Management of wilt of pea (*Pisum sativum* L.) caused by *Fusarium oxysporum* f. sp. *pisi* (W.C. Snyder and H.N. Hansen, 1940) through non-chemical approaches

THESIS

Submitted to

Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur

In partial fulfilment of the requirements for the Degree of

MASTER OF SCIENCE

In

AGRICULTURE
(Plant Pathology)

By

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2019

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This is to certify that the thesis entitled **"Management of wilt of pea (*Pisum sativum* L.) caused by *Fusarium oxysporum* f. sp. *pisi* (W.C. Snyder and H.N. Hansen, 1940) through non-chemical approaches"** submitted in partial fulfilment of the requirement for the degree of **MASTER OF SCIENCE (Ag.)** in Department of Plant Pathology of Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur is a record of the bonafide research work carried out by **Mr. Rahul Yadav** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and the Director of Instructions. All the assistance and help received during the course of the investigation has been acknowledged by him.

Place: Jabalpur

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Place: Jabalpur **(Rahul Yadav)**

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ABBREVATIONS

Cm	=	Centimeter
Mm	=	Millimete
G	=	Gram
Ib	=	Pound
Psi	=	pound pressure inch
PDA	=	potato dextrose agar
Ml	=	Milliliter
Lit	=	Liter
No.	=	Number
i.e.	=	that is
Sp.	=	Species
^{0}C	=	Degree Celsius
Etc	=	et cetra
et al	=	co-worker
Eg	=	as for example
BOD incubator	=	Biological oxygen demand incubator
Ha	=	Hectare
%	=	Percent
@	=	at the rate of
DAS	=	days after sowing
Viz.	=	Namely

INTRODUCTION

Pea (*Pisum sativum* L.) is one of the major pulse crops of temperate region of the world and was originally cultivated in the Mediterranean basin (Sardana *et al*., 2007). It is the fourth leading legumes in terms of consumption in the world and an important field and vegetable crop of India. The crop belongs to family leguminosae and is believed to be native of Europe and West Asia. It is a rich source of proteins, amino acids, sugars, carbohydrates, vitamins A and C, calcium and phosphorus, besides having a small quantity of iron. The protein content of pea cultivars is 26 -33 per cent.

The major green pea producing countries in the world are China, India, UK, USA and Egypt. Total area of pea in the country is 545.89 thousand hectares with the production of 5451.62 thousand metric tonnes and productivity 9.98 metric tonnes per hectare. Madhya Pradesh contributes an area of 106.51 thousand hectares with the production of 1113.47 thousand metric tonnes and productivity 10.45 metric tonnes per hectare (Anon, 2017).

The crop is attacked by a number of diseases such as damping-off (*Pythium spp*, *Fusarium spp* etc.), seedling rot (*Rhizoctonia solani*), bacterial blight (*Pseudomonas syringae* pv. *pisi*), *Aschochyta* blight (*Aschochyta* spp.), powdery mildew (*Erysiphe pisi*) and *Fusarium* root rot (*Fusarium solani* f. sp. *pisi*) etc.

Among the diseases, wilt, caused by *Fusarium oxysporum* f. sp. *pisi,* is one of the major limiting factors for successful production of pea and is found to be most predominant and destructive. It causes severe damage to the crop and thus reduces potential yields. During 1981-82 and 1982-1983 in Northern region of Madhya Pradesh, incidence of wilt of pea ranged from 12.5 to 30.25 and from 19.57 to 37.39 per cent respectively (Sharma *et al.,* 1989). In India, Fusarium wilt has been reported to cause 14 to 95 per cent losses (Kumar *et al.,* 1993). The disease is soil borne and often associated with root rot and is responsible to cause losses in yield up to 93 per cent (Sharma *et al.,* 2006).

Though the disease can be managed by soil and seed treatments using chemicals but these are uneconomical due to their high cost. These chemicals are known to leave harmful residues on plant and have deleterious effect on the existing ecosystem.

Development of resistance varieties through conventional breeding approaches is a time consuming procedure. Management of disease through botanicals is economically feasible and ecofriendly means of plant disease control. A perusal of literature available on this disease revealed that no detailed and systematic work has been carried out employing botanicals to control *Fusarium oxysporum* causing wilt.

Plant derivatives possessing pesticial properties are gaining worldwide importance as alternative / supplement for the existing pesticides because of low cost, less environmental hazards and no risk of resistance by pathogens.

However the botanical agents have gained more importance in recent years due to their advantages like maintenance of ecological balance, less expensive, help to achieve pollution free environment, reduced residues and health hazards, easy movebility and remain effective over long periods (Affokpon *et al*., 2011).

Looking to the facts, the present investigation on “study the efficacy of botanicals, plant biproducts and biocontrol agents” was conducted with the following objectives.

1) Collection, isolation, purification and pathogenicity of *Fusarium oxysporum* f. sp*. pisi.*
2) Management of wilt of pea through botanicals, plant biproducts and biocontrol agents.

REVIEW OF LITERATURE

2.1 Causal Organism(s)

Fusarium wilt of pea was reported to be caused by *Fusarium oxysporum* f. sp. *pisi* race 1 (Ken Knight, 1944). This race was responsible for the most serious foot and wilt disease of pea (Schreuder, 1951). Kerling (1952) indicated that this pathogen had been carried on or in the seed and had accumulated in the soil and was reported to cause vascular disease of peas.

Fusarium oxysporum f. sp. *pisi* race 2 was reported as a causal agent of wilt (Walker and Hare, 1942). Similar report was also given by Labruyere and Riepma (1954).

The fungus infected the seed surface and generally transmitted by the soil (Sawaryn, 1961). The pathogen was found in the rhizoplane of peas (Rintelen, 1973).

When the pea seeds are infected with *Fusarium oxysporum* f. sp. *pisi,* the disease development and intensity were not always related to symptoms expression on the seedlings (Manneuruccy and Gambogi, 1976).

Maheshwari *et al.* (1980) observed *Fusarium oxysporum* f. sp. *pisi* in the cotyledons of diseased pea seeds. The fungi spread to the root and stem through xylem vessels.

The pathogen is soil-borne and survives as thick-walled chlamydospores, which remain viable in soil for more than 10 years (Kraft 1995). *Fusarium oxysporum* penetrates pea roots and infects the vascular system at any growth stage (Infantino *et al*., 2006)

2.2 Geographical distribution

Fusarium wilt of pea (*Pisum sativum* L.) is one of the most destructive diseases of pea and is of worldwide occurrence. *Fusarium* wilt (race 1) of peas was discovered by Jones and Linford in Wiscosin in 1924 (Hagedorn, 1984, Haglund and Kraft, 2001).

2.3 Disease incidence

Sharma *et al.* (1989) observed incidence of wilt of pea ranged from 12.5 to 30.25 per cent in Northern region of Madhya Pradesh.

Sharma *et al.* (2006) reported that wilt caused by *Fusarium oxysporum* f. sp. *pisi* is one of the most devastating diseases of pea, limiting the realization of its full yield potential. The disease along with root rot has been reported to cause yield losses up to 93 per cent in India. Sharma (2000) reported that, the disease has been found widespread in the North-Western Himalaya region where pea is grown as a major off-season cash crop.

Sharma (2011) collected wilt affected pea plants and confirmed the cause of disease, establishing koch's Postulates and proved that the wilt diseases are the major cause of losses ranged from 20 – 80 per cent.

Kuldhar *et al.* (2013) screened pea varieties / lines / germplasm to find out resistance against wilt incited by *Fusarium oxysporum* f. sp. *pisi.* Among the pea varieties tested, Arkel was found most susceptible with significantly highest mean wilt incidence (32.66%), and was followed by Latur local-1 (29 %) and Latur Local-2 (28%). Soldier was found moderately susceptible with mean wilt incidence of 19 per cent. Thus pea variety Soldier may be preferred, so as to minimize the yield losses due to wilt incidence.

Merzoug *et al. (*2014*)* reported pathogenic variability in 52 isolates of *Fusarium oxysporum* f. sp. *pisi*, collected from different pea-growing areas of western Algeria, and reported that the disease was prevalent in all the fields and Races 1 and 2 were more common with 61.5 and 19.2 per cent of occurrence, respectively.

Rao (2014) reported that the disease incidence ranged between 3.6 to 14.42 per cent in Eastern Uttar Pradesh.

2.4 Symptomatology

First symptom of pea wilt is pale colouration of the leaflets. The plant growth is checked, upper part of plant may be more rigid than normal and

leaves loose turgidity beginning with the lower ones and progressing upward. The plant wilts and the stem shrivels. Few rootlets of the plant are injured and with light yellow to orange brown discolouration of the vascular system of tap root which extends up in the stem for several internodes (Harter, 1938).

According to Buxton (1955) true wilt appears as stunting and rolling of the leaf margins and stipules, the upper parts become pale, develop a greyish bloom and chlorosis follows. The leaves become somewhat brittle and are readily removed. The upper part of plant may wilt and dries the vascular discolouration is pale yellow to deep orange brown.

Hepple (1963) stated that first symptoms appears as wilting of lower leaves and no apparent discolouration except in cotyledonary vascular bundels.

Isacc and Rogers (1974) observed that first wilting of old leaves (4 to 6 week old seedlings) showed yellowing symptoms, reduction of root system, stunting of growth and shredding of basal stem portion. The pods of infected plants were smaller than those of healthy ones.

Lin (1991) observed wilting at flowereing stage and was accompained by leaf yellowing and vascular discolouration. The stunted plant growth yellowing and wilting symptoms were also noticed in severely affected pea plants (Bodker *et al.,* 1993).

Fusarium oxysporum f. sp. *pisi* penetrates pea root and infects vascular system at any growth stage of the crop (Infantino *et al.,* 2006). Infected plants often shows an orange or dark red discolouration in the vascular tissue of the root and lower parts of the stem. Above ground symptoms consist of leaf yellowing that wilt and curl downward during the flowering to pod-fill stages. Early infection often results in seedling death, which may be obscured by the growth of adjacent plants.

2.5 *In vitro* evaluation of leaf extracts

Verma and Dohroo (2003) tested extracts (at 25% concentration) of 12 plants *viz.,* agave (*Agave Americana*), broccoli leaves (*Brassica oleracea*

var. *italic*), eucalyptus (*Eucalyptus globulus*), neem (*Azadirachta indica*), pine (*Pinus roxburghii*), ginger (*Zingiber officinale*), turmeric rhizome (*Curcuma longa*), chilli seeds (*Capsicum frutescens*), tulsi seeds (*Ocimum sanctum*), cotton (*Gossypium indicum*), sarson cake (*Brassica campestris*) and garlic cloves (*Allium sativum*) at 25 per cent concentration against *F. oxysporum* f. sp. *pisi* and found that garlic extract resulted in cent per cent inhibition, followed by sarson (24.53%).

Sharma and Gaur (2003) tested tulsi and jatropha plant extracts against wilt pathogen and reported that plant extracts of these two plants completely checked mycelial growth of *Fusarium oxysporum.*

According to Sharma and Kumar (2009) tested extracts of three weed plants, namely, *Capparis decidua, Lantana camara* and *Tridax procumbens*, all the plants showed antifungal property against *Fusarium oxysporum*. Acetone extracts of *Datura stramonium* has been reported to have antifungal activity against several fungi including *Fusarium oxysporum* (Gaire *et al.*, 2013).

Khaleel *et al.* (2014) reported that, extracts of *Allium sativum, Azadirachta indica*, *Zingiber officinale*, *Calatropis procera*, *Moringa oleifera* and *Parthenium hysterophorus* showed fungitoxic effect against *F. oxysporum* f. sp. *pisi.*

In vitro antifungal assay was conducted against *F. oxysporum* f. sp. *lycopersici,* using plant extracts of fifteen plants. Out of the plants tested extracts of three plants *viz., Solanum indicum* (78.33%), *Azadirachta indica* (75.00%), *Oxalis latifolia* (70.33 %) proved to be potential in inhibiting the growth of the *F. oxysporum* f. sp. *lycopersici.* (Kumar *et al.,* 2015).

Kumar and Dabbas (2016) tested leaf extracts *viz.,* Neem (*Azadirachta indica*), Datura (*Datura stramonium*), Ginger (*Zingiber officinale*), Mustard (*Brassica nigra*), Parthenium (*Parthenium hysterophorus*), Garlic (*Allium sativum*), Pudina (*Mentha spicata*), Lantana (*Lantana camara*), Arandi (*Ricinus communis*), Tulsi (*Ocimum tenuiflorum*), Onion (*Allium cepa*), Eucalyptus

(*Eucalyptus obliqua*) and Madar (*Calotropis gigantea*). They reported that leaf extracts of Neem, Datura, Ginger, Mustrad and Parthenium have proved to be the most effective in inhibiting the fungal growth of wilt causing fungus of pea.

Ghante *et al.* (2019) tested 12 botanicals (each @ 10 and 20%) *viz., Allium cepa, Lantana camara, Osmium sanctum, Gliricidia sepium, Azadirachta indica, Allium sativum, Bougainveilliea spectapbilis, Moringa oleifera, Eucalyptus globulus, Pongamia pinnata, Vinca rosea* and *Asparagus racemosus in vitro* for their antifungal activities against *Fusarium oxysporum* f. sp. *udum* using poison food technique and found that *Bougainveilliea spectapbilis* most effective inhibiting the test fungus.

2.6 Influence of botanicals as soil treatment

Dubey (2002) in his experiment with soil application of karanj (*Pongamia pinnata*) and subabul (*Leucaena lencoocephala*) leaf extraxts separately (5 and 10 %) and in integration (2.5 and 5 %) against web blight of urd and mungbeen caused by *Thanatephorus cucumeris* found that karanj leaf extract showed superiority over subabul leaf extract in all the respects.

Sunfuentes *et al.* (2002) while working with disease of *Vigna radiata* observed suppression of *Rhizoctonia solani* with Eucalyptus bark and leaf extract in pot under glass house conditions.

Shahraj *et al*. (2007) while working with plant extracts and *Rhizoctonia solani* observed that use of aqueous extract of leaves, stem, bark and fruits from *Eucalyptus* sp. at a concentration of five per cent as soil amendment increased in germination, shoot and root length and the weights of mungbean plants significantly. They reported that all the plant parts of *Eucalyptus* sp. were equally effective in reducing infection of *Rhizoctonia solani* in mungbean.

Rizvi *et al.* (2012) reported that when the botanicals namely *Argemone mexicana, Calotropis procera, Solanum xanthocarpum,* and *Eichhornia echinulata* are applied in soil in combination with normal as well as

deep ploughing against soil-inhabiting fungi infesting chickpea reduced frequency of parasitic fungi such as *Macrophomina phaseolina, Fusarium oxysporum, Rhizoctonia solani, Phyllosticta phaseolina,* and *Sclerotium rolfsii.*

Thakre and Bhatt (2016) conducted pot experiment under glass house condition where leaf powder of nine plants extracts *i.e.* Neem (*Azadirachta indica*), Karanj (*Pongamia pinnata*), Babul (*Acacia nilotica*), Nilgiri (*Eucalyptus tereticornis*), Jatropha (*Jatropha curcas*) Ashok (*Polyalthia longifolia*), Tulsi (*Ocimum sanctum*), Bougainvillea (*Bougainvillea sp.*) and Mehndi (*Lawsonia alba*) were mixed with soil @ 40 g/kg soil. All the botanicals showed antifungal properties as soil amendment against *Fusarium oxysporum* f. sp. *vigni* Minimum plant mortality was recorded on neem amended soil.

2.7 Influence of oil cakes as soil treatment

Four oil cakes (ground nut, mustard, sesamum and binola seed), tested against groundnut and mustard at two per cent concentration were found most effective in reducing pathogen *Fusarium oxysporum* f. sp. *lycoparsici* population (>70%) and disease incidence on tomato. However, groundnut was found superior over mustard as it not only reduced higher disease index (77.1%) but improved plant growth also. Cotton seed cake was the least effective (Raj and Kapoor, 1996).

Dubey (2002) in his experiment with soil application of karanj cake @ five per cent showed best performance. It increased seed germination and grain yield of mungbean and decreased seedling mortality and disease intensity web blight.

Oilcakes were used *In vitro* to control *Fusarium oxysporum* on bottle gourd and cucumber. Significant effect on seed germination, plant height and seedling mortality was observed, where infested seeds of bottle gourd and cucumber were sown in non-sterilized soil amended with mustard, neem and castor cakes in all ratios and neem and castor cakes at the ratios of 1:20 and 1:30 respectively. Mustard and neem cakes were found most effective in

enhancing seed germination and plant height as well as minimizing seedling mortality in bottle gourd (Sultana *et al.,* 2013).

Rafi *et al*. (2016) reported that effects of soil amendment with oil seed cakes including mustard (*Brassica campestris* L.), cotton (*Gossypium hirsutum* L.), almond (*Prunus amygdalus* L.) and black seed (*Nigella sativa L*.) reduced the root infecting fungi like *Macrophomina phaseolina, Fusarium spp.*

2.8 *In vivo* effect of leaf extract as seed treatment

Singh *et al.* (1980) found that growth of *Fusarium oxysporum* f. sp. *ciceris* was inhibited by neem leaf extract treated seed sown in wilt infested soil.

Hashmi (1992) in his findings observed that seed treatment with neem leaf extract (80 ppm) controlled *Fusarium oxysporum* in lentil.

Mishra and Tripathi (1994) reported efficacy of leaves of *Clematis gouriana* to control the wilt disease of chickpea by seed treatment as well as through soil amendment.

Rahman *et al.* (1999) reported that seed treatment with neem (*Azadirachta indica)* extract @ 0.3 per cent was effective against seed borne infection (including *Fusarium spp*) of wheat.

2.9 *In vivo* effect of bio-control agents.

In view of the adverse effects of fungicides to the environment and increasing interest in sustainable Agriculture, biological control has been tried as an attractive possibility for management of soil borne plant pathogens (Bapat and Shar, 2000).

Sharma and Rana (2000) observed that extracts of easily available botanicals and bio-agents, when used as seed treatment, can frame an important component of cost effective integrated disease management system.

Manczinegr *et al*. (2002) reported that *Trichoderma harzianum, T. viride* and *T. polysporum* have a strong antagonistic effect against soil borne pathogens.

Singh *et al.* (2002) reported that supplementing the soils with fungal or bacterial antagonists reduced incidence of *Fusarium* wilt.

Khan and Khan (2003) found that application of *Trichoderma harzianum* decreased the incidence and severity of *Fusarium* wilt by 43 and 48 per cent, respectively in comparison to control. Other bio-agents were also found effective in decreasing the incidence and severity of wilt by 42 and 39 per cent. *Pseudomonas flourescens* and *Bacillus subtilis* reduced the disease incidence by 22 and 17 per cent, respectively.

Anjaiah *et al.* (2003) reported that inoculation of pigeonpea and chickpea seeds with *Pseudomonas aeruginosa* significantly reduced the *Fusarium* wilt in naturally infested soil.

MATERIAL & METHODS

The experiments were carried out in the Department of Plant Pathology, college of Agriculture, J. N. K. V. V., Jabalpur (M.P.).

3.1 Collection of seeds

Pea seeds (var. JP 885) collected from Breeder Seed Production Unit, Department of Genetics and Plant Breeding, J.N.K.V.V., Jabalpur. The seed were cleaned from inert matter labeled and stored in the paper bags followed by polythene bags and used during the course of investigation.

3.2 Location

The experiments were conducted in the Department of Plant Pathology, J.N.K.V.V., College of Agriculture, Jabalpur. Laboratory and under glass house facilities were utilized during experimentation.

Field experiments were carried out in the experimental area of Department of Plant Pathology, J.N.K.V.V. Jabalpur (M.P.).

3.3 Preparation of soil composite

Sandy loam soil was collected from the experimental area of J.N.K.V.V., Jabalpur (M.P.). The soil, so collected, was thoroughly washed with three to four changes of water so as to remove the soluble leachetes and air dried. The soil was mixed with well decomposed dried and pulverized farm yard manure (FYM) in a ratio of 3:1. This soil composite was used throughout the experiments after sterilization.

3.4 Sterilization of soil composite.

The soil composite was sterilized with four per cent commercial formaldehyde by sealing the heap of soil with polyethylene sheet for 15 days. Later the soil was spread in a thin layer and exposed to direct sunlight to allow complete evaporation o f remnants of formaldehyde. Soil composite was then stored in a clean aluminum tray, covered with polyethylene sheet and was utilized as and when needed.

3.5 Sterilization of pots

Ten centimeter diameter earthen pots of 500 cm^3 capacity were used during the experimentation. Pots were sterilized by exposing them in fumes of four percent formaldehyde solution in a closed container and stored in clean and dry place covered with sterilized polythene sheet. These pots were used throughout the experimentation. A regular supply of sterilized pots was maintained during the course of investigation.

3.6 Cleaning and sterilization of glasswares

3.6.1 Glassware

Petri dishes, flasks, test tubes, pipettes, which were used during investigations were first cleaned by detergent, washed with tap water and then dipped overnight in cleaning solution (Potassium dichromate 80 g, Distilled water 300 ml and sulphuric acid 400 ml) followed by rinsing in distilled water four times and dried in air. The glass wares were sterilized in hot air oven at 180 ^{0}C for three hours.

3.6.2 Metallic equipment

Forceps, inoculation needle, scissors and cork borers were sterilized by dipping them in alcohol and passed over flame of a spirit lamp. The plastic wares *viz.,* beakers and measuring cylinders were cleaned and sterilized by a cotton swab with 95 per cent alcohol.

3.7 Irrigation of pots

Optimum moisture was regulated in each pot by providing 50 ml. purified water free from all biological entities chemicals and physical impurities.

3.8 Medium used

Potato Dextrose Agar (PDA) was used during the course of study and for isolation and maintenance of culture. The ingredients of the medium were as follows:

Potato (peeled and sliced)	-	200 g
Dextrose	-	20 g
Agar-agar	-	20 g
Distilled water	-	1000 ml.

Two hundred grams of peeled and sliced potatoes were washed and boiled in 500ml. of distilled water for 30 min. The extract so prepared was filtered through cheese cloth. Twenty gram Agar-agar strips, washed in distilled water and 20 g dextrose was added to the extract and total volume was made to1000 ml by adding distilled water to the suspension and was kept in water bath so as to obtain complete dissolution of the ingredients. Fifty ml medium was then poured into 250 ml conical flasks. These conical flasks were plugged with cotton plug and sterilized at 121.6 ^{0}C and 1.05 kg/cm^2 pressure for 30 minutes in an autoclave before use.

3.9 Collection of diseased plants

Diseased plants of pea showing characteristic symptoms of wilt, (*Fusarium oxysporum* f. sp. *pisi)* were collected from experimental area of Department of Plant Pathology J.N.K.V.V., Jabalpur. The infected plants were uprooted carefully so as to get whole root system intact. The roots were washed with tap water and dried with the help of blotter paper to remove traces of water.

3.10 Isolation and purification of *Fusarium oxysporum* f. sp. *pisi*

Isolations were made from the roots of wilted plants showing characteristic symptoms as described earlier. The sample were cut into small pieces (5-10 mm long), by sterilized knife. These were then surface sterilized with 1:1000 mercuric chloride ($Hgcl_2$) for 30 seconds followed by three changes of sterilized water before keeping them on Petri plate containing Potato Dextrose Agar, (PDA). The inoculated plates were incubated at 24±1 ^{0}C for six days and examined for the growth of fungus. The growing colony of *Fusarium oxysporum* f. sp. *pisi* was identified under research binocular microscope and after identification, small portion of the fungus was transferred to a Petri plate

containing PDA and allowed to grow for seven days. On the growth of fungus it was identified as per Booth's monograph under research binocular microscope and pure culture was developed following hyphal tip method. The pure culture so obtained was maintained on PDA slants and used as and when required (Plate 1 A and B).

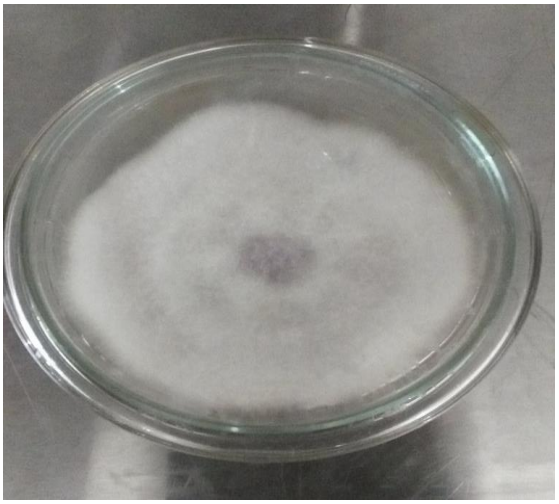


Plate 1: (A) Pure culture of test fungus *Fusarium oxysporum* f. sp. *pisi*

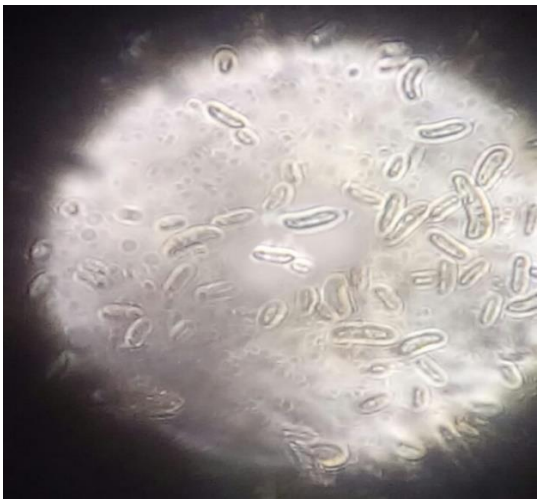


Plate 1: (B) Macro and micro conidia of test fungus *Fusarium oxysporum* f. sp. *pisi*

Plate 1: (C) Culture of test fungus on crushed maize seeds

3.11 Pathogenicity test

The pathogenicity of the *Fusarium oxysporum* f. sp. *pisi* was tested on pea plants under glass house conditions. The inoculum for pathogenicity was prepared from culture of *Fusarium oxysporum* f. sp. *pisi* maintained on PDA. The following method was employed to mass multiply the organism.

Maize seeds were crushed in bulk and moistened with water (Plate 1 C). One hundred gram of crushed seeds were filled in 250 ml flasks and sterilized in an autoclave. After cooling the flasks, these were inoculated with five mm disc of seven days old culture of the *Fusarium oxysporum* f. sp. *pisi* and incubated at 24±1 ^{0}C. On sufficient growth of the fungus, the culture was mixed with the sterilized pot soil @ 10g / 500g soil (Tripathi, 1998). The pots were kept for seven days to allow the multiplication, development and spread of the fungus in pot soil. Surface sterilized pea seed were sown in pots. Each pot received five seed. The pots were irrigated with 50 ml of sterilized tap water. The pots were observed for germination and emergence of seed and mortality of the seedlings. The plants showing wilt symptoms were removed carefully, the fungus was re-isolated from such plants and identified.

3.12 Collection of Bio-agents

Commercial formulations of antagonistic microorganisms *viz*., *Trichoderma viride* and *Pseudomonas fluorescens* were obtained from Department of Plant Pathology, J.N.K.V.V., Jabalpur.

3.13 Collection of plant leaves

Plant leaves of commonly available trees having medicinal values *viz.*, Neem (*Azadirachta indica*), Karanj (*Pongamia pinnata*), Datura (*Datura stramonium*), Tulsi (*Ocimum tenuiflorum*), Aak (*Calotropis gigantia*), Mehndi (*Lawsonia inermis*), Eucalyptus (*Eucalyptus globulus*), Jatropha (*Jatropha curcas*), Parthenium (*Parthenium hysterophorus*) and Ashok (*Saraca asoca*), were collected from the botanical garden of College of Agriculture, Jabalpur. The leaves were dried at room temperature. After complete drying, the leaves were

ground in fine powder and stored in a clean and dry place for further use as and when needed. Required quantity of leaf powder was kept aside for further use and rest was used for making extract. The powder and extract were used immediately to carryout experiments.

3.13.1 Botanicals (Leaf extracts)

The leaf extracts of following plants were used in the present Investigations.

S. No.	Common name	Botanical name
1	Neem	*Azadirachta indica*
2	Karanj	*Pongamia pinnata*
3	Datura	*Datura stramonium*
4	Tulsi	*Ocimum tenuiflorum*
5	Aak	*Calotropis gigantia*
6	Mehndi	*Lawsonia inermis*
7	Eucalyptus	*Eucalyptus globules*
8	Jatropha	*Jatropha curcas*
9	Parthenium	*Parthenium hysterophorus*
10	Ashok	*Saraca asoca*

3.14 Method of extraction and preparation of soil amendment

Leaf extract was prepared by boiling ten gram powder of each plant leaf in 100 ml distilled water so as to obtain extract (Plate 2 A). The decoction was filtered through cotton wool to obtain clear extract. The extracts were used immediately after cooling to carryout experiments (Saramangala *et. al.,* 1993).

Fresh plant leaves, collected, were thoroughly washed in running tap water so as to remove undesirable contents and drying in hot air oven at 60 ^{0}C till complete dryness. Leaves were ground with the help of pestle and mortar

in to a fine powder (Plate 2 B). Required quantity of leaf powder was kept aside for evaluation purpose. The powders were used immediately to carryout experiments.

Plate: 2 (A) Leaf extracts of botanicals

1) Neem (*Azadirachta indica*)
2) Karanj (*Pongamia pinnata*)
3) Datura (*Datura stramonium*)
4) Tulsi (*Ocimum tenuiflorum*)
5) Aak (*Calotropis gigantea*)
6) Mehndi (*Lawsonia inermis*)
7) Eucalyptus (*Eucalyptus globulus*)
8) Jatropha (*Jatropha curcas*)
9) Parthenium (*Parthenium hysterophorus)*
10) Ashok (*Saraca asoca*)

Plate: 2 (B) Leaf powders of botanicals

1) Neem (*Azadirachta indica*)
2) Karanj (*Pongamia pinnata*)
3) Datura (*Datura stramonium*)
4) Tulsi (*Ocimum tenuiflorum*)
5) Aak (*Calotropis gigantea*)
6) Mehndi (*Lawsonia inermis*)
7) Eucalyptus (*Eucalyptus globulus*)
8) Jatropha (*Jatropha curcas*)
9) Parthenium (*Parthenium hysterophorus)*
10) Ashok (*Saraca asoca*)

Plate 2: (A) Leaf extracts of botanicals

Plate 2: (B) Leaf powders of botanicals

3.15 Sowing of pea seeds

Good, bold and healthy seeds of pea (var. JP 885) obtained from Department of Genetics and Plant Breeding, were used throughout the experiments. The seeds were surface sterilized by mercuric chloride as described earlier and were sown in pots containing 500 g sterilized soil composite. The pots were watered with sterilized tap water as and when needed.

3.16 Bioassay

The experiment was conducted under laboratory conditions to evaluate the efficacy of plant leaves against *Fusarium oxysporum* f. sp. *pisi* .The extracts of ten plant leaves *viz*. Neem (*Azadirachta indica*), Karanj (*Pongamia pinnata*), Datura (*Datura stramonium*), Tulsi (*Ocimum tenuiflorum*), Aak (*Calotropis gigantia*), Mehndi (*Lawsonia inermis*), Eucalyptus (*Eucalyptus globulus*), Jatropha (*Jatropha curcas*), Parthenium (*Parthenium hysterophorus*) and Ashok (*Saraca asoca*) were evaluated using poisoned food technique (Nene et. al., 1979). Water deficit Potato Dextrose Agar (PDA) was prepared and 100 ml medium was poured in 250 ml capacity Earlemayer flask. Required quantities (10, 20 and 30 %) of extracts were mixed with this water deficit medium and autoclaved as per the method described earlier. The medium along with leaf extracts was then poured in to sterile Petri plate. After solidification, the plates were inoculated with three mm disc of test fungus *i.e. Fusarium oxysporum* f. sp. *pisi* and were incubated at 24 ±1 ^{0}C. Observations on colony diameter were recorded at 48, 96 and 144 hours after inoculation.

3.17 Efficacy of plant leaf powders as soil treatment

The experiment was conducted in pots under glass house condition. The test fungus *Fusarium oxysporum* f. sp. *pisi* was grown on crushed maize grain and mixed with the soil @ 10 g/kg soil as per the method described earlier. At the same time the leaf powders were also incorporated @ 5, 10 and 15 g/kg soil along with fungus. The soil mixture was then filled in sterilized pots.

Surface sterilized seeds of pea were sown in each pot. Each pot received five seeds. Each treatment was replicated three times and randomized over glass house bench. The pots were irrigated with sterilized tap water as and when required. The glass house temperature during the period of experimentation ranged between 18-30 ^{0}C. The experiment was allowed to run for 60 days. The pots were observed for seed germination, seedling mortality and appearance of wilt symptoms. The data recorded was subjected to statistical analysis following complete randomized design (CRD).

3.19 Efficacy of oil cakes against *Fusarium oxysporum* f. sp. *pisi*

The experiment was conducted in pots under glass house condition. The test fungus *Fusarium oxysporum* f. sp. *pisi* was grown on crushed maize grain and mixed with the soil @ 10 g/kg soil as per the method described earlier. At the same time the oil cakes (Neem, Mustard, Castor, Cotton and Linseed) was incorporated (@ 5, 10 and 15 g/kg soil) along with fungus. The soil mixture was then filled in sterilized pots. Surface sterilized seeds of pea were sown in each pot. Each pot received five seeds. Each treatment was replicated four times. The pots were irrigated with sterilized tap water as and when required. The glass house temperature ranged between 18-30 ^{0}C during the period of experimentation. The experiment was allowed to run for 60 days. The pots were observed for seed germination, seedling mortality and appearance of wilt symptoms. The data so obtained was analyzed following complete randomized design (CRD).

3.18 Efficacy of bio-control agents and plants leaves extract as seed treatment against *Fusarium oxysporum* f. sp. *pisi*

The experiment was conducted under field conditions. Apparently healthy surface sterilize seeds of pea (JP 885) were coated with bio-agents *viz*., *Trichoderma viride* @ 5 g/kg seed and *Pseudomonas fluorescens* @ 5 g/kg seed separately. Good and bold surface sterilize seeds of pea (JP 885) were soaked in 5 and 10 per cent leaf extract of neem and eucalyptus for 6 and 12 hours respectively. There were seven treatments replicated four times. Each plot

received 192 seed (32 g). Observations on seed germination, seedling mortality and appearance of wilt symptoms were recorded. The data obtained was analyzed following Randomized block design (RBD).

RESULTS

4.1 Test of Pathogenicity

The experiment was conducted under glass house condition using infested soil. The fungus, *Fusarium oxysporum* f. sp. *pisi*, was grown on crushed maize grain soaked in water. Ten days old fungal culture grown on this medium was mixed with the sterilized pot soil and sterilized seeds of pea were sown and allow to grow till the appearance of symptoms (Plate 3 A and B).

The symptoms appeared 17 days after sowing of seeds in the infested soil. The symptoms further developed and plants were wilted within 21 days. The root did not show any external rotting. Such root when splited vertically from the collar region showed brown discoloration in vascular tissues. *F. oxysporum* f. sp. *pisi* was reisolated on PDA plates from the roots of diseased plants.

4.2 Effect of leaf extracts on the mycelial growth of *Fusarium oxysporum* f. sp. *pisi*

The experiment was conducted under laboratory conditions in plates containing Potato Dextrose Agar. Observations were recorded 48, 96 and 144 hours after inoculation employing 10, 20 and 30 per cent concentrations of leaf extracts.

It is evident from the data presented in the Table 1 and Fig 1 that ten per cent concentration of Karanj (*P. pinnata*) was observed superior over control and rest of the treatments after 48 hours of inoculation. Minimum (4.66 mm) mycelial growth was recorded in this treatment against maximum in control (08.16 mm). This was followed by Neem (*A. indica*) and Mehndi (*L. inermis*) where 05.00 mm mycelial growth was noted. This concentration did not show any significant mycelial inhibition when the observations were recorded.

At 20 per cent concentration of leaf extract minimum (4.5 mm) radial growth of the test fungus was noted in Karanj, Neem and Parthenium followed by Jatropha (5.33 mm). Maximum (08.16 mm) radial growth was recorded in control.

(A) Infected plants

(A) Healthy plants

Plate 3: Test of pathogenicity of *Fusarium oxysporum* f. sp. *pisi* under pot condition

Eucalyptus (*E. globulus*), Datura (*D. stramonium*) and Ashok (*S. asoca*) stood next in order of their efficacies where 5.5, 5.5, and 5.66 mm radial growth of *Fusarium oxysporum* f. sp. *pisi* was noted. Mehndi (*L. inermis*) remained at par in inhibiting of mycelial growth of the test fungus.

At 30 per cent concentration, Parthenium (*P. hysterophorus*) observed to be significantly superior over rest of the treatments and control. This treatment showed minimum (3.83 mm) radial growth of *Fusarium oxysporum* f. sp. *pisi* followed by *Lawsonia inermis and Pongamia pinnata* (4.33 mm). Rest of the treatments however, remained at par with each other but superior over control at 48 hours after inoculation.

The data presented in the Table 2 and Fig 2 indicated that after 96 hours of inoculation *L. inermis* and *P. pinnata* at ten percent concentration remained statistically superior over control where the mycelium growth of *F. oxysporum* f. sp*. pisi* was recorded to be 18.00 and 18.16 mm against control (25.00 mm).These treatments were followed by Neem (19.5 mm) that was again superior over control.

At 20 per cent concentration Mehndi (*L. inermis*) remained effective and superior over rest of the treatments and control. Minimum (15.00 mm) radial growth of the *Fusarium oxysporum* f. sp. *pisi* was recorded in this treatment as against maximum (25 mm.) radial growth in control.

Minimum 10.16 mm radial growth of *Fusarium oxysporum* f. sp. *pisi* was observed at 30 per cent concentration of Neem (*A. indica*) leaf extract followed by Mehndi (10.33 mm) and *Datura stramonium* (14.33 mm). Rest of the treatments remained at par with each other in the inhibition of test fungus as against maximum (25 mm) in control.

The data presented in the Table 3, Plate 4 and Fig 3 revealed that after 144 hours at ten per cent concentration Mehndi (*L. inermis*) leaf extract amended medium showed minimum (31 mm) radial growth of the test fungus against maximum (43.83 mm) in control.

Table 1: Effect of leaf extracts on mycelial growth of *Fusarium oxysporum* f. sp. *pisi* at 48 hrs

S. No.	Treatments	Mean radial growth (mm)*					
		Concentration (per cent)					
		10	% Inhibition	20	% Inhibition	30	% Inhibition
1	Neem	5.00	38.72	4.5	44.85	4.16	49.01
2	Karanj	4.66	42.89	4.5	44.85	4.33	46.93
3	Datura	6.16	24.50	5.5	32.59	5.33	34.68
4	Tulsi	7.16	12.25	6.16	24.50	5.83	28.55
5	Aak	7.66	06.12	6.5	20.34	6.33	22.42
6	Mehndi	5.00	38.72	4.66	42.89	4.33	46.93
7	Eucalyptus	6.16	24.50	5.5	32.59	5.16	36.76
8	Jatropha	6.33	22.42	5.33	34.68	5.16	36.76
9	Parthenium	5.33	34.68	4.5	44.85	3.83	53.06
10	Ashok	6.83	16.29	5.66	30.63	5.00	38.72
11	Control	8.16		8.16		8.16	
		Treatments (A)		Concentrations (B)		Interaction (A × B)	
	SEm±	0.235		0.122		0.406	
	CD at 5 %	0.664		N/A		1.149	

*Each value is a mean of three replications.

Eucalyptus (*E. globulus*) and *P. pinnata* leaf extracts recorded 33.66 and 34.33 mm radial growth of the test fungus respectively. Datura (*D. stramonium*) stood second in order of efficacy where 32.5 mm radial growth was recorded. Rest of the treatments however less effective in mycelial growth inhibition but superior over control.

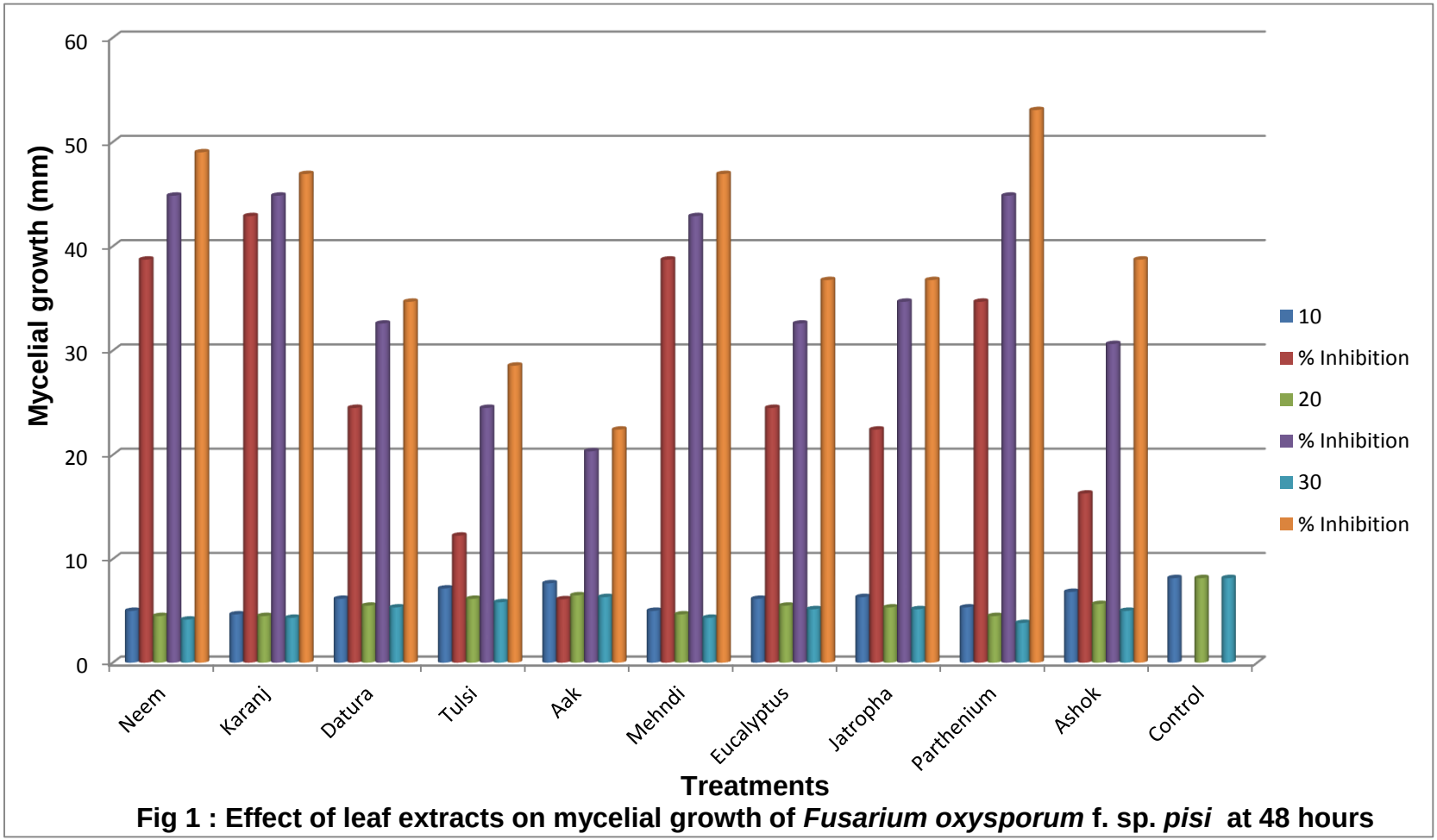


Fig 1 : Effect of leaf extracts on mycelial growth of *Fusarium oxysporum* f. sp. *pisi* at 48 hours

Table 2: Effect of leaf extracts on mycelial growth of *Fusarium oxysporum* f. sp. *pisi* at 96 hrs

S. No.	Treatments	Mean radial growth (mm)*					
		Concentration (per cent)					
		10	% Inhibition	20	% Inhibition	30	% Inhibition
1	Neem	19.50	22.00	16.66	33.36	10.16	59.36
2	Karanj	18.16	27.36	16.16	35.36	14.16	43.36
3	Datura	21.00	16.00	17.83	28.68	14.33	42.80
4	Tulsi	23.83	04.68	22.33	10.68	21.66	13.36
5	Aak	22.66	09.36	21.66	13.36	19.83	20.68
6	Mehndi	18.00	28.00	15.00	40.00	10.33	58.68
7	Eucalyptus	20.00	20.00	18.00	28.00	15.33	38.68
8	Jatropha	22.16	11.36	20.16	19.36	16.83	32.68
9	Parthenium	21.83	12.68	19.00	24.00	14.16	43.36
10	Ashok	20.83	16.68	19.33	22.68	17.5	30.00
11	Control	25.00		25.00		25.00	
		Treatments (A)		Concentrations (B)		Interaction (A × B)	
	SEm±	0.339		0.177		0.587	
	CD at 5 %	0.959		0.501		1.661	

*Each value is a mean of three replications.

Twenty per cent concentration of Mehndi (*L. inermis*) leaf extract inhibited the mycelial growth of *Fusarium oxysporum* f. sp. *pisi* significantly followed by Datura (*D. stramonium*).Radial growth of the test fungus was 24.5 and 26.5 mm respectively against maximum (43.83 mm) in control. This was followed by Neem (32 mm), Eucalyptus (32.5 mm) and *P. pinnata* (33 mm). Rest of the treatments remained at par with each other but superior over control.

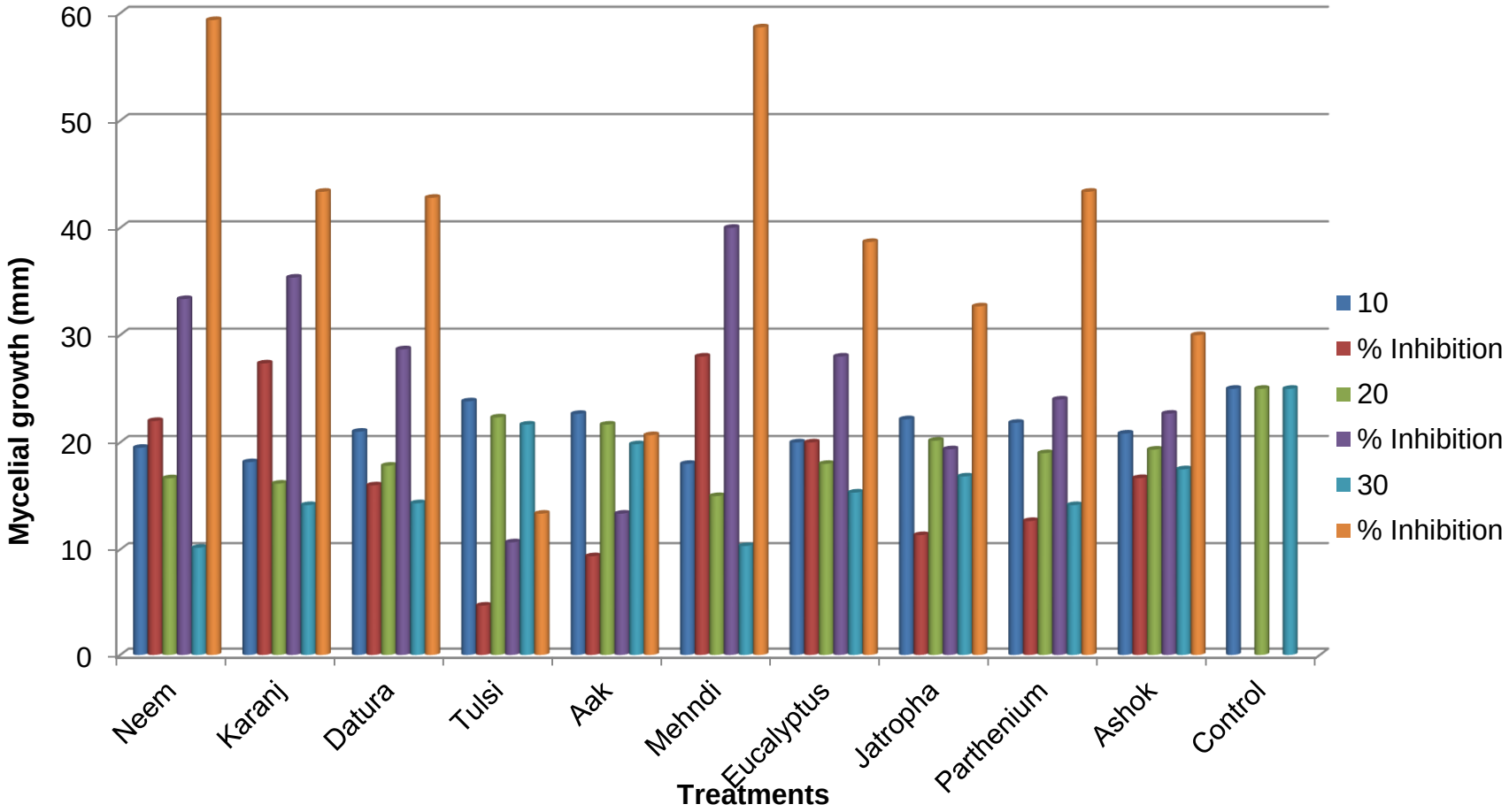


Fig 2: Effect of leaf extracts on mycelial growth of *Fusarium oxysporum* f. sp. *pisi* at 96 hours

Minimum (20.5 mm) radial growth of *Fusarium oxysporum* f. sp. *pisi* was noted in Mehndi (*L. inermis*) leaf extract followed by Neem (22 mm), Datura (26.33 mm), *P. pinnata* (29 mm) and Eucalyptus (29.33 mm) against maximum (43.83 mm) in control after 144 hours at 30 per cent concentration.

Table 3: Effect of leaf extracts on mycelial growth of *Fusarium oxysporum* f. sp. *pisi* at 144 hrs

S.No.	Treatments	Mean radial growth (mm)*					
		Concentration (per cent)					
		10	% Inhibition	20	% Inhibition	30	% Inhibition
1	Neem	37.00	15.58	32.00	26.99	22.00	49.80
2	Karanj	34.33	21.67	33.00	24.70	29.00	33.83
3	Datura	32.50	25.84	26.50	39.53	26.33	39.92
4	Tulsi	40.00	08.73	40.00	08.73	35.00	20.14
5	Aak	39.33	10.26	39.16	10.65	34.00	22.42
6	Mehndi	31.00	29.27	24.50	44.10	20.50	53.22
7	Eucalyptus	33.66	23.20	32.50	25.84	29.33	33.08
8	Jatropha	37.16	15.21	35.16	19.78	33.16	24.34
9	Parthenium	40.66	07.23	35.83	18.25	29.50	32.69
10	Ashok	35.33	19.39	33.50	23.56	33.00	24.70
11	Control	43.83		43.83		43.83	
		Treatments (A)		Concentrations (B)		Interaction (A × B)	
	SEm±	0.343		0.179		0.595	
	CD at 5 %	0.971		0.507		1.683	

*Each value is a mean of three replications.

Plate: 4 Effect of Leaf extracts against *Fusarium oxysporum* f. sp. *pisi*

T_1 - Neem (*Azadirachta indica*)

T_2 - Karanj (*Pongamia pinnata*)

T_3 - Datura (*Datura stramonium*)

T_4 - Tulsi (*Ocimum tenuiflorum*)

T_5 - Aak (*Calotropis gigantea*)

T_6 - Mehndi (*Lawsonia inermis*)

T_7 - Eucalyptus (*Eucalyptus globulus*)

T_8 - Jatropha (*Jatropha curcas*)

T_9 - Parthenium (*Parthenium hysterophorus)*

T_{10} - Ashok (*Saraca asoca*)

4.3 Effect of leaf powders as soil amendments on mortality of pea.

The experiment was conducted under glass house condition in pots were the leaf powders of plants *viz.,* Neem, Karanj, Datura, Tulsi, Aak, Mehndi, Eucalyptus, Jatropha, Parthenium, and Ashok were mixed with soil @ 5, 10, and 15 g/kg soil along with an unamended control. The observations were recorded at 30 and 45 days after sowing.

The data presented in the Table 4 and fig 4 revealed that all treatments were significantly superior over control in managing the disease up to 45 days, the maximum time period involved in the experimentation.

It is evident from the data that the leaf powders of Neem, Karanj and Mehndi were effective in managing the wilt of pea plants within 30 days at 5 g/kg dose. The plant mortality was minimum (0.66) in neem followed by Karanj and Mehndi (1.00) against control where maximum (3.00) plants were killed

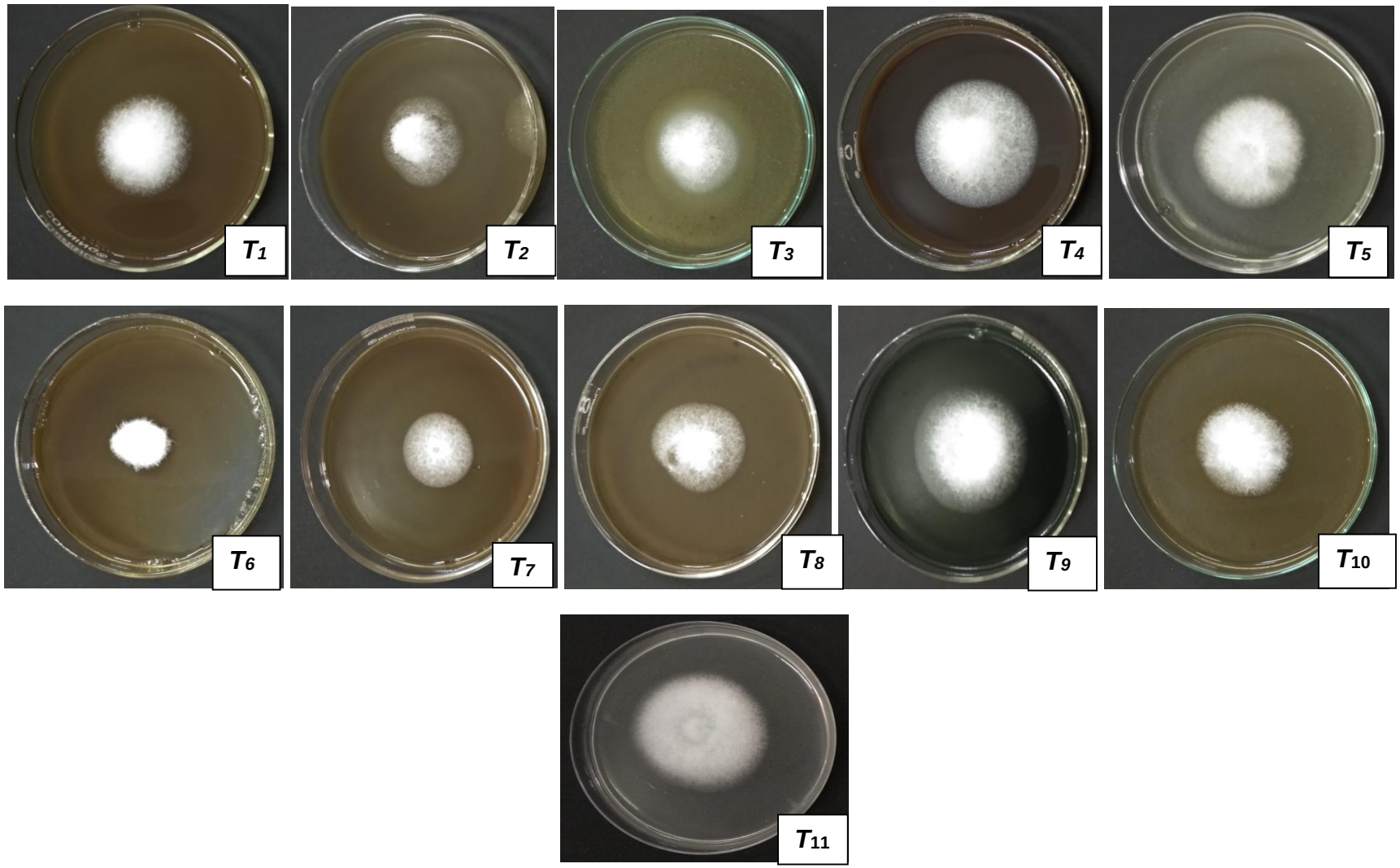


Plate 4: Effect of leaf extracts against *Fusarium oxysporum* f. sp. *pisi*

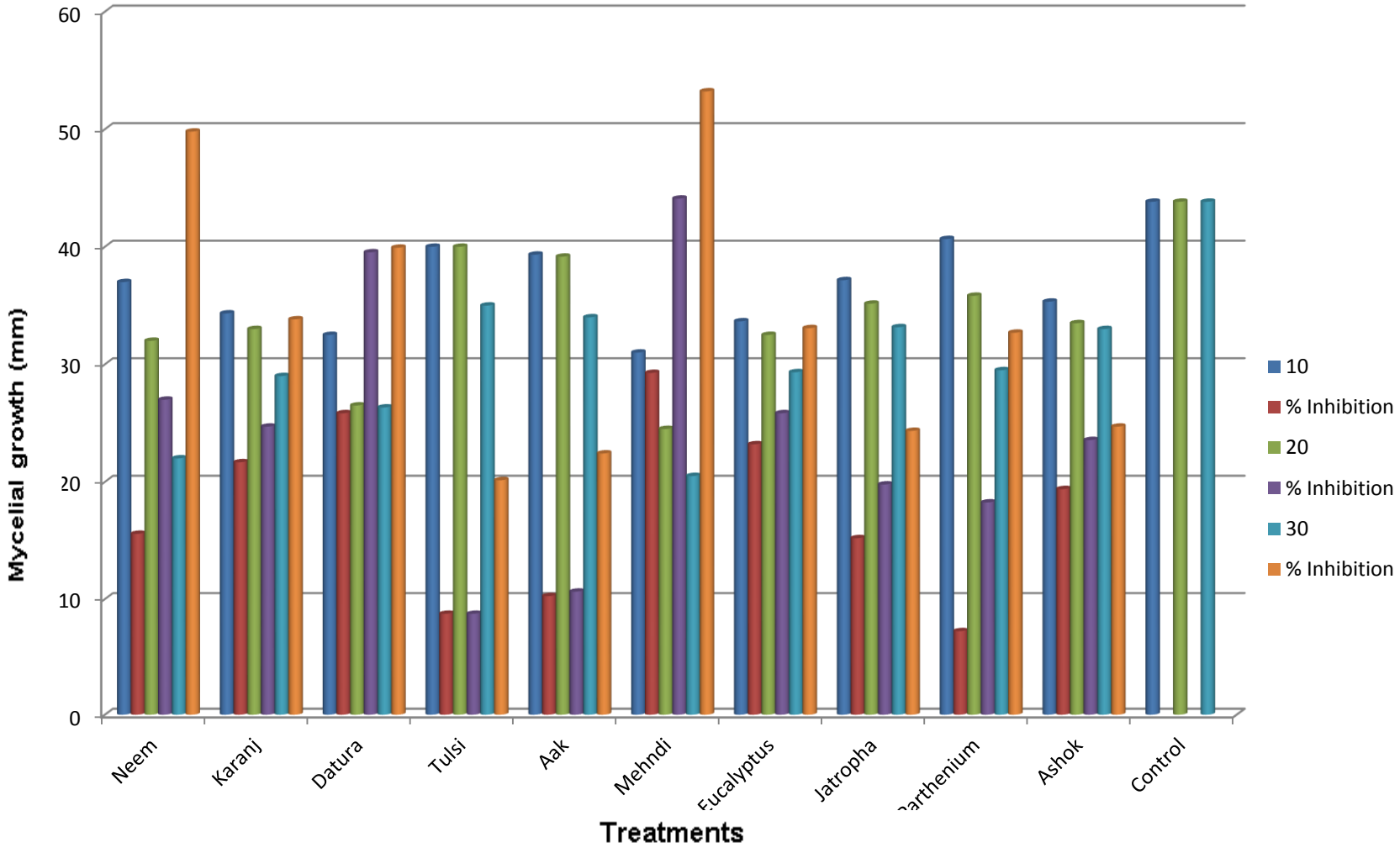


Fig 3 : Effect of leaf extracts on mycelial growth of *Fusarium oxysporum* f. sp. *pisi* at 144 hours

within 30 days of plant growth. These treatments were followed by Tulsi and Eucalyptus (2.00), Jatropha (2.33), Parthenium and Datura (2.66). These were remained at par in their efficacies in managing the disease.

Minimum (0.33) plant mortality was noted in pots where neem powder was incorporated with the soil @ 10 g/kg followed by Karanj (1.00). Effect of Mehndi was observed to significantly superior over control where 1.33 plants were noted to be killed. Maximum plant mortality was observed in control (3.00).

The data in the Table 4, Fig 3 indicated that neem at 15 g/kg dose showed minimum (0.33) plant mortality followed by Karanj (0.66). Mehndi, Tulsi and Eucalyptus was observed to significantly superior over control where 1.66 plants were noted to be killed. Jatropha recorded less plant mortality (2.00) against maximum (3.00) in control within 30 days. Rest of the treatments were found superior in managing the disease within the course of investigation.

The data presented in Table 5, Plate 5 and Fig 5 indicated that minimum (1.33) plant mortality was recorded within 45 days where Neem (*A. indica*) leaf powder was incorporated @ 5 g/kg soil followed by *P. pinnata* (1.66) and Mehndi (2.00) against maximum (3.66) in un amended control. These treatments were followed by Eucalyptus (2.33) and Jatropha (2.66). Rests of the treatments were at par with each other but superior over control.

Incorporation of 10 g/kg soil dose of Neem leaf powder recorded minimum plant mortality was recorded (1.00) followed by *P. pinnata* (1.33) against maximum (3.66) in control. Rest of the treatments were at par among themselves in reducing the plant mortality but showed their superiority over unamended control pots.

Minimum (1.00) plant mortality observed at 15 g/kg dose of Neem leaf powder followed by Karanj (1.33), Mehndi (1.66). Maximum (3.66) plant mortality was recorded in control. Eucalyptus (2.00), Jatropha and Datura (2.33) and Parthenium and Aak (2.66) remained statistically at par among themselves but showed superior efficacy over control after 45 days of plant growth.

Table 4: Effect of leaf powders as soil amendments on mortality of pea (30 DAS)

S. No.	Treatments	Seedling mortality*					
		Doses					
		5 g	% Control	10 g	% Control	15 g	% Control
1	Neem	0.66	78.00	0.33	89.00	0.33	89.00
2	Karanj	1.00	66.66	1.00	66.66	0.66	78.00
3	Datura	2.66	11.33	2.33	22.33	2.33	22.33
4	Tulsi	2.00	33.33	1.66	44.66	1.66	44.66
5	Aak	3.00	0.00	2.66	11.33	2.66	11.33
6	Mehndi	1.00	66.66	1.33	55.66	1.66	44.66
7	Eucalyptus	2.00	33.33	1.66	44.66	1.66	44.66
8	Jatropha	2.33	22.33	2.33	22.33	2.00	33.33
9	Parthenium	2.66	11.33	2.66	11.33	2.33	22.33
10	Ashok	3.00	0.00	2.66	11.33	2.66	11.33
11	Control	3.00		3.00		3.00	
		Treatments (A)		Concentrations (B)		Interaction (A × B)	
	SEm±	0.268		0.140		0.464	
	CD at 5 %	0.758		N/A		1.314	

*Each value is a mean of three replications.

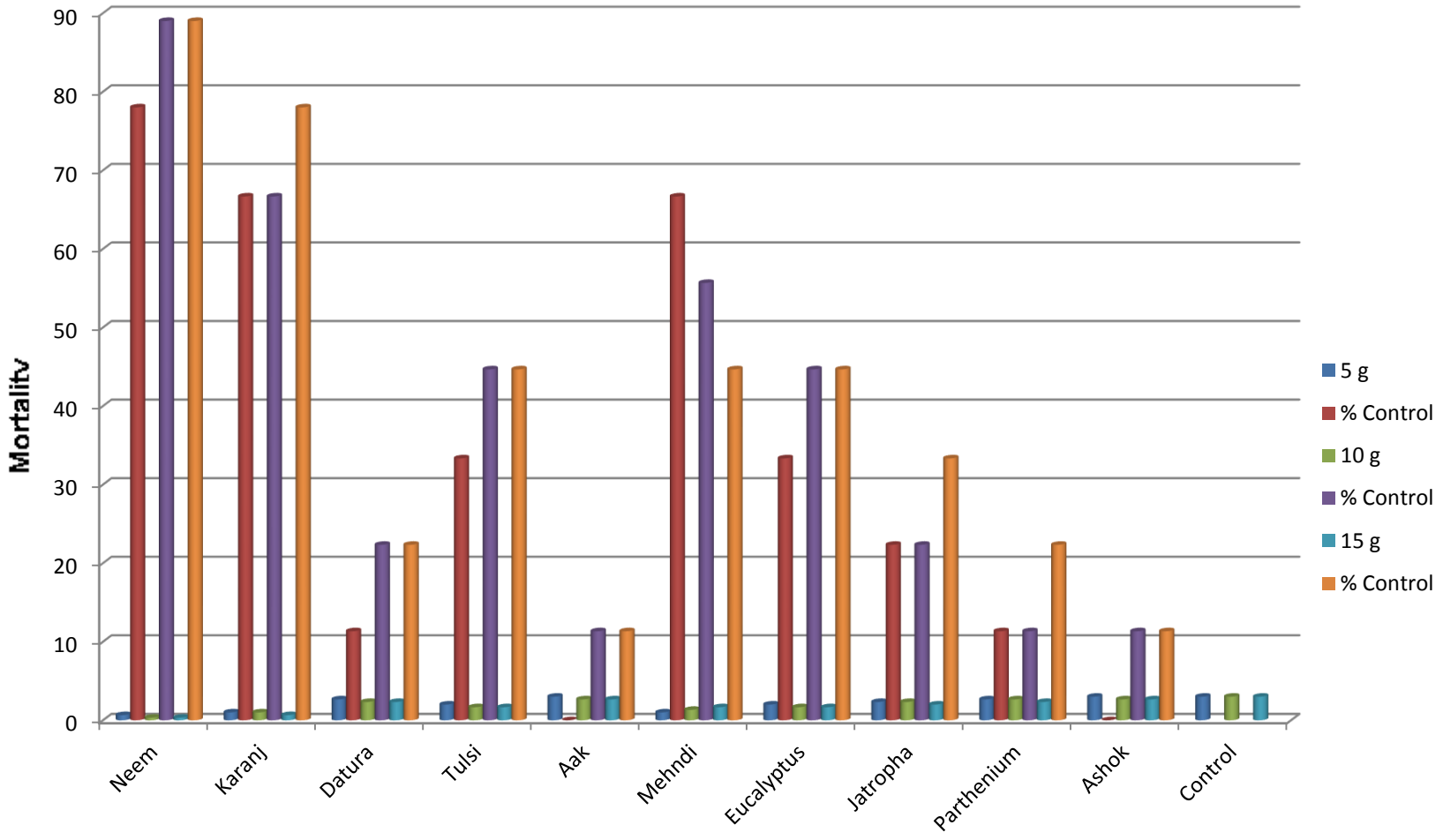
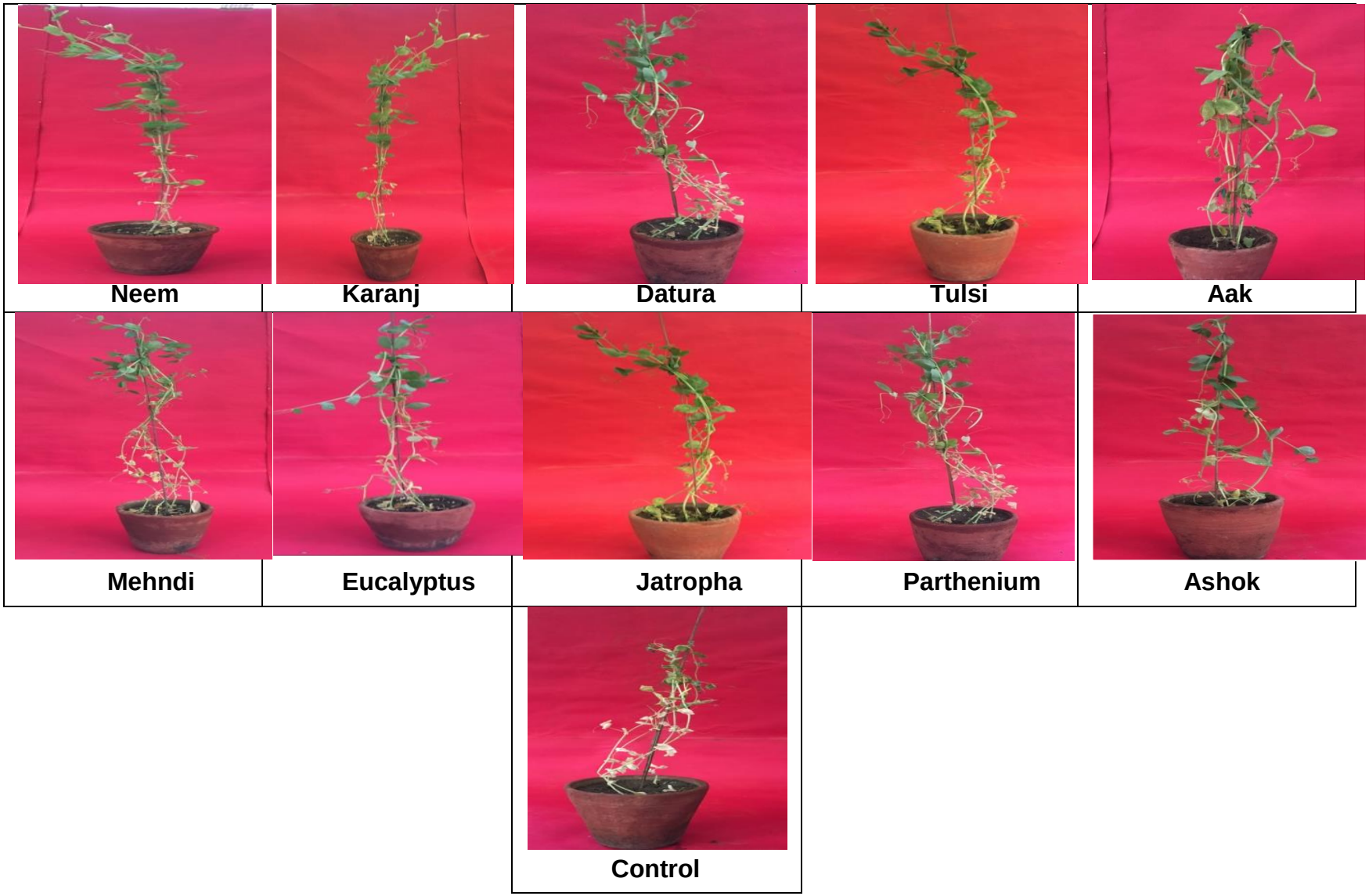


Fig 4 : Effect of leaf powders as soil amendments on mortality of pea (30 DAS)

Table 5: Effect of leaf powders as soil amendments on mortality of pea (45 DAS)

S. No.	Treatments	Seedling mortality*					
		Doses					
		5 g	% Control	10 g	% Control	15 g	% Control
1	Neem	1.33	63.66	1.00	72.67	1.00	72.67
2	Karanj	1.66	54.64	1.33	63.66	1.33	63.66
3	Datura	3.00	18.03	2.33	36.33	2.33	36.33
4	Tulsi	2.33	36.33	2.00	45.35	2.00	45.35
5	Aak	3.33	9.01	3.00	18.03	2.66	27.32
6	Mehndi	2.00	45.35	1.66	54.64	1.66	54.64
7	Eucalyptus	2.33	36.33	2.00	45.35	2.00	45.35
8	Jatropha	2.66	27.32	2.33	36.33	2.33	36.33
9	Parthenium	3.00	18.03	2.66	27.32	2.66	27.32
10	Ashok	3.66	0.00	3.33	9.01	3.00	18.03
11	Control	3.66		3.66		3.66	
		Treatments (A)		Concentrations (B)		Interaction (A × B)	
	SEm±	0.262		0.137		0.453	
	CD at 5 %	0.740		N/A		1.283	

*Each value is mean of three replications.



Pate 5: Effect of leaf powders as soil amendments on mortality of pea

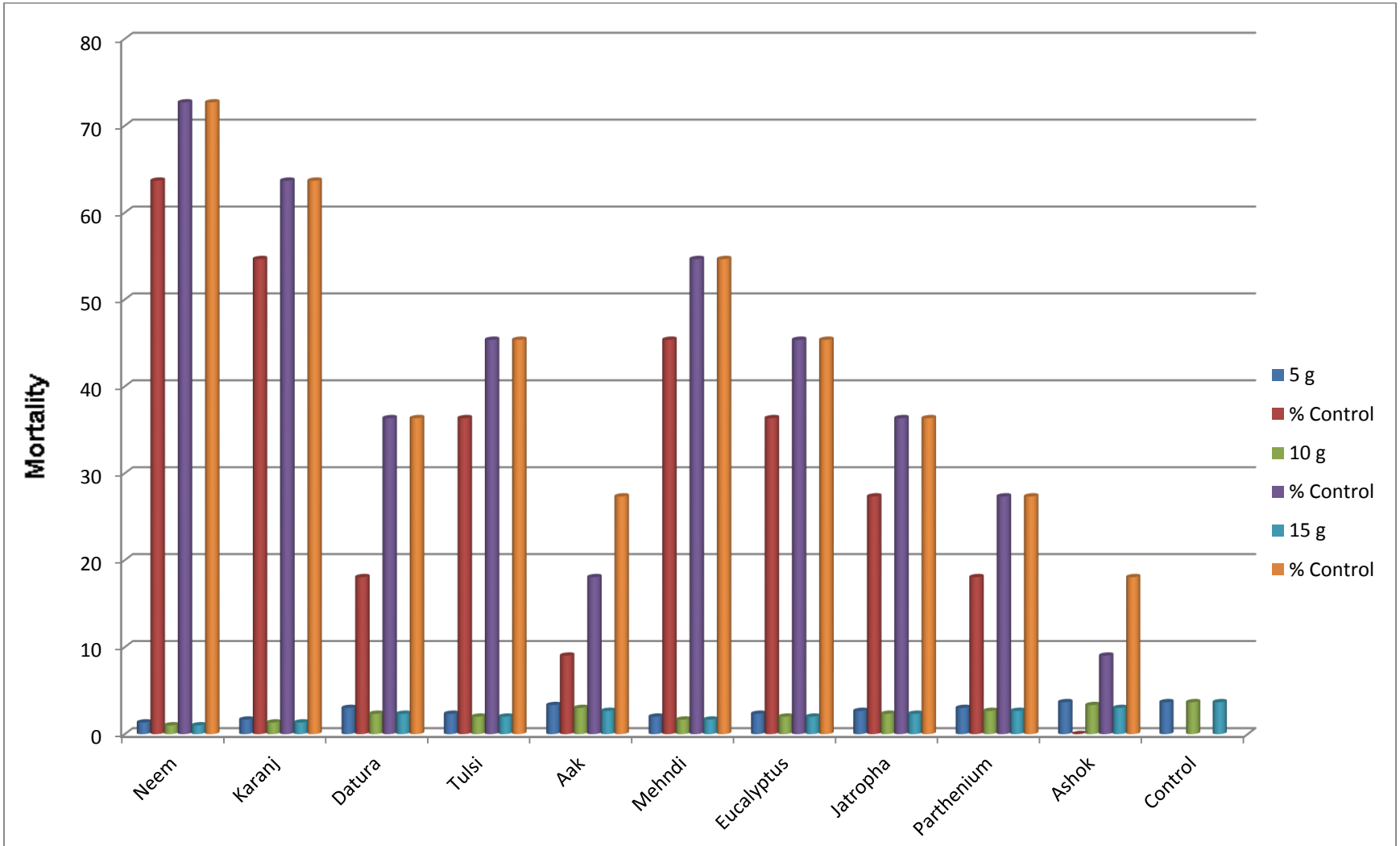


Figure 5 : Effect of leaf powders as soil amendments on mortality of pea (45 DAS)

4.4 Effect of oil cakes as soil amendments on mortality of pea

The data presented in Table 6 and Fig 6 revealed that five g dose of Neem after 30 days of plant growth showed its efficacy where minimum (1.25) mortality was recorded. This was followed by Linseed (1.50) and mustard (1.75) which were remained at par among themselves. Maximum mortality (2.50) was recorded in control.

At ten gram dose of cake minimum (1.00) plant mortality was recorded in Neem amended soil whereas maximum (2.50) was recorded in control. Linseed, Mustard and castor cakes recorded 1.25, 1.50 and 2.0 wilted plants respectively. Rest of the treatments remained at par among themselves in keeping the plants healthy up to 30 days.

Minimum to no wilted plants were recorded in 15 g dose 30 days after sowing in the pots treated with Neem cake. This was followed by Linseed (0.75) against maximum (2.50) in untreated control. Mustard cakes recorded 1.25 wilted plants. Rest of the treatments however, statistically inferior in their efficacies but superior over control 30 days after sowing when the observations were recorded.

The data presented in the Table 7, Plate 6 and Fig 7 indicated that the cakes of each plant was effective in keeping the plants healthy up to 45 days under pot conditions.

In Table 7 (45 DAS), plant mortality minimum (1.75) was recorded in 5 g/kg soil dose of Neem cake followed by Linseed (2.0). Mustard and cotton stood next in order of their efficacies where 2.25 and 2.5 plant mortality was noted where maximum (3.0) plants were noted to be killed in control.

Minimum (1.50) plant mortality was observed at ten g dose of Neem cake followed by Linseed where 1.75 plants dried within 45 days against maximum (3.0) in control. Rest of the treatments were inferior to Neem and Linseed but superior over control when the observations on 45^{th} day were recorded.

Table 6: Effect of oil cakes as soil amendments on mortality of pea (30 DAS)

S. No.	Treatments	Seedling Mortality*					
		Doses					
		5 g	% Control	10 g	% Control	15 g	% Control
1	Neem	1.25	50.00	1.00	60.00	0.50	80.00
2	Mustard	1.75	30.00	1.50	40.00	1.25	50.00
3	Castor	2.25	10.00	2.00	20.00	1.50	40.00
4	Cotton	2.25	10.00	2.25	10.00	1.75	30.00
5	Linseed	1.50	40.00	1.25	50.00	0.75	70.00
6	Control	2.50		2.50		2.50	
		Treatments (A)		Concentrations (B)		Interaction (A × B)	
	SEm±	0.221		0.157		0.383	
	CD at 5 %	0.629		0.445		N/A	

* Each value is a mean of four replications.

At 15 g/kg soil Neem cake incorporated remained effective and superior over rest of the treatments and control. Minimum (1.0) mortality was recorded in this treatment as against maximum (3.0) in control. Maximum (2.25) mortality was noted in cotton over treatments on 45th day of plant growth.

4.5 Effect of botanicals and bio-agents as seed treatment on mortality of pea

The experiment was conducted *in vivo* conditions where the seeds were soaked in five and ten per cent concentrations of Neem and Eucalyptus with soaking duration of 6 and 12 hours respectively. Pea seeds were coated with bio-agents *T. viride and P. flourescens* @ 5 g/kg seeds.

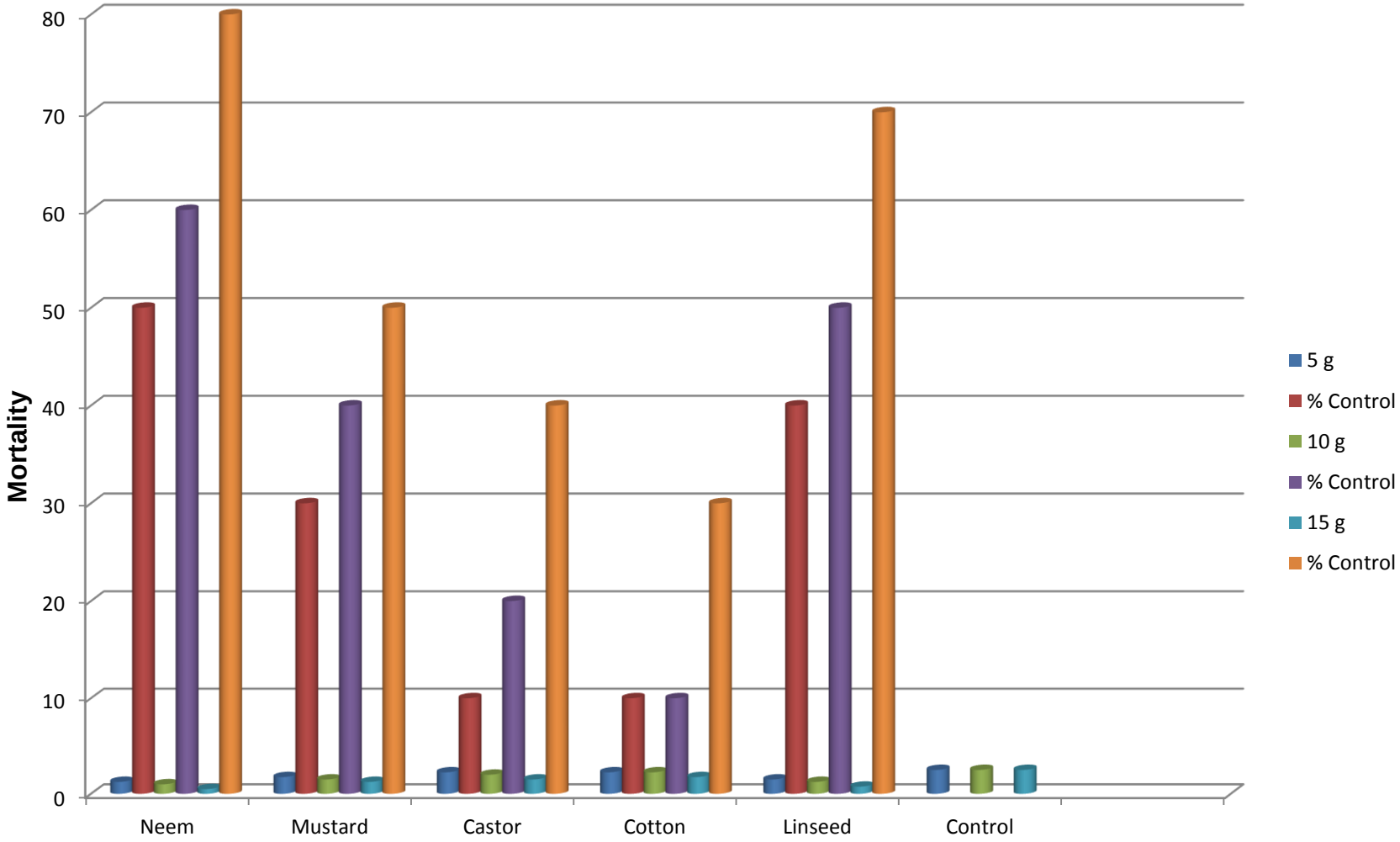


Fig 6 : Effect of oil cakes as soil amendments on mortality of pea (30 DAS)

Table 7: Effect of oil cakes as soil amendments on mortality of pea (45 DAS)

S.No.	Treatments	Seedling Mortality*					
		Doses					
		5 g	% Control	10 g	% Control	15 g	% Control
1	Neem	1.75	41.66	1.50	50.00	1.00	66.66
2	Mustard	2.25	25.00	2.00	33.33	1.75	41.66
3	Castor	2.75	08.33	2.25	25.00	2.00	33.33
4	Cotton	2.50	16.66	2.50	16.66	2.25	25.00
5	Linseed	2.00	33.33	1.75	41.66	1.50	50.00
6	Control	3.00		3.00		3.00	
		Treatments (A)		Concentrations (B)		Interaction (A × B)	
	SEm±	0.281		0.199		0.487	
	CD at 5 %	N/A		0.565		N/A	

* Each value is a mean of four replications.

It is evident from the data presented in the Table 8 and Fig 8 that *P. flourescens* was found more effective as compared to other treatments.

After 30 days of plant growth *P. flourescens* showed its efficacy where minimum (6.5) mortality was recorded. This was followed by *T. viride (*10.25) and in Neem and Eucalyptus (@ 10%) 10.5 and 14.0 dead plants were recorded respectively. Maximum mortality was (24.75) recorded in control.

P. flourescens again showed its efficacy on the mortality of pea (11.5) followed by *T. viride* (13.50) after 45 days of plant growth. This was followed by Neem (10 %) where 14.50 plant mortality was noted. In Eucalyptus

Plate 6: Effect of oil cakes as soil amendments on mortality of pea

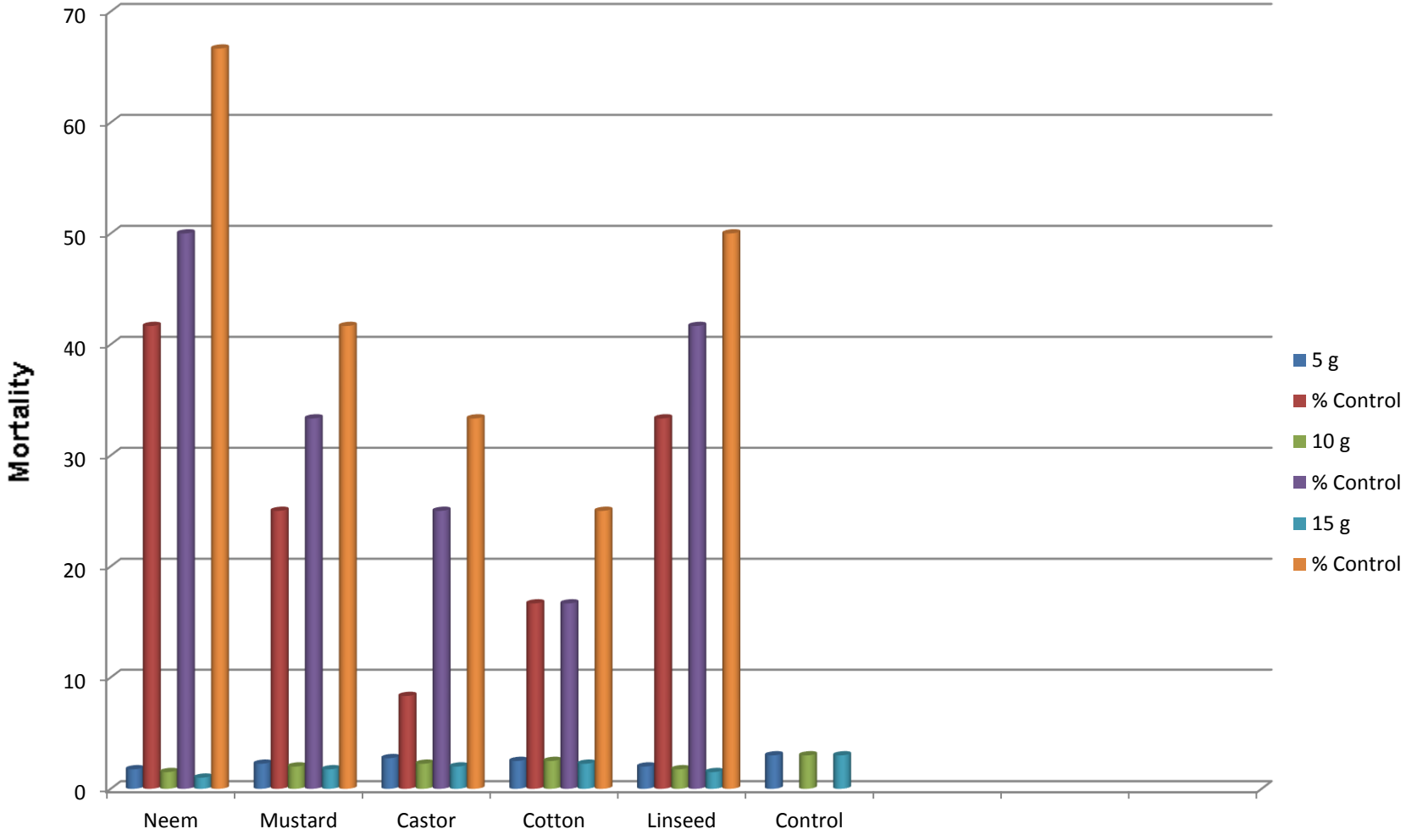


Fig 7 : Effect of oil cakes as soil amendments on mortality of pea (45 DAS)

(10%), Neem (5%) and Eucalyptus (5%) 17.25, 22.25 and 22.75 mortality was noted respectively against maximum (28.25) in control.

Table 8: Effect of botanicals and bio-agents as seed treatment on mortality of pea

S. No.	Treatments	Concentrations & Doses	Seedling Mortality*			
			Day After Sowing			
			30	% Control	45	% Control
1	*Trichoderma viride*	5 g	10.25	58.58	13.50	52.21
2	*Pseudomonas fluorescens*	5 g	06.50	73.73	11.50	59.29
3	Neem	5 %	19.00	23.23	22.25	21.23
4	Neem	10 %	10.50	57.57	14.50	48.67
5	Eucalyptus	5 %	19.25	22.22	22.75	19.46
6	Eucalyptus	10 %	14.00	43.43	17.25	38.93
7	Control		24.75		28.25	
	SEm±		1.426		1.457	
	CD at 5 %		4.270		4.363	

* Each value is a mean of four replications.

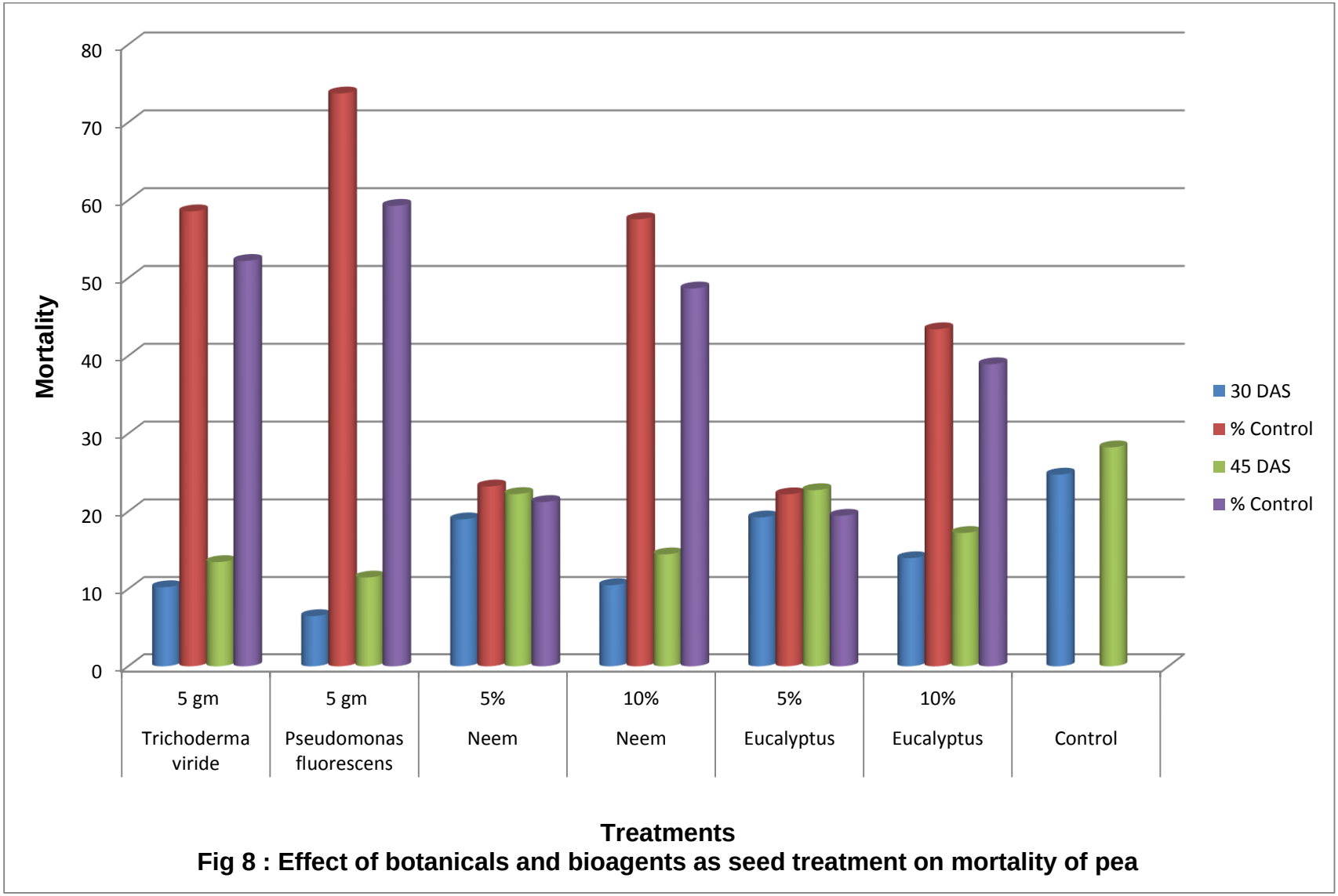


Fig 8 : Effect of botanicals and bioagents as seed treatment on mortality of pea

DISCUSSION

5.1 Test of pathogenicity

The experiment on confirmation of pathogenicity of *Fusarium oxysporum* f. sp. *pisi* on pea was conducted under pot conditions after growing the test fungus on crushed maize seed moistened with water and mixing it with the sterilized pot soil.

The symptoms appeared within 17 days and plant mortality was noted on 21th day. These results are in accord with the findings of Sharma (2011), on pea. The fungus infected the seedlings, brown lesions were observed on the roots and cotyledons. Characteristic brown rot was observed in hypocotyl and epicotyl regions of seedlings. The upper part of plant may wilt and dried. The vascular discolouration is pale yellow to deep orange brown.

Infantino *et al.* (2006) reported that *Fusarium oxysporum* f. sp. *pisi* penetrates pea roots and infects the vascular system at any growth stage of the crop. Infected plants often show orange or dark red discolouration in the vascular tissue of the root and lower parts of the stem. Above ground symptoms consist of leaves yellowing that wilt and curl downward during the flowering to pod-fill stages. Early infection often results in seedling death, which may be obscured by the growth of adjacent plants.

5.2 *In vitro* evaluation of leaf extracts

The experiment was conducted under laboratory condition where the water deficit potato dextrose agar (PDA) medium was amended with leaf extracts of various test plants in three concentrations (10, 20 and 30 %) and data on mycelial growth was recorded 48,96 and 144 hours after inoculation. The neem leaf extract showed better results in all concentrations. At 48 hours its efficacy increased as the concentration increased up to 30 per cent. Neem (*Azadirachta indica*) leaf extract contains azadirachtin, monoterpenes and salanin which show toxic effect against the *Fusarium oxysporum* f. sp. *pisi.* This was also demonstrated by Sharma *et al*. (2005).

Leaf extracts of all the plants were found toxic to the mycelial growth of *Fusarium oxysporum* f. sp. *pisi*, however the difference in their toxication varied. Neem (*Azadirachta indica*) was observed to be inhibitory at ten per cent concentration within 48 hours and later its toxicity declined. Similarly Eucalyptus (*Eucalyptus globulus*), Jatropha (*Jatropha curcas*), Aak (*Calotropis gigantia*), Parthenium (*Parthenium hysterophorus*), Datura (*Datura stramonium*), Ashok (*Saraca asoca*) and Karanj (*Pongamia pinnata*) were noted to be toxic to the mycelial growth but their efficacy declined as the time lapsed. However Karanj (*Pongamia pinnata*) was recorded to be effective in reducing the mycelial growth of the test fungus. The result are in accord with the findings of Verma and Dohroo (2003).

The leaf extracts of Mehndi *(Lawsonia inermis)* was also found effective in reducing the mycelial growth of *Fusarium oxysporum* f. sp. *pisi*. The mycelial growth was noted to be reduced at 30 percent concentration.

Tulsi (*Ocimum tenuiflorum*) leaf extract exhibited least antifungal activity against the *Fusarium oxysporum* f. sp. *pisi* discuss results for its effectiveness. Similar results were also noted by Gnanasekaraan *et al.* (2015).

5.3 Influence of leaf powders as soil treatment on plant mortality

The experiment was carried out under pot conditions. In the present investigation leaf powders of plants were used as soil amendment. The result obtained during the course of study indicated that all the treatments were significantly effective in managing *Fusarium oxysporum* f. sp. *pisi* and increasing the plant stand. Out of the powders tested *A. indica* showed minimum plant mortality up to 45th day when the experiment was terminated. These results are in the conformity with the findings of Bhasker *et al.* (2005) on Amorphophallus and potato infected by *Rhizoctonia solani* and *Alternaria solani.*

Karanj stood next in the order of efficacy and provided better plant growth. The results are in accord with the findings of Goswami *et al.* (2007) on pigeon pea and Dubey (2002) on Urdbean. Application of Karanj may improve soil conditions including nutritional status of soil supporting crop health.

The investigations indicated that Mehndi (*Lawsonia inermis*) and Eucalyptus (*Eucalyptus globulus*) leaf powders remained at par in their efficacies. Better plant stand was recorded in *Jatropha curcas.* Followed by Eucalyptus (*Eucalyptus globulus*) and Mehndi (*Lawsonia inermis*). The results are in confirmation with the findings of Shahraj *et al.* (2007).

The performance of these plant leaves may be due to the presence of antifungal substances which inhibited the growth of the pathogen but not so Inhibitory against natural antagonistic micro biota especially fungal antagonists present in soil (Dubey and Patel, 2000).

5.4 Influence of oil cakes as soil amendment on plant mortality

Minimum numbers of wilted plants were recorded with the soil treated Neem oil cake at 45thday of plant growth. Linseed (*Linum usitatissimum*) stood next in the order of efficacy and providing better plant growth. Better result due to soil treatment with Neem cake were also recorded by Mishra *et al.* (2005) while working with *Rhizoctonia solani* on mungbean.

Better plant stand was recorded in cotton (*Gossypium arboreum*). Followed by castor (*Ricinus communis)* and mustard (*Brassica nigra*). The results are in confirmation with the findings of Rafi *et al*. (2016) who reported that effects of soil amendment with oil seed cakes including mustard (*Brassica campestris* L.) and cotton (*Gossypium hirsutum* L.), reduced the root infecting fungi like *Macrophomina phaseolina, Fusarium spp.*

5.5 Influence of leaf extracts as seed treatment on plant mortality

The seeds of pea were soaked in five and ten percent concentrations of leaf extracts for six and 12 hours and sown in field.

It is evident from the data recorded after 30 and 45 days that the best plant stand was recorded where the seeds were treated with neem extract. Minimum numbers of wilted plants were noted in this treatment. The results are in confirmation with the findings of Bowers and Loeke (2000) on chrysanthemum infected with *Fusarium oxysporum* f. sp. *chrysanthemi.*

Better plant stand was noted in neem (*Azadirachta indica)* @ ten per cent followed by Eucalyptus (*Eucalyptus globulus*) at the same concentration. The result are in confirmation with the findings of Rahman *et al.* (1999) who reported that seed treatment with neem (*Azadirachta indica)* extract @ 0.3 % was effective against seed borne infection of various pathogen including *Fusarium spp*. in wheat.

Sharma and Rana (2000) suggested that plant extracts are easily available and biochemical agents used as seed treatment can frame an important component of cost effective and eco-friendly integrated disease management system.

5.6 Influence of biocontrol agents as seed treatment on plant mortality

Bio-control of the organism at a crucial stage, where successful examples of using fungal antagonists in management are need to warrant continuing support from the public and the industry.

In the present investigation *Trichoderma viride* and *Pseudomonas fluorescens* were tested *in vivo* conditions. *Pseudomonas fluorescens* was found most effective and showed minimum plant mortality, while *Trichoderma viride* was the least effective in the management of *Fusarium oxysporum* f. sp. *pisi* under field conditions. The results are in accord with the findings of Anjaiah *et al.* (2003) who reported that inoculation of pigeonpea and chickpea seeds with *Pseudomonas aeruginosa* significantly reduced *Fusarium* wilt in naturally infested soil.

Manczinegr *et al.* (2002) reported that *T. harzianum* and *T. viride* have a strong antagonistic properties against soil borne pathogens. Chand and Singh (2005) reported efficacy of fungal bio-control agents and plant extracts of eco-friendly management of chick pea wilt

SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

6.1 Summary

The present investigations deal with the evaluation of commonly available botanicals *viz.,* Neem (*Azadirachta indica*), Karanj (*Pongamia pinnata*), Datura (*Datura stramonium*), Tulsi (*Ocimum tenuiflorum*), Aak (*Calotropis gigantia*), Mehndi (*Lawsonia inermis*), Eucalyptus (*Eucalyptus globulus*), Jatropha (*Jatropha curcas*), Parthenium (*Parthenium hysterophorus*) and Ashok (*Saraca asoca*) under laboratory and field conditions.

Pathogenicity of *Fusarium oxysporum* f. sp. *pisi* on pea was confirmed by growing the fungus on crushed maize seeds moistened with water and then mixing with pot soil. The symptoms appeared within 17 days and the plants were killed within 21 days after sowing the test fungus was recovered from the roots of diseased plants.

Evaluation of plant leaf extracts was done under laboratory conditions using poisoned food technique. Neem leaf extract within 48 hours and Mehndi (*Lawsonia inermis*) within 96 and 144 hours in all the concentrations were found to be effective in reducing the mycelial growth of *Fusarium oxysporum* f. sp. *pisi.*

The plant leaves were also tested as soil amendment under pot condition where the leaf powders were mixed with the soil pre infested with *Fusarium oxysporum* f. sp. *pisi.* The plants grown on neem amended soil remained healthy up to 45 days where minimum mortality was recorded. *P. pinnata* and *L. inermis* stood next in order of their efficacies in managing the wilt disease of pea.

An experiment was also conducted to evaluate the oil cakes as soil Treatment. Neem cake is most effective with minimum plant mortality. Linseed (*Linum usitatisimum*) and Mustard (*Brassica nigra*) were superior over rest of the treatments.

Evaluation of plant leaves as seed treatment was also done in field conditions. Out of the two plants leaf extracts tested Neem (10 %) showed its superiority over rest of the treatment and *E. globulus* (10 %) was noted to be inferior to neem but superior over rest of the treatments.

Pseudomonas flourescens was found to be more effective as compared to *Trichoderma viride* and recorded minimum wilt appearance and plant mortality under field conditions.

6.2 Conclusions

The wilt symptoms on pea appeared within 17 days and the plants were killed 21 days after sowing .The root did not show any external rotting. Such root when splited vertically form the collar region downward showed brown discoloration of vascular tissues.

Mehndi leaf extract was found superior under *in vitro* conditions in reducing the mycelial growth of *Fusarium oxysporum* f. sp. *pisi.*

Minimum number of wilted plants were also noted in pots where *A. indica* leaf powder was amended on soil.

Minimum numbers of wilted plants were recorded in soil treated with neem oil cake and was found superior over rest of the treatments.

Pseudomonas flourescens was found to be most effective with minimum mortality when used as seed treatment to manage wilt of pea.

6.3 Suggestions for further work

Research efforts need to be directed to test more number of botanicals and bio-control agents having wilt management properties. Efforts are also required to standardize methods and strategies for introducing and monitoring populations of bio-control agents and exploring their antagonistic potentials. More botanicals, bio-control agents and oil cakes need to be tested and promising ones may be ascertained for their quantitative performance under field condition.

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APPENDICES

Analysis of Variance Table 1 (*in vitro*)

(48 hours)

Source of Variation	DF	SS	MSS	F-Calculated	Significance
Treatment (A)	10	24.712	2.471	4.993	0.00002
Concentrations (B)	2	0.247	0.124	0.250	0.77953
Intraction A X B	20	112.530	5.627	11.368	0.00000
Error	66	32.667	0.495		
Total	98	170.157			

Analysis of Variance Table 2 (*in vitro*)

(96 hours)

Source of Variation	DF	SS	MSS	F-Calculated	Significance
Treatment (A)	10	415.803	41.580	40.259	0.00000
Concentrations (B)	2	11.227	5.614	5.435	0.00653
Interaction A X B	20	995.439	49.772	48.190	0.00000
Error	66	68.167	1.033		
Total	98	1,490.636			

Analysis of Variance Table 3 (*in vitro*)

(144 hours)

Source of Variation	DF	SS	MSS	F-Calculated	Significance
Treatment (A)	10	766.444	76.644	72.265	0.00000
Concentrations (B)	2	62.788	31.394	29.600	0.00000
Intraction A X B	20	2,490.268	124.513	117.398	0.00000
Error	66	70.000	1.061		
Total	98	3,389.500			

Analysis of Variance Table 4 (soil)

(30 day)

Source of Variation	DF	SS	MSS	F-Calculated	Significance
Treatment (A)	10	16.667	1.667	2.578	0.01064
Concentrations (B)	2	0.545	0.273	0.422	0.65757
Intraction A X B	20	48.121	2.406	3.722	0.00003
Error	66	42.667	0.646		
Total	98	108.000			

Analysis of Variance Table 5 (soil)

(45 day)

Source of Variation	DF	SS	MSS	F-Calculated	Significance
Treatment (A)	10	14.970	1.497	2.430	0.01568
Concentrations (B)	2	0.061	0.030	0.049	0.95204
Intraction A X B	20	43.939	2.197	3.566	0.00005
Error	66	40.667	0.616		
Total	98	99.636			

Analysis of Variance Table 6 (soil)

(30 day)

Source of Variation	DF	SS	MSS	F-Calculated	Significance
Treatment (A)	5	7.903	1.581	2.688	0.03058
Concentrations (B)	2	10.361	5.181	8.811	0.00049
Intraction A X B	10	7.639	0.764	1.299	0.25459
Error	54	31.750	0.588		
Total	71	57.653			

Analysis of Variance Table 7 (soil)

(45 day)

Source of Variation	DF	SS	MSS	F-Calculated	Significance
Treatment (A)	5	7.069	1.414	1.490	0.20847
Concentrations (B)	2	9.528	4.764	5.020	0.01001
Intraction A X B	10	5.472	0.547	0.577	0.82576
Error	54	51.250	0.949		
Total	71	73.319			

Analysis of Variance Table 8 (*in vivo*)

(30 day)

Source of Variation	DF	SS	MSS	F-Calculated	Significance
Replication	3	89.821			
Treatment	6	980.429	163.405	20.087	0.00000
Error	18	146.429	8.135		
Total	27	1,216.679			

(45 day)

Source of Variation	DF	SS	MSS	F-Calculated	Significance
Replication	3	49.143			
Treatment	6	874.857	145.810	17.170	0.00000
Error	18	152.857	8.492		
Total	27	1,076.857			

CURRICULUM VITAE

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The author of this thesis Mr. Rahul Yadav S/O Shri. Devaram Yadav and Shrimati Sunita Yadav, born on 20th July 1992 at Khargone (Madhya Pradesh). He joined the following institutions and successfully completed the degree of M.Sc. (Ag.) during the year 2018-19 with 6.68 OGPA out of 10-point scale.

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3	Govt. H. S. School, gogawa, Khargone	12th	2011
4	Govt. H. S. School, gogawa, Khargone	10th	2009

For the partial fulfilment of the master's degree programme, He was allotted a research problem on **"Management of wilt of pea (*Pisum sativum* L.) Caused by *Fusarium oxysporum* f. sp. *pisi* (W.C. Snyder and H. N. Hansen, 1940) through non-chemical approaches"** which was successfully conducted by him and being submitted in the form of the thesis.