem (@2%) was noticed least colony diameter (6.25, 13.12, 15.27 and 18.00mm) at 48, 72, 96 and 120 hrs after incubated plates, respectively.

All the essential oils were found least colony diameter of *Alternaria brassicae* at 4% concentration. Among oil treatments, neem was recorded minimum colony diameter but Bordeaux mixture (@ 0.3%) was found best reduction of colony diameter (6.52, 8.42, 10.47 and 12.02mm) at 48, 72, 96 and 120 hrs after incubation.

Bordeaux mixture @ 0.4% was found best reduction of mycelial colony diameter (6.25, 7.97, 9.75 and 14.75mm) as compared with all the essential oils at 48, 72, 96 and 120 hrs after incubation, respectively. While, neem oil @ 6% was recorded least colony diameter among all the essential oils.

Population of *Xanthomonas campestris* pv. *campestris* was reduced in the poured petriplates with neem leaf extract @ 25, 50 and 75 % concentrations at 48 hrs (8.66, 6 and 2, respectively) and 72 hrs (23.33, 10 and 7, respectively) after incubation. While, it is found maximum % inhibition over Bordeaux mixture and control also.

Neem oil was observed maximum population reduction of *X. campestris* and inhibition % over control at the rate of 2, 4 and 6% concentrations on 48 hrs (6, 5.7 and 2.7, respectively) and 72 hrs (15, 9.7 and 8.7, respectively) after incubation.

Per cent disease intensity of black leaf spot (*Alternaria brassicae*) was reduced maximum in the treated plots with Bordeaux mixture (15.23, 16.33, 19.93 and 21.94) at 45, 60, 75 and 90 days after sowing of cabbage seeds, respectively. While in the botanical extracts, neem was found maximum reduction of disease intensity at different days of interval.

Per cent disease intensity of black rot (*Xanthomonas campestris* pv. *campestris*) was reduced in the sprayed plots with neem leaf extracts (5.86, 12.10, 14.50 and 17.20%) at 45, 60, 75 and 90 days after sowing of cabbage seeds.

Cabbage head diameter (cm) was increased in the bordeaux mixture treatment (17.06, 25.13) at 90 and 120 days after sowing. While, neem leaf extract was found maximum head diameter among all the botanical extracts.

Maximum yield of cabbage head was observed in the chemical treatment (1504.72 g) as comparison from all the botanical extracts. However, neem extracts was noticed maximum yield (1429.86 g) in all the botanical extracts.

Maximum reduction of % disease intensity of black spot was recorded in the sprayed plots with *Azadirachta indica* (7.96, 18.20 and 21.35%) at 45, 75 and 90 days after sowing of cabbage seeds. However, Bordeaux mixture was very effective and found minimum per cent of black spot disease intensity (13.23%) at 60 DAS.

Syzygium aromaticum oil was found maximum reduction of black rot disease intensity (3.23, 8.80, 11.73 and 14.73%) followed by all the essential oil at 45, 60, 75 and 90 DAS. While, maximum disease intensity was recorded in the untreated plots (17.26, 27.56, 38.66 and 50.16%).

Maximum cabbage head diameter (cm) was observed in the sprayed plots with *Azadirachta indica* oil (14.10, 25.20) at 90 and 120 days after sowing of cabbage seeds. While, lowest head diameter was recoded without treated plots (7.43 and 14.66 cm) at 90 and 96 DAS, respectively.

The edible head weight (g) at harvesting time, it was recorded that maximum in the Bordeaux mixture (1782.06 g) and *Azadirachta indica* (1740.46) followed by all other essential oils including with control.

CONCLUSION

From the perusal results of the above objectives it's concluded that Bordeaux mixture was found least mean colony diameter and maximum % inhibition of *Alternaria brassicae* in food poison technique at 0.2, 0.3 and 0.4% concentrations. However, neem leaf extract and oil was superior in the minimum colony diameter and highest % inhibition of *A. brassicae* among all the botanical extracts. Colony of *Xanthomonas campestris* pv. *campestris* was reduced in the treated petriplates with neem leaf extract and neem oil. Highest reduction of black spot (*Alternaria brassicae*) disease intensity, cabbage head diameter and yield were recorded in the treated plots with Bordeaux mixture (21.94%) while, minimum black rot disease intensity was recorded in the neem leaf extract and oil of *Syzygium aromaticum*. The leaf extract is eco-friendly, economic and technically viable field formulation, which will be Bio-ecologically compatible for management of various plant pathogens.

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APPENDIX

***In vitro* efficacy of botanical extracts at 25 % concentration on mycelial growth of *Alternaria brassicae* at different hrs of interval**

At 48 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal
Treatments	12	636.109	53.009	224.049
Error	39	9.225	0.236	-
Total	51	-	-	-

At 72 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal
Treatments	12	5096.079	424.676	1080.903
Error	39	15.325	0.398	-
Total	51	-	-	-

At 96 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal
Treatments	12	11003.506	916.959	1125.986
Error	39	31.760	0.813	-
Total	51	-	-	-

At 120 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal
Treatments	12	20883.013	1740.254	994.218
Error	39	68.265	1.753	-
Total	51	-	-	-

***In vitro* efficacy of botanical extracts at 50 % concentration on mycelial growth of *Alternaria brassicae* at different hrs of interval**

At 48 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal
Treatments	12	333.090	27.757	91.437
Error	39	11.840	0.305	-
Total	51	-	-	-

At 72 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal
Treatments	12	4485.617	373.803	621.804
Error	39	23.445	0.601	-
Total	51	-	-	-

At 96 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal
Treatments	12	12390.026	1032.508	1821.850
Error	39	22.105	0.567	-
Total	51	-	-	-

At 120 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal
Treatments	12	17383.397	1448.613	4031.111
Error	39	14.015	0.353	-
Total	51	-	-	-

In vitro **efficacy of botanical extracts at 75 % concentration on mycelial growth of** ***Alternaria brassicae*** **at different hrs of interval**

At 48 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Treatments	12	707.872	58.985	213.564	1.187
Error	39	10.775	0.272	-	-
Total	51	-	-	-	-

At 72 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Treatments	12	4167.840	347.320	1525.824	3.615
Error	39	8.875	0.226	-	-
Total	51	-	-	-	-

At 96 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Treatments	12	11621.227	968.434	1454.754	9.146
Error	39	25.965	0.667	-	-
Total	51	-	-	-	-

At 120 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Treatments	12	19806.797	1650.563	3615.397	1.845
Error	39	17.805	0.455	-	-
Total	51	-	-	-	-

***In vitro* efficacy of essential oils at 2 % concentration on the radial growth and per cent inhibition of *Alternaria brassicae* at different hrs of interval**

At 48 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Treatments	13	823.773	63.364	501.926	1.149
Error	42	5.305	0.122	-	-
Total	55	-	-	-	-

At 72 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Treatments	13	7664.965	589.619	1329.590	1.668
Error	42	18.625	0.444	-	-
Total	55	-	-	-	-

At 96 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Treatments	13	13875.641	1067.350	2149.041	7.126
Error	42	20.860	0.496	-	-
Total	55	-	-	-	-

At 120 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Treatments	13	19224.548	1478.812	3387.970	5.096
Error	42	18.335	0.434	-	-
Total	55	-	-	-	-

***In vitro* efficacy of essential oils at 4 % concentration on mycelial radial growth of *Alternaria brassicae* at different hrs of interval**

At 48 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Treatments	13	964.872	74.220	179.724	1.983
Error	42	17.345	0.419	-	-
Total	55	-	-	-	-

At 72 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Treatments	13	5159.055	396.852	711.078	8.037
Error	42	23.440	0.550	-	-
Total	55	-	-	-	-

At 96 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Treatments	13	12873.103	990.230	1830.340	2.059
Error	42	22.725	0.540	-	-
Total	55	-	-	-	-

At 120 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Treatments	13	20500.553	1576.965	2577.398	1.578
Error	42	25.695	0.618	-	-
Total	55	-	-	-	-

***In vitro* efficacy of essential oils at 6 % concentration on radial growth of *Alternaria brassicae* at different hrs of interval**

At 48 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Treatments	13	1158.863	89.146	116.558	1.371
Error	42	32.125	0.768	-	-
Total	55	-	-	-	-

At 72 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Treatments	13	2691.238	207.011	91.254	1.925
Error	42	95.285	2.266	-	-
Total	55	-	-	-	-

At 96 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Treatments	13	12084.231	929.557	1272.328	4.186
Error	42	30.685	0.735	-	-
Total	55	-	-	-	-

At 120 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Treatments	13	18950.352	1457.715	2180.347	5.268
Error	42	28.080	0.665	-	-
Total	55	-	-	-	-

***In vitro* efficacy of selected botanical extracts at 25 % concentration on number of colonies of *X. c.* pv. *campestris* at different hrs of interval**

At 48 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Treatments	12	25800.561	2150.040	401.209	2.581
Error	26	139.333	5.359	-	-
Total	38	-	-	-	-

At 72 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Treatments	12	67537.333	5628.111	222.619	5.074
Error	26	657.333	25.280	-	-
Total	38	-	-	-	-

***In vitro* efficacy of selected botanical extracts at 50 % concentration on no. of colonies of *Xanthomonas campestris* pv. *campestris* at different hrs of interval**

At 48 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Treatments	12	31777.745	2648.142	693.137	2.209
Error	26	99.333	3.825	-	-
Total	38	-	-	-	-

At 72 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Treatments	12	80075.026	6672.918	310.183	7.147
Error	26	559.333	21.518	-	-
Total	38	-	-	-	-

***In vitro* efficacy of selected botanical extracts at 75 % concentration on no. of colonies of *Xanthomonas campestris* pv. *campestris* at different hrs of interval**

At 48 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Treatments	12	20517.745	1709.819	51.259	5.684
Error	26	867.333	33.359	-	-
Total	38	-	-	-	-

At 72 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Treatments	12	29678.561	2473.216	71.710	8.850
Error	26	896.666	34.481	-	-
Total	38	-	-	-	-

***In vitro* efficacy of essential oils at 2 % concentration on no. of colonies of *Xanthomonas campestris* pv. *campestris* at different hrs of interval**

At 48 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Treatments	13	12805.730	985.057	37.851	2.789
Error	28	728.666	26.028	-	-
Total	41	-	-	-	-

At 72 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Treatments	13	19666.305	1512.790	39.617	1.543
Error	28	1069.333	38.194	-	-
Total	41	-	-	-	-

***In vitro* efficacy of selected essential oils at 4 % concentration on no. of colonies of *Xanthomonas campestris* pv. *campestris* at different hrs of interval**

At 48 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Treatments	13	13526.287	1040.485	55.171	1.971
Error	28	528.000	18.851	-	-
Total	41	-	-	-	-

At 72 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Treatments	13	20019.610	1539.976	63.037	3.347
Error	28	684.000	24.425	-	-
Total	41	-	-	-	-

***In vitro* efficacy of selected essential oils at 6 % concentration on no. of colonies of *Xanthomonas campestris* pv. *campestris* at different hrs of interval**

At 48 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Treatments	13	16343.648	1257.202	85.163	5.844
Error	28	413.333	14.769	-	-
Total	41	-	-	-	-

At 72 hrs after incubation :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Treatments	13	23922.287	1840.178	133.711	1.259
Error	28	385.333	13.769	-	-
Total	41	-	-	-	-

Field efficacy of selected botanical extracts at 75% concentration on disease intensity of black spot (*Alternaria brassicae*) of cabbage at different days of interval

At 45 DAS :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Replications	2	1.834	0.917	2.496	0.109
Treatments	12	57.817	4.815	13.067	9.654
Error	24	8.842	0.367	-	-
Total	38	-	-	-	-

At 60 DAS :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Replications	2	6.760	3.385	22.521	3.118
Treatments	12	1010.405	84.202	560.775	2.473
Error	24	3.605	0.151	-	-
Total	38	-	-	-	-

At 75 DAS :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Replications	2	9.726	4.863	8.153	0.009
Treatments	12	1701.306	141.774	237.917	6.702
Error	24	14.300	0.599	-	-
Total	38	-	-	-	-

At 90 DAS :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Replications	2	12.653	6.321	8.584	0.005
Treatments	12	2898.120	241.510	327.867	1.481
Error	24	17.679	0.736	-	-
Total	38	-	-	-	-

Field efficacy of selected botanical extracts at 75 % concentration on disease intensity of black rot (*X. campestris* pv. *campestris*) of cabbage at different days interval

At 45 DAS :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Replications	2	2.582	1.291	6.617	0.001
Treatments	12	207.925	17.329	88.638	7.449
Error	24	4.697	0.194	-	-
Total	38	-	-	-	-

At 60 DAS :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Replications	2	6.260	3.130	4.799	0.016
Treatments	12	511.373	42.615	65.334	2.539
Error	24	15.653	0.652	-	-
Total	38	-	-	-	-

At 75 DAS :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Replications	2	3.299	1.644	3.006	0.062
Treatments	12	704.126	58.671	107.094	8.187
Error	24	13.147	0.549	-	-
Total	38	-	-	-	-

At 90 DAS :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Replications	2	0.419	0.204	0.140	0.862
Treatments	12	1099.440	91.622	64.736	2.825
Error	24	33.966	1.413	-	-
Total	38	-	-	-	-

Field efficacy of selected botanical extracts at 75 % concentration on head diameter of cabbage at different days after sowing

At 90 DAS :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Replications	2	7.093	3.546	0.997	0.381
Treatments	12	338.948	28.243	7.920	9.916
Error	24	85.503	3.567	-	-
Total	38	-	-	-	-

At 120 DAS :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Replications	2	0.420	0.210	0.351	0.700
Treatments	12	600.219	50.012	84.105	1.366
Error	24	14.273	0.597	-	-
Total	38	-	-	-	-

Field efficacy of selected botanical extracts at 75% concentration on edible head weight (gm) of cabbage at 120 DAS

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Replications	2	755.142	377.571	0.726	0.494
Treatments	12	2791468.017	232622.332	449.518	3.452
Error	24	12419.994	517.499	-	-
Total	38	-	-	-	-

Field efficacy of essential oils at 6% concentration on disease intensity of black spot (*Alernaria brassicae*) of cabbage at different days of interval

At 45 DAS :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Replications	2	11.865	5.932	6.805	0.002
Treatments	13	248.091	19.083	21.878	9.882
Error	26	22.670	0.872	-	-
Total	41	-	-	-	-

At 60 DAS :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Replications	2	20.324	10.162	15.015	4.626
Treatments	13	484.447	37.260	55.070	1.544
Error	26	17.598	0.676	-	-
Total	41	-	-	-	-

At 75 DAS :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Replications	2	2.263	1.136	1.583	0.224
Treatments	13	955.900	73.530	102.874	6.182
Error	26	18.583	0.717	-	-
Total	41	-	-	-	-

At 90 DAS :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Replications	2	43.117	21.558	1.456	0.257
Treatments	13	2044.361	157.255	10.588	2.664
Error	26	386.399	14.861	-	-
Total	41	-	-	-	-

Field efficacy of essential oils at 6 % concentration on disease intensity of black rot (*Xanthomonas campestris* pv. *campestris*) of cabbage at different days interval

At 45 DAS :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Replications	2	4.881	2.440	11.807	0.002
Treatments	13	501.605	38.583	186.454	3.173
Error	26	5.384	0.209	-	-
Total	41	-	-	-	-

At 60 DAS :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Replications	2	2.961	1.480	12.908	0.001
Treatments	13	692.368	53.256	463.569	2.564
Error	26	2.981	0.118	-	-
Total	41	-	-	-	-

At 75 DAS :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Replications	2	3.672	1.831	9.525	0.007
Treatments	13	690.871	53.143	275.668	2.095
Error	26	5.013	0.197	-	-
Total	41	-	-	-	-

At 90 DAS :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Replications	2	2.830	1.415	0.739	0.487
Treatments	13	1305.314	100.409	52.221	2.983
Error	26	49.990	1.926	-	-
Total	41	-	-	-	-

Field efficacy of essential oils at 6 % concentration on head diameter (cm) of cabbage at different days intervals

At 90 DAS :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Replications	2	0.730	0.360	0.991	0.383
Treatments	13	148.247	11.404	31.084	1.617
Error	26	9.536	0.367	-	-
Total	41	-	-	-	-

At 120 DAS :

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Replications	2	2.791	1.390	4.129	0.027
Treatments	13	400.240	30.780	90.921	2.941
Error	26	8.808	0.336	-	-
Total	41	-	-	-	-

Field efficacy of essential oils at 6 % concentration on edible head weight (gm) of cabbage at 120 DAS

Source of variation	D. F.	S. S.	M. S. S.	F cal	F prob
Replications	2	963.935	481.962	0.287	0.758
Treatments	13	7157065.441	550543.496	330.933	1.985
Error	26	43253.817	1663.602	-	-
Total	41	-	-	-	-