

“Studies on Alternaria blight of Sesame”

THESIS

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In

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(PLANT PATHOLOGY)

By

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CERTIFICATE – I

*This is to certify that the thesis entitled “**Studies on Alternaria Blight of Sesame**” submitted in partial fulfillment 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. Narender Kumar** 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.

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*This is to certify that the thesis entitled “**Studies on Alternaria Blight of Sesame**” submitted by **Mr. Narender Kumar** to the Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture** in the Department of Plant Pathology has been, after evaluation, approved by the External Examiner and by the Student’s Advisory Committee after an oral examination on the same.*

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Declaration and Undertaking by the Candidate

I, **Narender Kumar S/o Shri Shudev Singh** certify the work embodied in thesis entitled “**Studies on Alternaria Blight of Sesame**” is my own first hand bonafide work carried out by me under the guidance of **Dr. Sushma Nema**, Professor (Plant Pathology) at Department of Plant Pathology, College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur and place during 2014-2015.

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INTRODUCTION

Sesame (*Sesamum indicum* L.) otherwise known as sesamum or benniseed, member of the family Pedaliaceae, is one of the most ancient oilseeds crop known to mankind. Sesame plays an important role in human nutrition. It is one of the oldest oil crop and thought to have originated in Africa (Brar and Ahuja 1979; Ram et al. 1990). It is widely grown in tropical and subtropical regions. Its production is often concentrated in marginal and sub marginal lands (Ashri 1998). Most of the sesame seeds are used for oil extraction and the rest are used for edible purposes (El Khier et al. 2008). Sesame is grown primarily for its oil-rich seeds. Before seeds were appreciated for their ability to add nutty flavour or garnish foods, they were primarily used for oil and wine (Ghandi 2009). After the extraction of oil, the cake is mostly used for livestock feed or often as manure. Its colour varies from cream-white to charcoal-black but it is mainly white or black. Other colours of some sesame seed varieties include, yellow, red or brown (Natrland 2002). The lighter varieties of sesame which are considered to be of higher quality are generally more valued in the West and Middle East, while both the pale and black varieties are prized in the Far East. There are numerous varieties and ecotypes of sesame adapted to various ecological conditions (Nzioku et al. 2010).

Sesame (*S. indicum*) is grown in 53 countries having different agro climatic conditions. The major world producers include India, Sudan, China and Burma, who contribute about 60% of the total world production. (El Khier et al. 2008). It is the third most important oil seed crop in India. India ranks first in the world in area (24%) and second in production (26%) of sesame. The area under sesame cultivation in India was 1666.6 thousand hectares, with a total production of 674.9 thousand tonnes giving an average yield of 404.9 kg/ha (Anon. 2014). In Madhya Pradesh during 2013-14 area and production were declined to 39.2 thousand hectares, 10.1 thousand tonnes as compared to 2012-13. In Madhya Pradesh during 2013-14 the area under sesame cultivation was 256 thousand hectares; with a total production of 107

thousand tonnes giving an average yield of 418 kg/ha. In 2013-14 area and production were declined to 58.5 thousand hectares and 50.7 thousand tonnes as compared to 2012-13. In Madhya Pradesh major sesame growing districts are Chattarpur, Tikamgarh, Gwalior, Shivpuri, Singrauli, Sidhi, Panna, Shahdol, Morena and Katni.

Sesame seed is rich in fat, protein, carbohydrates, fibre and some minerals. The oil seed is renowned for its stability because it strongly resists oxidative rancidity even after long exposure to air (Global Agri Systems, 2010). The oil fraction shows a remarkable stability to oxidation. This could be attributed to endogenous antioxidants namely lignins and tocopherols (Elleuch et al. 2007; Lee et al. 2008).

Prevalence of disease constitutes a major setback in sesame production worldwide. Diseases affect plants right from the planting to harvesting and storage of the produce and account for world estimated losses of 7 million tonnes. Due to insufficient attention given to sesame crop, its cultivation process is still traditional, old and tiresome. The management practices starting from planting to harvesting and processing demand high labour. Moreover, diseases and insect pests are the important constraints in the sesame production system.

Sesame is vulnerable to a number of diseases caused by a number of pathogens. Fungal diseases as *Alternaria* leaf spot caused by *Alternaria sesami* (Cook 1981), *Cercospora* leaf spot by *Cercospora sesami* (Hansford 1931), damping off / root rot by *Macrophomina phaseolina*, virus and phytoplasma diseases as mosaic, leaf curl and phyllody inflict appreciable losses (Roy 1931). There are also two bacterial pathogens associated with sesame are Bacterial blight by *Xanthomonas campestris* pv. *sesami* (Sabet and Dowson, 1960) and Bacterial leaf spot caused by *Pseudomonas syringae* pv. *Sesame* (Young et al. 1978).

The pathogen *Alternaria sesami* causing sesame blight attacks all parts of the plant at all stages. Foliar symptoms as small, dark brown water soaked, round to irregular lesions, with concentric rings, 1 mm to 2 cm in diameter appear on the leaves and under excessive atmospheric and soil humidity the

spot increases in size and number . The lesions may also appear on the midrib and veins of the leaves. In case of the severe occurrence of the disease the plant may die.

Therefore keeping in view all factors objectives of the present study were-

1. To study the symptomatology, morphological and cultural characters of *Alternaria sesami*.
2. To find out the resistant sources of sesame against *Alternaria sesami*.
3. Evaluation of fungicide and plant extracts against *Alternaria sesami*.

REVIEWS OF LITRATURE

A comprehensive review of literature is an important for better understanding of concepts defined and methods of analysis employed in any research project. Past research helps in identifying the conceptual issues as well as it facilitates in concluding the findings and also gives the idea of methodology relevant to the study. Hence, in this chapter an attempt has been made to review the research work carried as symptomatology, morphological and cultural characteristic, pathogenicity, association of pathogen on seed, survival, effect of media, pH, fungicides, botanicals and screening of sesame germplasm lines.

2.1 Symptomatology, morphological and cultural characteristic

Alternaria leaf spot on *Sesame orientale* was first described by Kvashina (1928) from Northern Caucasus. He reported that *Alternaria* sp. formed rounded or irregular frequently brown spots on the ventral surface of leaves of sesame, pale towards the margins and measuring 1.5 to 5 mm. The conidiophores were olive-brown, 1 to 4 septate, 26 to 48 µm long and 4.4 to 5.2 µm in wide. The spores varied in shape, flask shaped with short beak to clavate with a very long beak (5 to 6 times the length of spore). Spores were olive brown with 1 to 14 transverse and 1 to 4 longitudinal septa, and measures 28 to 362 × 5.2 to 24 µm. Kawamura (1931) observed a similar fungus on *S. indicum* in Japan and named it *Macrosporium sesami* Kawamura. Since at that time the genera *Macrosporium* and *Alternaria* were known to be the same. Day (1948) recorded *Alternaria* sp. for the first time in India on Gingally.

Mohanty and Behera (1958) reported that the pathogen of sesame blight closely resembled with *Macrosporium sesami* with the difference that the conidia developed in chains. The fungus accordingly renamed as *Alternaria sesami*.

Leppik and Sowell (1964) published a review on the symptoms and morphological characteristics of the disease on sesame.

Berry (1960) reported that damping off seedlings grown in the green house were sprayed with a culture suspension of *A. sesami* and found dark brown to black water-soaked lesions followed by shrinkage of stem tissue and leaves. The spores of all isolates were similar and borne singly or occasionally. Ellis (1970) characterised *Alternaria sesami*. Ngabala (1971) described spread, mode of infection, morphology and control of *Alternaria sesami* on sesame.

Malaguti et al. (1972) described identification, morphology and culture of *Alternaria sesamicola* on sesame. They further illustrated blight and stem canker or severe necrosis on foliage in addition to the characteristic zonate spots.

Malaguti (1973) discussed economic importance and the conditions favouring for development of pathogens *C. sesami*, *Cylindrosporium sesami*, *A. sesamicola* and bacterial *X. sesami* and *P. sesami*.

Mehta and Prasad (1976) described conidial morphology and germination of *Alternaria sesami*.

Singh et al. (1980) reported that the dormant mycelium of *A. sesami* usually occurred in the sub epidermal layers of the seed coat and occasionally in the endosperm and embryo. Rajpurohit (1981) documented on morphology and taxonomy of *Alternaria sesami*.

Simmons (2004) cultured colonies of *Alternaria sesami* on V-8 agar, Potato carrot agar (PCA) and Hay agar exhibited rapid growth of colonies and they were 2.5 cm radius within 7 days. Colonies were composed of almost conidiophores and conidia. Sporulation was densely crowded on V-8, PCA and Hay agar media. Concentric rings of mycelia growth and sporulation were evident in V-8 medium.

He further observed that conidiophores were simple or sparingly branched; raised directly from hyphae on or beneath the agar surface. Size varied from ca. 60-225 x 5.0-6.5 μm and gradually enlarged near the apex into a clavate conidiogenous cell, which usually produced a single conidium. Juvenile conidia were narrow-ellipsoid, tapering to a pointed apex and transverse distoseptation was evident in young conidia which were large ca.

45 x 8 µm in size. Young conidia remained ellipsoid or widen and became ovoid. A long, flexuous and filamentous beak formed during early stages. Transverse and longitudinal distoseptum formation was abundant and remains obvious throughout enlargement of conidia; transverse eusepta were inserted in less than half of the distosepta. Colour of conidium was dull brown with darker septa. Wall appeared smooth. The apical beak ranged from 200-325 x 2.5 µm; rarely produces a single lateral branch near the body apex. Secondary conidium production at the tip of a beak is still uncommon.

11 isolates of *A. solani* causing early blight of tomato showed cultural variability in respect of radial growth, growth rate per day and pigmentation on Potato dextrose agar medium. Average thickness of conidiogenous hyphae and colony character were variable on PDA (Pandey et al. 2005). Similarly, variability in pigmentation of conidiophores and colony character (ranging from brown to olivaceous brown) was also observed (Varma et al. 2007, Kumar et al. 2008).

The isolates of *A. solani* showed morphological variability in terms of size of conidia, length of beak and number of transverse and longitudinal septa. Conidiophores were solitary or in small groups, straight or flexuous and brown in colour. The conidia were solitary, straight or slight flexuous, muriform and ellipsoidal tapering to a beak, pale or olivaceous brown (Varma et al. 2007). Similarly, Kumar et al. (2008) found that isolates of *A. solani* showed variability in mycelial growth pattern which were circular / irregular, smooth / rough, concentric and without zonation.

Cultural characteristics of 80 isolates of *A. burnsii* revealed the differences in colony colour, colony type and growth habit. Among these, 32 isolates showed dark brown to black colour growth, 19 isolates showed typical black, 20 isolates were of olive green and 9 isolates showed dirty white coloured colonies (Pipaliya and Jadeja 2008a).

Savitha et al. (2013) collected 14 isolates of *Alternaria* sp. from Raichur, Gulbarga, Dharwad, Bidar, Bangalore, Hyderabad and Coimbatore districts comprising of eight isolates of *Alternaria alternata* and six isolates of *Alternaria sesami*, studied for cultural, morphological, physiological,

pathogenic and genetic variabilities. The conidiophores of *Alternaria* sp. bearing conidia were solitary, straight, muriform with yellowish brown. The isolates ALT₁, and ALT₂₁ showed maximum conidial size ranging from 37.77 to 44.40 x 6.66 to 9.62 µm and from 37.77 to 44.40 x 6.66 to 9.62 µm respectively. The isolate ALT₄ showed maximum beak length measuring from 14.80 to 19.24 µm. The colour of isolates varied from light grey to dark brown. 6 isolates showed irregular margins whereas 8 showed raised growth on the culture.

Sahani and Mahapatra (2014) studied symptoms of *Alternaria* blight of sesame.

2.2 Pathogenicity

Mc Donald (1964) established pathogenicity for *Alternaria sesami*.

Yu et al. (1982) observed *Alternaria sesami*, *A. sesamicola*, *A. longissima* in seed samples of sesame in Korea. *A. sesamicola* was predominant in some samples with 30-68 per cent seed infection. Occurrence of *A. sesami* and *A. longissima* was 1-3%. A detailed description of the 4 species was communicated on the basis of morphology of conidia supported by photographs and diagrammatic sketches. Based on the original descriptions and the isolates studied it was concluded that *A. sesami* and *A. sesamicola* are 2 distinct species. *A. longissima* generally considered as a saprophyte. Later they found for the first time it as pathogen usually produces zonatespots symptoms on leaves and capsules. *Alternaria sesami* and *Alternaria sesamicola* affected seed germination and plant stand.

Alternaria sesami, *Cercospora sesamicola*, *Colletotrichum gloeosporioides*, *Helminthosporium halodes*, *Fusarium semitectum*, *Macrophomina phaseolina* and *Pestalotiopsis mayumbensis* were found closely associated with the disease and their pathogenicity was established. *A. sesami*, *C. sesamicola*, *C. gloeosporioides* were most virulent. None of the sesame cultivars tested showed resistance.

2.3 Detection of *Alternaria sesami* with sesame seeds

Kushi (1977) noticed that *Alternaria sesami* was associated with 10 seed samples, out of 26 samples of sesame seeds. She further noticed that for the detection of seed borne fungi by agar plate method. Czapek-Dox agar medium was the best for growth of *M. phaseolina*, *A. sesami* and *C. cassiicola* while Richards's medium and Asthana and Hawker's medium were good for the sporulation of above three important seed borne fungi.

Kushi and Khare (1979) found that standard blotter method was most effective followed by selective medium and PDA containing PCNB. While in pretreated seeds, deep freezing blotter method or plain PDA were more effective. Similarly, Mathur and Kabeere (1975) observed that blotter method to be better for detection of fungi on sesame seeds in Uganda.

Esewi (1984) isolated *A. sesami* from diseased plants in Aydm, Turkey and found that seed infection was 16.5 to 63.5%.

Sulochana and BalaKrishnan (1997) noticed that the agar plate method provided better result than the standard blotter method for the detection of seed borne fungi in sesame. For the detection of *A. sesami* from sesame seeds, Oat meal agar method was found very effective as compared to paper towel or blotter methods as reported by Ojiambo et al. (1999).

Mahatma et al. (2001) reported that the agar plate method was slightly superior over modified blotter method for the detection of seed mycoflora of sesame. Wasnikar et al. (1987) reported nine fungi and two bacteria, viz. *A. sesami*, *Helminthosporium sesami*, *F. oxysporum* fsp *sesami*, *Myrothecium roridum*, *Penicillium* sp., *Curvularia lunata*, *Botrytis cinerea*, *Aspergillus* sp., *Pseudomonas syringae* pv *sesami* and *Xanthomonas campestris* pv *sesami* in 12 sesame varieties tested by standard blotter method. Wu (1988) detected 10 fungi on sesame seeds by blotter method and out of these, 5 were *Alternaria longissima*, *A. sesami*, *A. sesamicola*, *A. tenuis*, *A. alternata* and *Corynespora cassiicola* new reports on sesame in Taiwan.

Agarwal (2006) imported 2240 sesame germplasm lines from 29 countries and all the samples were observed for the presence of fungal spores and fructification. He observed twelve fungi from introduced

germplasm lines *Cercospora sesami*, *Alternaria longissima*, *A. sesami* and *Fusarium solani* were known seed borne fungi and were of high quarantine significance.

2.4 Survival of the pathogen *A. sesami* in seed and crop debris

A. burnsii the cause of cumin blight remains viable for more than 10 months in seed as well as in crop debris (Patel and Desai 1971).

Tripathi and Kaushik (1984) studied survival of *A. brassicae* causing blight in mustard, had been recorded for the survival even 10 - 11 months in plants debris buried under field conditions at the depth of 7.5 cm.

A. alternata causing blight of broadbean remained viable in diseased plant debris up to 11-13 months in laboratory and up to 10 - 12 month in soil respectively. It remained viable in infected seed up to 15 months in laboratory and up to 13 month in soil (Dubey and Patel 2000). However, Shrestha and Mathur (2003) have reported that *A. brassicae* survived in the seed for at least one year when stored at 5 to 10°C and act as sources of primary inoculum.

A. sesami causing blight of sesame could be survived in seed and crop debris under field conditions up to 11 months (Naik et al. 2007).

Sharma and Pandey (2013) observed 100% survival of *A. burnsii* on seed during April and May, which later decreased to 70% during October, November under laboratory condition. Whereas, in crop debris buried in soil, its survival was 40 to 60% during October and November.

2.5 Effect of Media and pH

Vitamins do not have significant effect on the growth of *Alternaria sesami*. Vigorous mycelial growth and sporulation was between pH 4-8, pH 5 was best for mycelial growth and pH 7 for sporulation (Samuel et al. 1972).

Mehta and Prasad (1976) reported that Oat meal agar and Richards' as best solid and liquid medium at 25°C for growth of *Alternaria sesami*.

Mohapatra et al. (1978) accounted effects of different culture media, pH, C and N sources on *A. sesami* growth.

Siddarmiah et al. (1981) reported that isolates from sesame leaves were differed from those of capsules in their growth on studied media. However, Potato dextrose agar and Tochinai's agar favoured growth of both the isolates. Cultural characters suggested the possibilities of physiological strains of the fungus.

Rajpurohit and Prasad (1981) studied effect of different media and pH on the growth and sporulation of *A. sesami*. Gupta et al. (1987) found that Czapek-Dox agar was best for the growth of *Alternaria porri* without sporulation.

Rotem (1994) reported that *Alternaria* sp. sporulates better on sugar poor media (V8 juice). Even though mycelial growth of cultures maintained on Czapek-Dox agar was high and sporulation was poor as compare to V-8 juice.

Saeed et al. (1995) reported that the *Alternaria alternata* grow well at pH 6.5 to 7. Hassain and Khutan (1997) reported that maximum growth of *A. porri* was at the pH 6.0 to 7.0. Rao and Vijaylakshmi (2000) studied the influence of pH on the growth of *Alternaria sesami*, nutritional requirements and ability to produce phytotoxic and antibacterial metabolites. Jash et al. (2003) found that the maximum growth of *Alternaria zinniae* pape developed leaf and flower blight of marigold at pH 6 to 6.5.

Hubbali et al. (2010) found growth of *A. alternata* under *in vitro* conditions at different pH levels and media. However, *A. Alternata* showed maximum growth at pH 6.0 to 6.5 pH. She described that host leaf extract medium supported maximum growth of *A. alternata* significantly followed by Potato dextrose agar. Further, the cultural characters viz. colony and substrate colour, margin, topography, zonation, pigmentation, colony diameter and sporulation were also described.

Madhavi et al. (2012) reported that Czapek-Dox agar medium supported good growth of *Alternaria porri* at pH 5.0.

2.6 Effect of fungicides

Siddarmiah et al. (1979) found the effect of fungicides *in vitro* against growth of isolates of *Alternaria sesami*.

Rajpurohit et al. (1984) evaluated 14 fungicides against *Alternaria sesami* and found that all of them were effective in controlling the spore germination of *Alternaria sesami* at 1000 ppm and 500 ppm concentration.

Patel (1993) studied the efficacy of non-systemic fungicides viz. Dithane M-45, Foltaf, Cumin-L, Thiram, Captaf and Kavach, systemic fungicides as Bavistin and Topsin-m against *A. burnsii* and observed that Mancozeb (2000ppm) completely inhibited fungal growth *in vitro* and was more effective (10.65% disease incidence) *in vivo* also. Similarly, inhibition of spore germination under lower and higher concentration of Mancozeb (500 and 2000 ppm) ranged between 76 and 100% respectively. The study indicated that all the isolates were sensitive to Mancozeb (Pipaliya and Jadeja 2008 and Vihol et al. 2009).

In a similar study Hexaconazole, Difenconazole, Tebuconazole and Iprodione at 2000 ppm gave 100% inhibition of fungal growth and conidial germination under *in vitro* condition against *A. alternata* causing leaf spot of apple (Kumar et al. 2005). However, Propiconazole and Hexaconazole were proved to be equally good and completely inhibited the growth of *A. alternata* even at minimum concentration of 50 ppm (Akbari and Parakhia 2007). Similarly, among the 9 fungicides evaluated 6 fungicides viz. Mancozeb, Thirum, Tarbendazim + Mancozeb (companion), Propiconazole, Hexaconazole and Copper oxychloride at their recommended dose completely inhibited the growth of *A. alternata* causing leaf blight of watermelon under *in vitro* condition (Umamaheshwari and Shankarlingam 2005).

Singh et al. (2006) tested the efficacy of different fungicides, viz. Hexaconazole at 1000, 500, 200, 100 and 50 ppm and Mancozeb, copper oxychloride, Chlorothalonil, Azoxystrobin and Propineb at 2500, 2000, 1000, 500 and 250 ppm against *Alternaria alternata* was studied *in vitro* using the poisoned food technique and found that Hexaconazole was the

most effective fungicide as it caused 100 per cent growth inhibition at all tested concentrations.

Mesta et al. (2009) reported that inhibition of mycelial growth and sporulation by systemic fungicides viz. Hexaconazole (63.12 % and 72.87 % respectively) and Propiconazole (61.80 and 76.53 % respectively) were most effective. Neem leaf extract with 38.49% inhibition of spore germination and 43.90% inhibition of mycelial growth was effective than all other plant extracts.

Sharma and Pandey (2011) evaluated the efficacy of fungicides viz. Mancozeb, Tebuconazole, Hexaconazole 5% + Captan, Carbendazim 12% + Mancozeb 63%, and Azoxystrobin at 3 different concentrations on mycelial growth and sporulation of *Alternaria burnsii*. Among the tested fungicides, Tebuconazole was found quite toxic at 0.03, 0.04 and 0.05% concentrations which completely inhibited the mycelial growth and sporulation of the pathogen. Whereas, Propiconazole at 0.20% concentration completely inhibited the mycelial growth and sporulation. The next best effective fungicide in order of efficacy was Hexaconazole 5% + Captan 70%, which showed 75.1 to 84.4% growth inhibition at 0.004 and 0.006% concentration respectively.

Thaware et al. (2011) tested different fungicides against *Alternaria alternata* under *in vitro* condition and found that Mancozeb (0.2%) and Propiconazole (0.05%) completely inhibited the growth of the test fungus. Madhavi (2012) found that Mancozeb was effective in controlling the growth of *A. porri*. Mishra and Gupta (2012) found that Mancozeb at 0.2% completely inhibited the growth of the pathogen followed by Azoxystrobin (0.1%) and Antracol (0.2%) under *in vitro* conditions.

11 fungicides evaluated viz. Mancozeb 75 WP, Chlorothalonil 75 WP, Copper oxychloride 50 WP, Thiram 75 WP, Captan 50 WP, Difenconazole WP, Hexaconazole 5 EC, Propiconazole 25 EC, Penconazole 10 EC and Metalaxyl + Mancozeb (Ridomil) 68 WP (at 500ppm) against *Alternaria carthami*. All the fungicides tested caused significant inhibition of mycelial growth of *A. carthami*. The test fungicides were ranged from 28.39% (Chlorothalonil) to 94.44% (Carbendazim). However, Carbendazim recorded

significantly highest (94.44%). The second and third best fungicides found were Mancozeb (85.43%) and Thiram (83.33%). These were followed by Hexaconazole and Difenconazole (77.16%). *Allium sativum* extract was found most fungistatic which recorded significantly highest (62.47%). The second and third best botanicals found were *Datura metal* (49.87%) and *Curcuma longa* (46.91%). Thus, all the botanicals tested were caused significant inhibition of mycelial growth of *A. carthami* (Taware et al. 2014).

2.7 Effect of botanicals

Shivpuri et al. (1997) evaluated Neem, Datura, Ocimum, Polyathia and Calotropis against *Alternaria brassicicola*. It was noticed that Ocimum and Polyathia were more effective than the rest of the botanicals. Similarly Seetharaman et al. (2001) evaluated chemical properties bearing inhibitory effect from several plants leaf extracts and found that 10 per cent extract of *Polyathia*, *Azadirachta* and *Ocimum* inhibited the mycelial growth of *Alternaria solani* 38.9, 37.7 and 33.7% respectively.

Chaudhary (2003) evaluated the efficacy of leaf extracts of *Eucalyptus globulus*, *Datura stramonium*, *Solanum xanthocarpum*, *Azadirachta indica*, *Lantana camara*, *Ricinus communis* and *Lawsonia inermis*; bulb extracts of *Allium sativum* and *Allium cepa* and rhizome extracts of *Zingiber officinale* against *Alternaria alternata in vitro*, bulb extracts of *Allium sativum* showed the highest inhibition properties against pathogen (56.19%) followed by *Allium cepa* (54.27%) bulb extracts.

Rajpurohit (2003) found that *Alternaria* leaf spot (*Alternaria sesami*) and phyllody (MLOs) are the important diseases of sesame in Rajasthan, India and tested plant products AFF3 and Neem Gold, the fungicide Mancozeb along with the *Trichoderma viride* against diseases. Mancozeb at 0.2% was highly effective against *Alternaria* leaf spot, whereas AFF3 and Neem Gold (6.5 ml/l) were effective against *Alternaria* leaf spot of sesame in field trial.

Patni and Kolte (2006) tested inhibitory effects of *Eucalyptus* leaf extract against *Alternaria alternata* and found that it significantly reduced the radial growth, sporulation and spore germination.

The antifungal activity of aqueous leaf extract of *Prosopis juliflora* (Sw.) DC. Showed antifungal activity with the help of poisoned food technique against *Alternaria alternata* and found significant antifungal activity at 24% concentration (Raghvendra et al. 2009).

Antifungal activity of ten plants extracts viz., *Allium sativum*, *Datura stramonium*, *Lantana camara*, *Allium cepa*, *Ocimum sanctum*, *Eucalyptus globules*, *Adhatoda vasica*, *Zingiber officinale*, *Azadirachta indica* and *Lawsonia inermis* against *Alternaria burnsii* and *Allium sativum* showed inhibition on the mycelium growth (Vihol et al. 2009).

Leaf extracts from 7 plant species against *A. tenuissima*, *in vitro* and *in vivo* condition resulted maximum inhibition of mycelial growth from leaf/bulb extracts of garlic (*Allium sativum*), neem (*Azadirachta indica*) and prosopis (*Prosopis juliflora*) at 15% (v/v) concentration (Raja 2010).

Natural plant extracts / products have the potential as safe alternatives for chemical fungicides in plant disease management. Methanol and pure methanol: water (50:50 v/v) extracts of Peppermint, Lavandula, Eucalyptus, Datura and Nettle were screened for their antifungal activity against *Alternaria sesami*, the causal agent of Alternaria leaf spot of sesame at 5, 10 and 15% concentrations in Shahrood Agricultural Research Center, Shahrood, Iran, during 2010. Poisoned food technique and spore germination assay were used to evaluate the antifungal potential of plant extracts against the pathogen. Fungicide mancozeb 80WP (0.2%) was used for better comparison. Results indicated 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. Among tested extracts, methanolic extracts of Peppermint (15%) and Eucalyptus (15%) were best in preventing the spore germination of the pathogen (Zaker 2010).

Sharma and Pandey (2011) evaluated the efficacy of Neem leaf extract on mycelial growth and sporulation of *Alternaria burnsii* and noticed that Neem leaf extract (15%) showed maximum (19.2%) inhibition of mycelial growth and poor sporulation.

Plant extracts amended PDA medium with poisoned food technique at different extracts *in vitro* @ 1.0, 2.0 and 5.0% was screened to measure per cent inhibition of radial growth, sporulation and spore germination of *Alternaria porri* (Ellis) Cif. The investigation revealed that maximum (97.05%) of mycelial growth was recorded in *Palma rosa* oil (5%) amended medium at 5 per cent concentration followed by 95.58% in *Ocimum sanctum* at the same concentration over check. *Eucalyptus*, *Palma rosa* and *Ocimum sanctum* oils showed minimum sporulation intensity 0.16, 0.26 & 0.39x10³/ml, respectively. *Ocimum sanctum* found most effective in reducing the spore germination (75.58%) under *in vitro* condition (Meena 2012).

Tapsya and Dubey (2012) found that significant reduction in mycelial growth of *Alternaria* blight of linseed with *Azadiracta indica* and *Acalypha indica* leaf extract. Waghmare (2012) reported that 25% leaf extract of *Polyalthia longifolia* reduced the mycelial growth of *Alternaria alternata* causing leaf spot of Gerbera. Mukhtar et al. (2012) evaluated aqueous extract of *Cuscuta reflexa* against five pathogenic fungi viz. *A. alternata*, *A. niger*, *Fusarium solani*, *F. oxysporum* and *Macrophomina phaseolina*. *Cuscuta* extracts exhibited significant antifungal activity against all test fungal isolates. However, extract was highly effective against *F. solani*, *F. oxysporum* and *M. phaseolina* and was least effective against *A. niger*. It was also found that 30% (w/v) concentration was significantly effective in reducing the mycelial growth of fungal isolates after 6 days of incubation.

2.8 Screening of germplasm / varieties against *Alternaria sesami*

Barboza et al. (1966) reported that all the varieties tested against *Alternaria sesami* were affected, yield being reduced by 15.7-55.2%. Incidence 94 days after the appearance of symptoms was a more reliable guide to the effect on yield than incidence 42 days later.

Jayaramah and Siddaramaiah (1981) found that under field conditions and in glasshouse inoculation, the sesame cultivars No. 4, J.T.-7, No.2 and E-8 were highly resistant to *Alternaria sesami*.

Dolls (1982) reported that none of the 22 varieties screened in the field was resistant, although X7732/10-2, wild Dhanduka 32, D7-11, E8 and 1978

K5222 were found to be moderately resistant. Dolls and Hedge (1984) found that incidence of *A. sesami* ranged from 32.38 to 72.21%.

Out of 963 sesamum cultivars grown under natural infection and examined 60 days after sowing, none was resistant to *A. sesami* but 12 were moderately resistant. Twelve showed a susceptible reaction when inoculated (Natrajan and Shanmuga 1983).

Sharmarao et al. (2003) evaluated 9 entries of sesame for their multiple resistance to diseases during 1998-99 and 1999-2000 in Bijapur, Karnataka, India. The genotypes MT-15, DS-14 and DS-10 recording 3.2, 4.0 and 4.0 disease grade, respectively, showed moderately resistant type of reaction to *Alternaria* blight. The genotypes MT-15, DORS-102, DS-14, and DS-10 showed multiple disease resistance against powdery mildew, *Alternaria* blight and bacterial leaf blight diseases. Similarly Nema et al. (2009) screened 68 varieties of sesame during *Kharif* 2004-06 and found that 13 varieties viz. N-8, JLT-7, JLT-26, Purva-1, Co-1, Paiyur-1, Thilthara, Thilarani, B-27, Rajeshwari, Krishna and Thilothama exhibited resistance reaction.

MATERIALS AND METHODS

The present investigation on “Studies on Alternaria blight of sesame” was carried out during 2014 - 15 at Department of Plant pathology, College of Agriculture, JNKVV, Jabalpur.

3.1 Location of site

The investigations were conducted at JNKVV, College of Agriculture, Jabalpur, that lies between 22° 49' and 22° 80' North latitude and 78° 21' and 80° 58' East longitude at an altitude of 411.78 meter above the mean sea level. The district is covered under the agro-climatic zone of Kymore plateau and Satpura hills of the state. The soil in the climatic zone is medium and deep clayey /black with hot sub humid climate having 1000 - 1500 mm rainfall, 90% in rainy season.

3.2 General laboratory procedure

3.2.1. Glassware cleaning

For all the laboratory experimental studies Corning and Borosil made glass wares were used. They were cleaned with detergent powder. After cleaning the glass wares with the detergent, they were thoroughly washed with running tap water and then rinsed in distilled water and dried before use whenever required.

3.2.2 Sterilization

The petri plates were sterilized in oven at 180°C for two hours. The inoculation needle and other metallic instruments were sterilized by dipping them in alcohol and heating over a flame surface, sterilization of plant parts and diseased materials were done by dipping them in 0.1 per cent mercuric chloride for 30 seconds to 1 minute followed by three washings in sterilized water. Media and water were subjected to sterilization by autoclaving at 1.1 kg/ cm² (121.6°C) for 20 minutes.

3.2.3 Composition of media

The following media was used during the course of investigation:

Oat meal agar medium

Powdered oat meal	30 g
Agar	20 g
Distilled water	1000 ml

Corn meal agar medium

Corn meal	100.0g
Agar	20.0g
Distilled water	1000ml

Potato dextrose agar medium

Peeled and sliced potato	200 g
Dextrose	20 g
Agar	20.00 g
Distilled water	1000 ml

Carrot agar medium

Peeled and sliced carrot	200 g
Dextrose	20 g
Agar	20 g
Distilled water	1000 ml

Rose bengal agar medium

Papaic digest of soybean meal	5 g
Dextrose	10 g
Monopotassium phosphate	1g
Magnesium sulphate	0.5 g
Rose bengal	0.05 g

Agar	15 g
Final pH (at 25°C)	7.2±0.2
Distilled Water	1000 ml

Asthana and Hawker's medium

Glucose	5.00 g
KNO ₃	3.50 g
KH ₂ PO ₄	1.75 g
MgSO ₄	0.75 g
Agar	20.00 g
Distilled water	1000 ml

Czapek-Dox agar medium

Sucrose	30.0 g
NaNO ₃	2.0 g
KH ₂ PO ₄	1.00 g
MgSO ₄ ·7H ₂ O	0.05 g
KCl	0.05 g
FeSO ₄ ·7H ₂ O	0.01g
Agar	20.00 g
Distilled water	1000.00 ml
pH	7.00

3.3 Isolation and identification

3.3.1 Isolation:

The diseased samples collected during the season from various locations were used for isolation in the laboratory. They were cut into small pieces and surface sterilized with 0.1 per cent HgCl₂ for 30 seconds. The pieces were then given three washings in sterile water and transferred into petri plates containing PDA. In each plate three pieces were kept in three replications. The plates were incubated at 28±2°C and examined for the

fungus growth after 4 days. The associated fungus was re-isolated, purified and identified.

3.3.2 Purification and maintenance of culture

The pathogen was purified by single spore isolation. The fungus was sub cultured on Potato dextrose agar petri plates. A spore suspension was prepared in sterilized distilled water from 15 days old cultures on PDA, was poured on plain agar in petri plates. The excess suspension was drained out and the petri plates were then incubated at room temperature. After 12 hours a single germinated conidium was marked with the help of dummy cutter and was transferred with a piece of plain agar to PDA slant and thus pure cultures were obtained. Such slants were preserved in the refrigerator at 4°C and sub cultured once in 30 days. Such culture was used throughout the study period.

3.3.3 Identification

The identification of the isolated fungus was done on the basis of spore shape, size and other morphological characters.

3.4 Pathogenicity Test

The following techniques were employed to confirm pathogenicity of *A. sesami* on plant parts.

I. Spray method:

Sesame seeds were sown in earthen pots containing sterilized soil and they were allowed to grow for one month. Five plants were maintained in each pot. Spore suspension along with mycelial bits obtained from 15 days old culture grown on PDA was sprayed with atomizer. The inoculations were done at 5 'O' clock in the evening. The plants were then covered with polythene hoods containing moist sterilized cotton within it for 48 hours, so as to create humidity. Plants of same age were sprayed with sterile water and the leaves were touched with bare needle which served as control.

II. Carborundum Injury:

Sesame seeds were sown in earthen pots containing sterilized soil and they were allowed to grow for one month. Five plants were maintained in each

pot. The leaves were gently rubbed with a fine carborundum powder. The wounded leaves were inoculated by spraying spore suspension.

The inoculations were done at 5 'O' clock in the evening. The plants were then covered with polythene hoods containing moist sterilized cotton within it for 48 hours, so as to create humidity. Plants of same age were sprayed with sterile water which served as control.

III. Pin-Prick method

Sesame seeds were sown in earthen pots containing sterilized soil and they were allowed to grow for one month. Five plants were maintained in each pot. Five cent pins were mounted on a cork covering 1 cm² area was dipped in spore suspension and one gentle prick was given in each leaf of potted plants. The length of the pin was adjusted in a way that it was not damage the other side of leaf. The inoculations were done at 5 'O' clock in the evening and plants were immediately covered with polythene hoods for 48 hours, so as to create humidity. Plants of same age were selected and one gentle prick was given in each leaf by cent pins dipped in sterile water which served as control. The inoculated plants were kept in net-house and development of symptoms was observed regularly.

3.5 Studies on symptomatology, cultural and morphological characters of *A. sesami*

3.5.1 Symptomatology:

Symptoms of the disease on various plants parts were examined critically at different stages of crop growth at 10 days interval to study their courses of development in the field.

3.5.2 Cultural characters:

For cultural characters the pathogen was studies on PDA medium. The 5 mm disc of pure culture of isolate was inoculated at the centres of the poured petri plates from ten days old actively growing culture. All inoculated plates were incubated at 28±2°C in BOD incubator. The growth rate was measured and colony characters and growth habit were visually observed

after 24 hours of incubation till the complete growth of the pathogen in petri plates.

3.5.3 Morphological characters:

Ten days old fungus was stained and observed under the microscope. Observations on number of horizontal and vertical septa and shape of conidia were recorded. Size of conidia of the pathogen was recorded with the help of ocular and stage micrometer.

Observations

A. Radial growth – Radial growth was recorded by measuring the diameter of colony cross wise and then average mean was taken.

B. Frequency of spore – For observing the frequency of spores of *Alternaria sesami*, slides were prepared from 3 different places from each plate and at least 10 microscopic fields/slide were observed. Therefore observation of sporulation was recorded after 10 days and categorised under following groups.

-	=	No sporulation (0 spore/ microscopic field)
+	=	Poor (0-10 spores/ microscopic field)
++	=	Moderate (10-50 spores/microscopic field)
+++	=	Good (50-100 spores/ microscopic field)
++++	=	Abundant (more than 100 spores/microscopic field)

3.6 Detection of *Alternaria sesami* on sesame seeds from different places

3.6.1 Collection of seed samples:

Seed samples of sesame were obtained from different 14 places viz. Nagour, Pali, Jaipur, Chittorgarh, Ajmer, Jalore, Jodhpur, Jabalpur, Khandwa, Morena, Rewa, Sidhi, Tikamgarh and Chattarpur. The seed were stored in polythene bags after proper labeling. The seed samples were examined for the detection of seed infection due to *Alternaria sesami*. Four hundred seeds of each sample were used for the detection of percent seed infection.

3.6.2 Methods for Detection of *Alternaria sesami*:

In order to find out the most effective method for the detection of *A. sesami* associated with seeds from different places, following methods were used -

- I. Blotter method (ISTA 1976)
- II. Agar plate method (Muskett and Malone 1941)
- III. Ragdoll method (ISTA 1976)

I. Blotter method:

Blotter method was employed for the detection of *A. sesami* associated with sesame seeds (ISTA 1976). Three circular pieces of white blotter paper of the size of plastic petri plate (10 cm) were placed in each after dipping them in sterilized water. Four hundred seeds of each-sample (from 14 different places) were taken for the detection of *A. sesami* without treatment with any chemical. 25 seeds were placed in each plate on the blotters in such a manner that sixteen formed an outer ring, eight an inner ring and one in the center. All the seeds were placed at equal distance from each other. The seeds were examined for the presence of associated *A. sesami* after seven days of incubation under stereoscopic binocular microscope.

II. Agar plate method:

In this method four hundred seeds of each-sample (from 14 different places) were taken for the detection of *A. sesami* without treatment with any chemical. Ten surface sterilized seeds of each sample were arranged in a petri plates containing solidified PDA in such a way that nine seeds formed an outer ring and one was placed in the center. The plates were incubated in an incubator at 28°C for seven days. The plates were examined for detection of percent seed infection with *Alternaria sesami* by preparing slides.

III. Ragdoll Method:

Same as the above methods, in this method also four hundred seeds were used. The paper towel (80 x 45 cm) was used for the detection of *A. sesami*. The related information of the sample was written in the center of the paper towel prior to its wetting with distilled water. Fifty seeds were arranged

on the paper towel in ten rows having five seeds in each row in half the length leaving 1 cm space on the other three sides. The paper towel was then rolled, wrapped and tightened with rubber band at the ends. This paper towel was placed vertically in an incubator at 28°C temperature for seven days. After incubation, paper towels were opened and seeds were examined for the association of *A. sesami*.

3.7 Survival of *A. sesami* in sesame seeds and plant debris:

Diseased seeds and plant debris (leaves, stems, branches and pods) were collected after harvesting of sesame and stored under two different conditions viz. refrigerator (Low temperature) and laboratory (High temperature). The isolation of the pathogen was made at an interval of 15 days on PDA and confirmed the survival of pathogen. The experiment was continued till the end of June (up to sowing).

3.8 Effect of media on mycelial growth and sporulation of *Alternaria sesami*:

The efficacy of various synthetic and non-synthetic media on growth of *A. sesami* was studied. Seven media viz. Oat meal agar, Corn meal agar, Potato dextrose agar, Carrot agar, Rose bengal agar, Asthana and Hawker's and Czapek-Dox agar were used. The different types of media were prepared as per the composition and placed in 250 ml conical flasks. The different types of media were then poured in the sterilized plates @ 20 ml per petri plate uniformly and allowed to solidify. Mycelial disc of five mm diameter was cut from 7 days old culture of pathogen and was inoculated in the centre of each plate. The inoculated plates were incubated at 28±2°C till the pathogen completely occupied the plate. Three replications were maintained for each media. An observation on radial growth (colony diameter) was recorded in cross way at 48 hours intervals till the completion of growth of pathogen (90 mm) in any plate. Colony characters and sporulation were also recorded at the end.

3.9 Effect pH on mycelial growth, sporulation and spore germination of *A. sesami*:

The pH for good growth and sporulation of the fungus was determined. Czapek-Dox synthetic media was used to study the effect of pH on the growth of *A. sesami*. Different pH levels from 4.5 to 7.5 with the difference of 0.5 were adjusted with Citrate phosphate buffer and the some cases by N/10 HCL or N/10 NaOH. Three replications were maintained, the inoculated plates were kept at $28\pm 2^{\circ}\text{C}$ for 8 days and observations on colony diameter were recorded at 2 days interval and sporulation was recorded in the end of the experiment.

3.10 Efficacy of plant extracts on the mycelial growth, sporulation and spore germination of *A. sesami*:

The present investigation was carried out to evaluate different plant species for the possible presence of fungal toxicant properties against *Alternaria sesami* by poisoned food technique.

The fresh plant samples of 100 g were washed in tap water then washed with alcohol and finally washed thrice using sterile distilled water. They were crushed in a sterile mortar and pestle by adding 100 ml of sterile distilled water. The extracts were collected by filtering through Whatman No. 1 filter paper. Finally, filtrate thus obtained from the sample was used as 100 per cent stock solution. To study antifungal mechanism of plant extracts. The stock solution 5 ml, 10 ml and 15ml was taken separately and was mixed with 50ml sterile molten PDA medium respectively, so as to get 10, 20 and 30 per cent concentrations. The plant extract amended PDA medium was poured aseptically into sterilized petri plates while the plates containing PDA alone served as control. All the plates were inoculated with 5 mm mycelial disc of 7 days old actively growing culture of *A. sesami*. Each treatment was replicated thrice, and incubated at $28\pm 2^{\circ}\text{C}$ till the pathogen completely occupied the control plate. To test the spore germination, two drops of each suspension at 10, 20 and 30 per cent concentration along with spores were placed in a cavity slide and incubated at $28\pm 2^{\circ}\text{C}$ for 24 hours and thereafter germination

percentages were recorded. The per cent inhibition over control was calculated according to formula given by Vincent (1947).

Table 1: The list of plant extracts used *in vitro*

S.No.	Botanicals	Common name	Plant parts used	Family
1	<i>Prosopis cineraria</i> (L.) Druce	Prosopis	Leaf	Fabaceae
2	<i>Azadirachta indica</i> Juss.	Neem	Leaf	Meliaceae
3	<i>Thuja compacta</i> L.	Thuja	Leaf	Cupressaceae
4	<i>Acacia nilotica</i> (L.) Willd.	Babool	Leaf	Fabaceae
5	<i>Cuscuta reflexa</i> Roxb.	Amarbel	Leaf	Convolvulaceae
6	<i>Parthenium hysterophorus</i> L.	Gajar grass	Leaf	Asteraceae
7	<i>Polyanthia longifolia</i> Sonn.	Ashoka	Leaf	Annonaceae

3.11 Effect of fungicides on the mycelial growth, sporulation and spore germination of *Alternaria sesami*:

The fungicides were weighed as per the desired concentration and mixed thoroughly in the 250 ml conical flasks containing warm PDA under aseptic conditions. The uniform mixture was then poured in the sterilized plates @ 20 ml per petri plate and allowed to solidify. Mycelial disc of five mm diameter was cut from 7 days old culture of pathogen and was inoculated in the centre of each plate. The inoculated plates were incubated at 28±2°C till the pathogen completely occupied the control plate. Three replications were maintained for each treatment, while the plates without fungicides served as control. An observation on radial growth (colony diameter) and sporulation was recorded in the end of the experiment. To test the spore germination, two drops of fungicide suspensions along with spores were placed in a cavity slide and incubated at 28±2°C for 24 hours and thereafter germination percentage were recorded.

Percent growth inhibition of pathogens was calculated as described by Vincent (1947).

$$I = C - T/C \times 100$$

Where,

I= Percent inhibition

C= Radial growth of control

T= Radial growth in treatment

Table 2: Details of fungicide screened against pathogen *in vitro*:

S.No.	Technical name	Tread name
1	Mancozeb	Dhanuka m- 45
2	Chlorothalonil	Jatayu
3	Hexaconazole 5% EC	Hexazol gold
4	Difenoconazole	Score
5	Propiconazole	Tilt
6	Azoxystrobin	Amistar
7	Tebuconazole	Folicure

3.12 Screening of sesame germplasm:

A trial was conducted for screening the germplasm lines, during *Kharif* 2014. Two hundred germplasm including susceptible check (Phule Til- 1) were sown in single row 40 cm apart. Disease severity was recorded at physiological maturity of the crop. Scoring of disease was done by using the scale given below (Mayee and Datar, 1986). Ten plants of each line and the three leaves per plant were chosen randomly to record disease severity. Plants were scored on a 0 (healthy) to 5 (severely infected) scale. The disease severity data from the three leaves were pooled to obtain an average value for each plant. Percent Disease Index was calculated using the formula which is given by Sherwood and Hagedorn (1958).

$$\text{PDI (\%)} = \frac{\text{Summation (No of plants in a disease scale category)}}{(\text{Total no plants}) \times (\text{Maximum disease scale category})} \times 100$$

Disease severity scale was used to record *A. sesami* symptoms on sesame leaves.

Disease scale	Disease symptoms on sesame leaves
0	No infection
1	1 – 10 % leaf area infected
2	11 – 25 % leaf area infected
3	26 – 50 % leaf area infected
4	51 – 70 % leaf area infected
5	71 – 100% leaf area infected

On the basis of disease severity (PDI) germplasm were categorized as Immune (0 PDI), Resistant (1-10 PDI), Moderately Resistant (11-25 PDI), Moderately susceptible (26-50 PDI), Susceptible (51 to 70 PDI) and Highly susceptible (71-100 PDI).

RESULT

The present investigation was carried out to study symptomatology, cultural and morphological characters of *Alternaria sesami* (Kaw.) Mohanty and Behera. Experiments were conducted to study the effect of different media and pH on mycelial growth and sporulation. The investigations were carried out on evaluation of plant extracts and fungicides under *in vitro*. Further the investigations were undertaken on detection of per cent seed infection and survival of *Alternaria sesami*. A field experiment was conducted to screen sesame germplasm against Alternaria blight. The results obtained are presented here as under -

4.1 Symptomatology of Alternaria blight of sesame:

The typical symptoms of Alternaria blight of sesame were studied during *Kharif* 2014-15.

Field symptoms of disease appeared as brown to dark, oval to irregular spots varying from 1 mm to 2 cm in diameter with pale margin and yellow halo. Spots were primarily observed on leaves; however, in severely infected plants, spots developed on stem and capsules also. On severely affected leaves, several spots coalesced to form large necrotic lesions, leading to blight and defoliation and sometimes death of the infected plant. The disease was most severe under moist conditions following wet weather.

In the initial stages of infection water soaked, small, brown, round /oval to irregular spots appeared on the leaf blade. Later on the spots enlarged and turned dark brown with concentric zonations demarcated with brown lines inside the spots on the upper surface. On the under surface the spots are greyish brown in colour. The dark brown water soaked lesions were also seen on the stem, midrib and also on the veins of the capsules. Small brown spots appeared on the capsules leading to formation of shrivelled and deformed seeds which reduced the quantity of yield as well as its quality. The pathogen was isolated and identified as *Alternaria sesami* (Kaw.) Mohanty and Behera.

4.2 Isolation and identification:

The pathogen was successfully isolated from infected leaves, stems and pods collected from sesame field of “Sesame and Niger project” and pure culture was maintained on potato dextrose agar as described in “Material and methods”.

The fungus was examined microscopically for the morphological and taxonomic characters such as shape, size, and colour of conidia and conidiophores.

4.3 Cultural and morphological characters of *Alternaria sesami*:

Colonies were characterized after 7 days of culture at 25°C. On PDA, colonies were raised, olive green and 58-62 mm in diameter with regular margins and immersed or partly superficial mycelium. Mycelium was light brown, slender, radiating branched and filamentous septate.

Conidiophores borne solitary or in small fascicles, simple or branched, smooth-walled, pale to light brown in colour, 0 - 3 septate with a single, small and swollen, terminal, pigmented conidiogenous site at the apex, up to 92 µm long and 2-6 µm wide and each bearing conidia singly.

Conidia were straight or slightly curved, obclavate, yellowish brown to dark brown in colour and measure 32-115 × 18-32 µm (excluding the beak). The conidia have 4-10 transverse septa and 1-4 longitudinal septa. The conidia have long and hyaline beak which may be simple or branched and from 25-210 × 2-4 µm in size.

4.4 Pathogenicity tests:

The pathogenic fungus was successfully isolated from leaves of naturally infected sesame plants. Thirty days old plants grown in sterilized earthen pots, filled with sterilized soil, were inoculated with spore suspension obtained from 10 day old culture of *Alternaria sesami*. For the pathogenicity of *Alternaria sesami* on sesame, three techniques viz. spore suspension spray method, pin-prick method and carborundum method were used on detached leaves and attached leaves.

Table 3: Efficacy of different methods of inoculation for testing the pathogenicity of *Alternaria sesami* on sesame leaves

S.No.	Method	Symptoms expressed after days			
		Attached leaves		Detached leaves	
		Initiation of spot	Full development of spot	Initiation of spot	Full development of spot
1.	Spore suspension spray	5	8	4	7
2.	Pin-prick	2	4	2	4
3.	Carborundum	3	5	2	4
4.	Control	-	-	-	-

In the present investigation it was found that the initiation and the full development of the spot was earlier on detached leaves as compared to attached leaves of sesame. The symptoms of *Alternaria sesami* appeared as small necrotic spots after 2 days of inoculation. The lesions enlarged and became water soaked and finally turn into dark brown colour.

Out of three methods tested for pathogenicity, pin prick method was found to be best, as in this case the initiation of spots was started 2 days after inoculation and within 4 days the spots developed fully on both attached and detached leaves.

In carborundum method the initiation of the disease was started 2 and 3 days after inoculation on detached leaves and attached leaves and full development of spots in 4 and 5 days respectively but in carborundum method because of rubbing, large necrotic areas were formed and typical symptoms did not appear. However in spray method the initiation of the disease occur in 4 and 5 days while the full development in 7 and 8 days on detached leaves and attached leaves respectively.

On artificial inoculation the symptoms on leaves developed like that of natural infection as brown to dark fuscous, oval to irregular spots with pale margin and yellow halo but the size of spot a bit smaller. Spots were primarily

observed on leaves; however, in severely infected plants, spots developed on stems also.

Isolation made from these diseased spots invariably yielded the fungus *Alternaria sesami*, identical with the type inoculated. Similar results were obtained in repeated trials and in this way Koch's postulate was proved.

4.5 Detection of *A. sesami* on seed samples from different places:

Alternaria sesami is common seed borne pathogen distributed frequently in different places of Rajasthan and Madhya Pradesh, from where seed samples collected, however the per cent seed infection varied from 2 to 19 recorded in agar plate, blotter and ragdoll methods. Higher counts (19%) of *A. sesami* were recorded in blotter method then the other two methods. Occurrence of infection was pronounced from the seeds procured from the Rajasthan; however agar plate and ragdoll methods detected quite large number of samples towards infection of *A. sesami* in Rajasthan as compared to Madhya Pradesh. (Table 4)

Table 4: Per cent infection of *Alternaria sesami* in sesame seeds

S.No.	Places	Per cent seed infection		
		Agar Plate	Blotter	Ragdoll
1	Nagour (Raj)	16	19	15
2	Pali (Raj)	14	15	12
3	Jaipur (Raj)	13	16	11
4	Chittorgarh Raj)	12	13	10
5	Ajmer (Raj)	14	16	13
6	Jalore (Raj)	9	10	8
7	Jodhpur (Raj)	16	18	15
8	Jabalpur (MP)	8	10	6
9	Khandwa (MP)	4	7	3
10	Morena (MP)	5	6	4
11	Rewa (MP)	3	4	2
12	Sidhi (MP)	4	5	3
13	Tikamgarh (MP)	4	6	2
14	Chattarpur (MP)	7	9	6

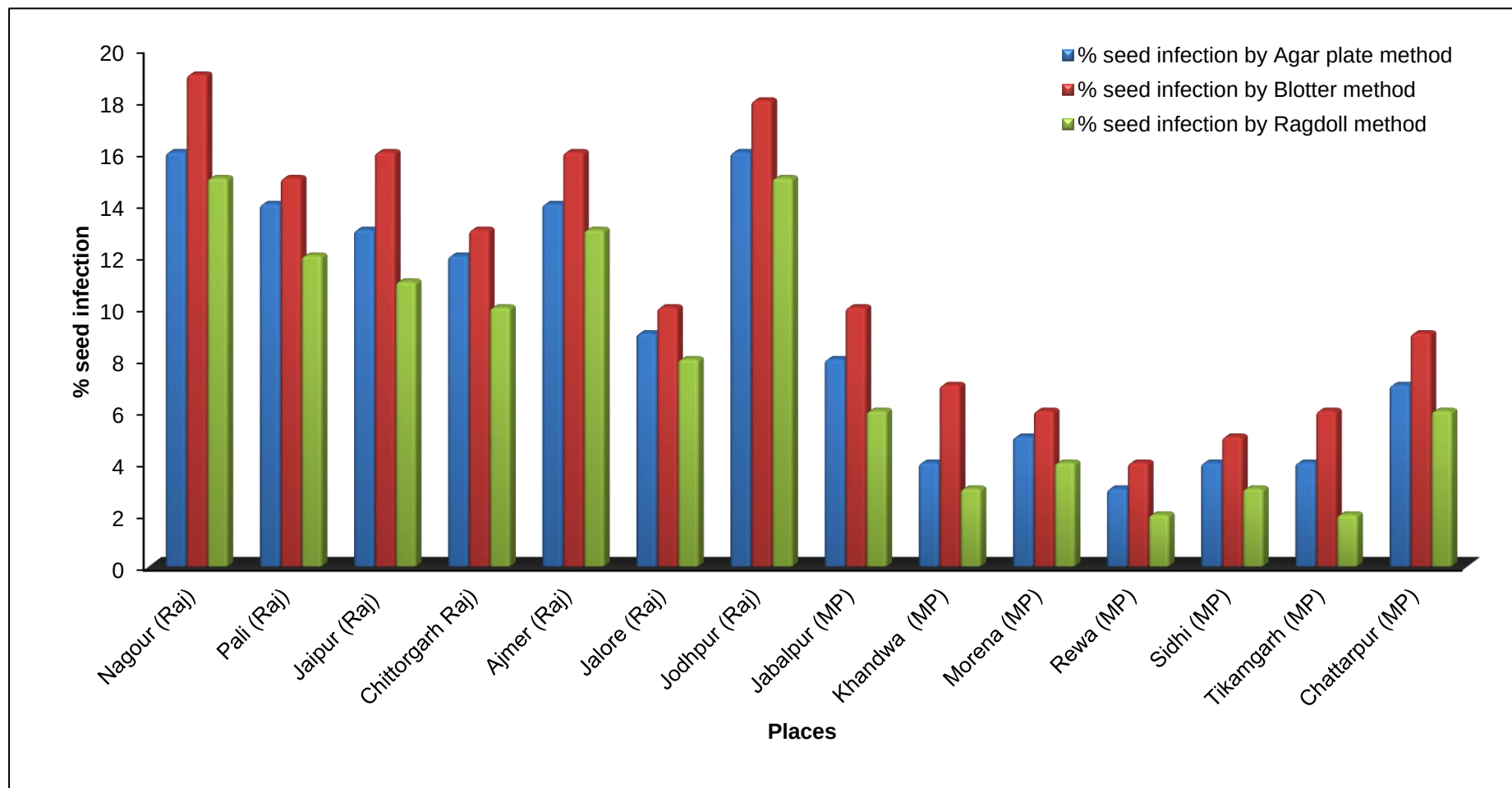


Fig. 1: Per cent seed infection of *A. sesami*

4.6 Survival of *Alternaria sesami* in seeds and plant debris

Survival of *A. sesami* was the maximum (12.8%) under low temperature even after 7 months from the harvest, whereas under high temperature 9.3% survival was noticed. *A. sesami* survived in both the store conditions (Table 5) i.e. from harvest (November) till the end of June (before sowing) in seeds as well as plant debris.

Table 5: Survival per cent of *Alternaria sesami* in seeds and plant debris under conditions of Refrigerator and Laboratory

S.No.	Date	Seeds		Plant Debris	
		Low temperature (Refrigerator)	High temperature (Laboratory)	Low temperature 4 ⁰ C (Refrigerator)	High temperature (Laboratory)
1	25 Nov.	40.5	39.7	Present	Present
2	10 Dec.	38.5	37.7	Present	Present
3	25 Dec.	37.6	36.9	Present	Present
4	9 Jan.	35.8	34.9	Present	Present
5	24 Jan.	34.9	32.2	Present	Present
6	8 Feb.	34.1	31.5	Present	Present
7	23 Feb.	31.9	30.8	Present	Present
8	10 Mar.	29.2	27.4	Present	Present
9	25 Mar.	25.3	23.5	Present	Present
10	9 April	23.8	19.0	Present	Present
11	24 April	21.7	15.1	Present	Present
12	9 May	18.8	12.9	Present	Present
13	24 May	15.5	11.4	Present	Present
14	8 June	13.4	9.9	Present	Present
15	23 June	12.8	9.3	Present	Present

4.7 Effect of media on mycelial growth and sporulation of *A. sesami*

Among the seven different media, the maximum (68.3 mm) mean radial growth was recorded on Potato dextrose agar medium followed by Oat meal agar (66.3 mm). Poor growth was recorded on Rose bengal agar medium (49.3 mm). Abundant sporulation on PDA and good sporulation was recorded on rest of the media. However, poor sporulation noticed on Czepek-Dox agar

medium. Colony characters also varied on different media used. On Oat meal agar the fungus showed good growth of dirty white to iron gray mycelium, felt type smooth growth and with smooth margins whereas on PDA olive green to iron grey mycelium, fair growth, felt type, smooth margins with distinct zonations was observed.

Table 6: Effect of media on mycelial growth and sporulation of *A. sesami*

S.No	Media	Mean radial growth (mm)* at 2 days interval				Sporulation at 8 days	Colony character
		2	4	6	8		
1	Oat meal agar	10.5	20.2	55.2	66.3	+++	Good growth of dirty white to iron grey mycelium, felt type growth smooth and with smooth margins and zonations diffuse type.
2	Corn meal agar	6.2	17.5	35.8	54.3	+++	Good growth of dirty white to iron grey mycelium, felt type slightly woolly growth with smooth margins.
3	PDA	10.5	23.0	35.0	68.3	++++	Olive green to iron grey mycelium, fair growth, felt type, margins smooth distinct zonations.
4	Carrot agar	5.2	18.3	26.7	54.5	+++	Good growth iron grey mycelium, felt type growth with smooth margins and zonations present.
5	Rose bengal agar	9.0	19.8	30.5	49.3	+++	Poor growth of aerial mycelium slightly submerges grey to light black in colour with smooth margins and zonations present.
6	Asthana and Hawker's	9.2	23.2	45.0	53.3	+++	Poor growth of aerial mycelium slightly submerges light black in colour with smooth margins and ill defined zonations.
7	Czepek-Dox agar	10.0	16.8	36.8	55.7	+	Good growth of aerial mycelium felt type, slightly woolly and direct white in centre, otherwise, olive green on the margins, margins smooth slight tendency to form sector.
S. Em±		1.08	0.92	1.28	2.19		
C. D. (P = 0.05%)		3.34	2.82	3.95	6.76		

*Mean of three replications

- Nil, + Poor, ++ Moderate, +++ Good and ++++Abundant

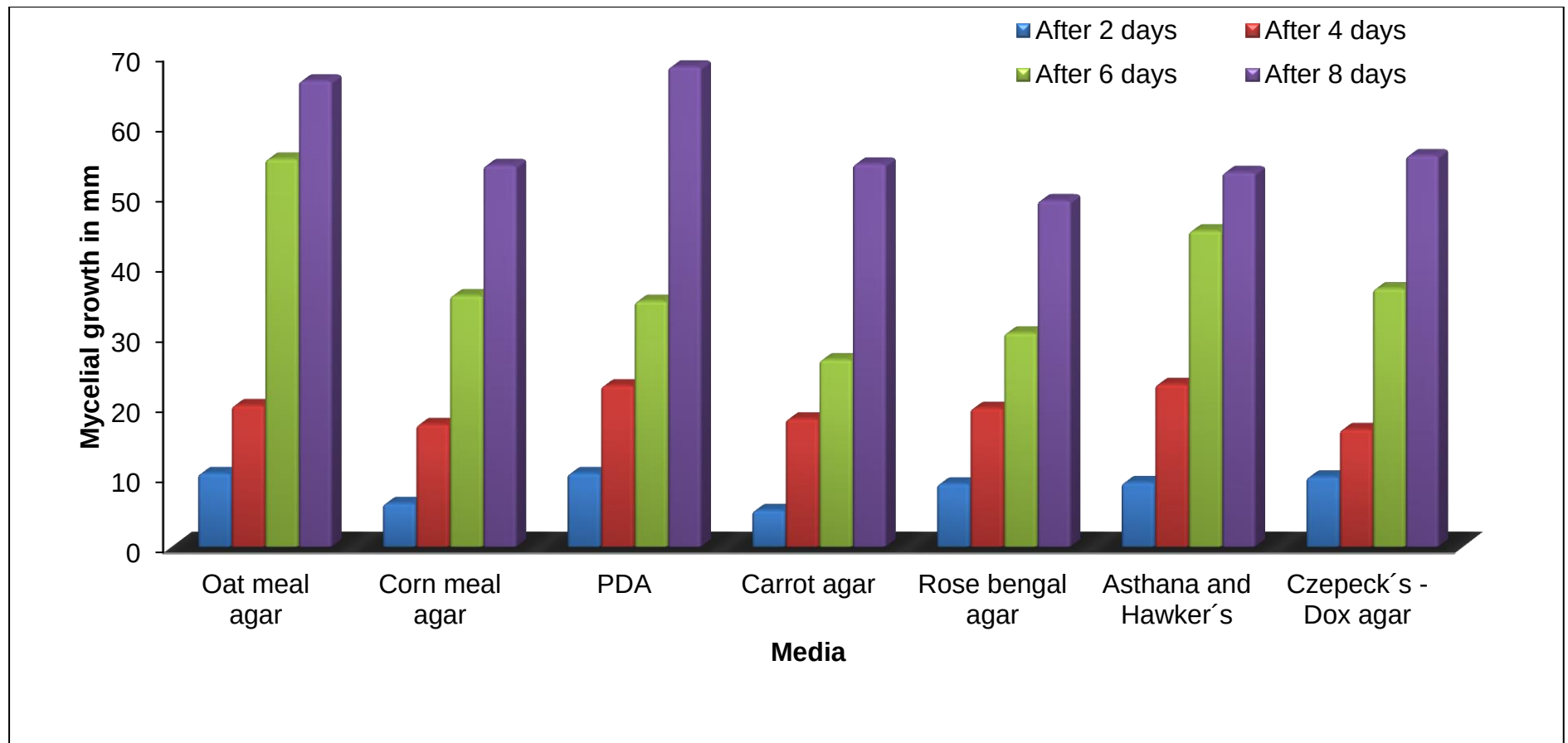


Fig. 2: Effect of media on mycelial growth of *A. sesami*

4.8 Effect of pH levels on mycelial growth and sporulation of *A. sesami*:

Among all the pH levels tested, the maximum mean radial growth was at on 6.5 (84.2 mm) followed by pH 6 (79.5 mm). The minimum mean mycelial growth was at pH 4.5 (62.0 mm). So ideal pH range for growth for mycelial growth from 6.0 to 6.5. On pH 6.0 and pH 6.5 abundant sporulation was recorded. While good sporulation was observed at pH 7.0 and 7.5. Poor sporulation was found on pH 4.5. This shows that fungus preferred acidic to neutral pH for mycelial growth and sporulation.

Table 7: Effect of pH on mycelial growth and sporulation of *A. sesami*

S.No.	pH	Mean radial growth (mm)* at 2 days interval				Sporulation at 8 days
		2	4	6	8	
1	4.5	21.8	31.3	54.8	62.0	+
2	5.0	24.8	34.3	69.2	70.0	++
3	5.5	23.7	42.0	71.5	73.2	++ (Zonations present)
4	6.0	23.0	44.5	78.3	79.5	++++
5	6.5	24.8	45.5	83.3	84.2	++++
6	7.0	19.3	37.0	56.7	78.8	+++ (Zonations present)
7	7.5	16.3	20.0	31.5	73.3	+++
S. Em±		0.83	0.85	0.37	0.35	
C.D. (P = 0.05%)		2.55	2.61	1.14	1.07	

*Mean of three replications

- Nil, + Poor, ++ Moderate, +++ Good and ++++Abundant

4.9 Efficacy of plant extracts on the mycelial growth, sporulation and spore germination of *A. sesami*:

Under *in vitro* conditions, the experiment was conducted in petri plates containing leaf extract amended PDA medium. Leaf extracts of seven plants

viz. Khejri, Neem, Thuja, Babool, Amarbel, Gajar grass and Ashoka were evaluated at three concentrations (10, 20 and 30%) for their efficacy against *Alternaria sesami* using poisoned food technique.

Leaf extracts of all the seven plants significantly reduced mycelial growth. The minimum mean mycelial growth of 46.7 mm was observed on Babool leaf extract amended medium followed by 50.5 mm and 54.7 mm with Neem and Gajar grass leaf extract amended medium respectively at 10% concentration. Whereas, Babool leaf extract amended medium showed minimum mycelial growth (31.7 mm) and it was followed by Neem leaf extract amended medium (38.0 mm) and Khejri leaf extract amended medium (40.0 mm) at 20% concentration. At 30% concentration, Babool leaf extract amended medium exhibited minimum (24.5 mm) radial growth followed by Neem (28.3 mm) and Gajar grass (29.3 mm) against maximum (90.0 mm) in control. Remaining treatments were found significantly superior over control.

Sporulation of *A. sesami* was poor at 30% concentration of Neem, Thuja, Babool, Gajar grass and Amarbel leaf extract amended media, whereas Ashoka and Khejri showed moderate sporulation.

Spore germination was also significantly inhibited by leaf extract of different plants at tested concentrations. On Gajar grass leaf extract it was minimum as 4.0, 7.5 and 19.0 per cent at 30, 20 and 10 per cent concentrations respectively. Whereas on Thuja leaf extract spore germination was 7.0, 9.5 and 20.0 per cent on 30, 20 and 10 per cent concentrations respectively.

Table 8A: Efficacy of plant extracts on mycelial growth and per cent growth inhibition of *A. sesami*

S.No.	Plants	Mean radial growth (mm)*			Percent growth inhibition at 30% concentration
		Concentration (%)			
		10	20	30	
1	Khejri	60.0	40.0	30.3	66.3
2	Neem	50.5	38.0	28.3	68.5
3	Thuja	69.5	50.7	40.3	55.2
4	Babool	46.7	31.7	24.5	72.8
5	Amarbel	73.3	46.0	32.5	63.9
6	Gajar grass	54.2	45.7	29.3	67.4
7	Ashoka	79.7	60.8	50.3	44.1
8	Control	90.0	90.0	90.0	-
S. Em±		0.80	0.71	0.61	
C. D. (P = 0.05%)		2.43	2.176	1.869	

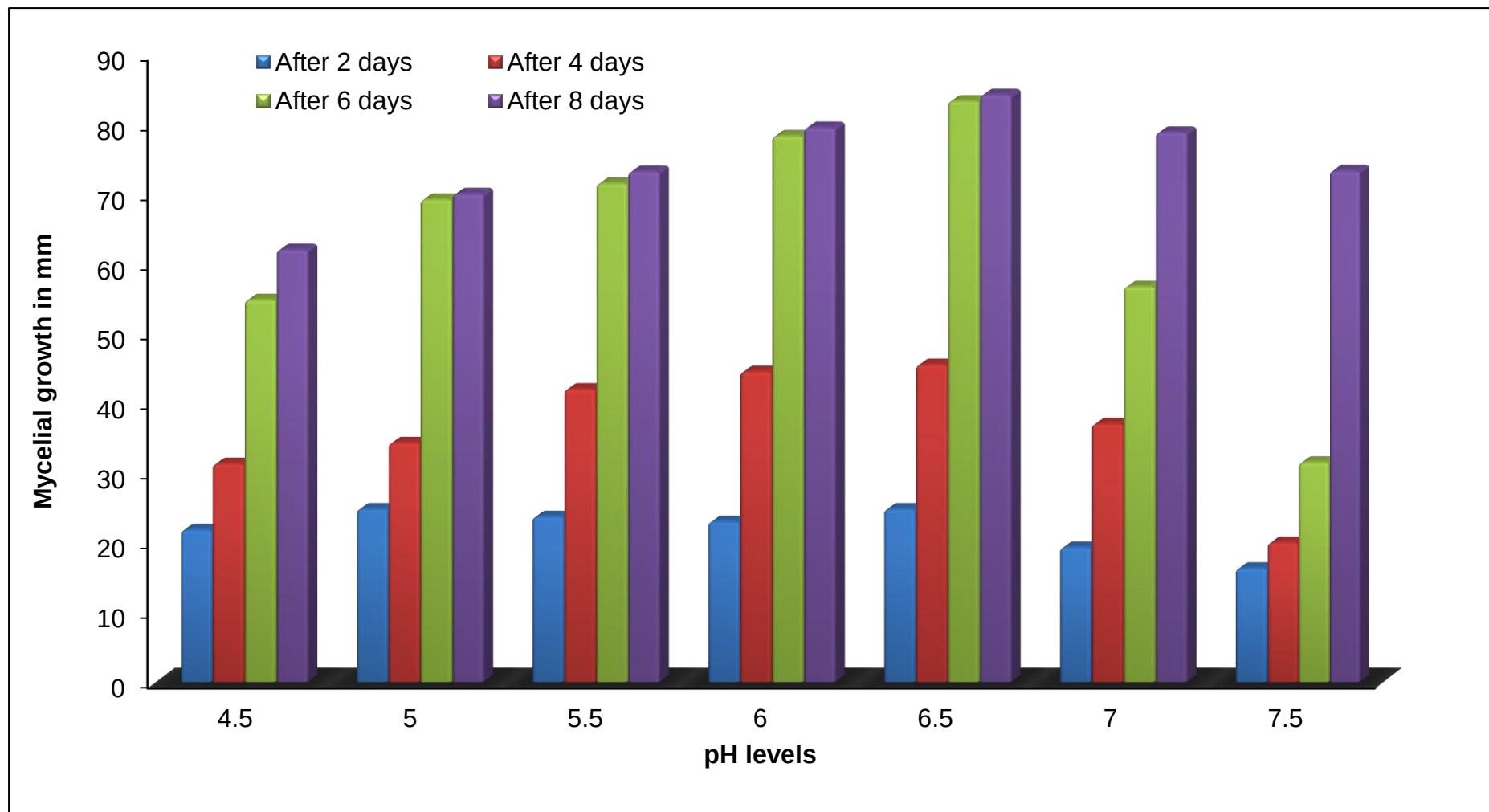


Fig.3: Effect of pH levels on mycelial growth of *A. sesami*

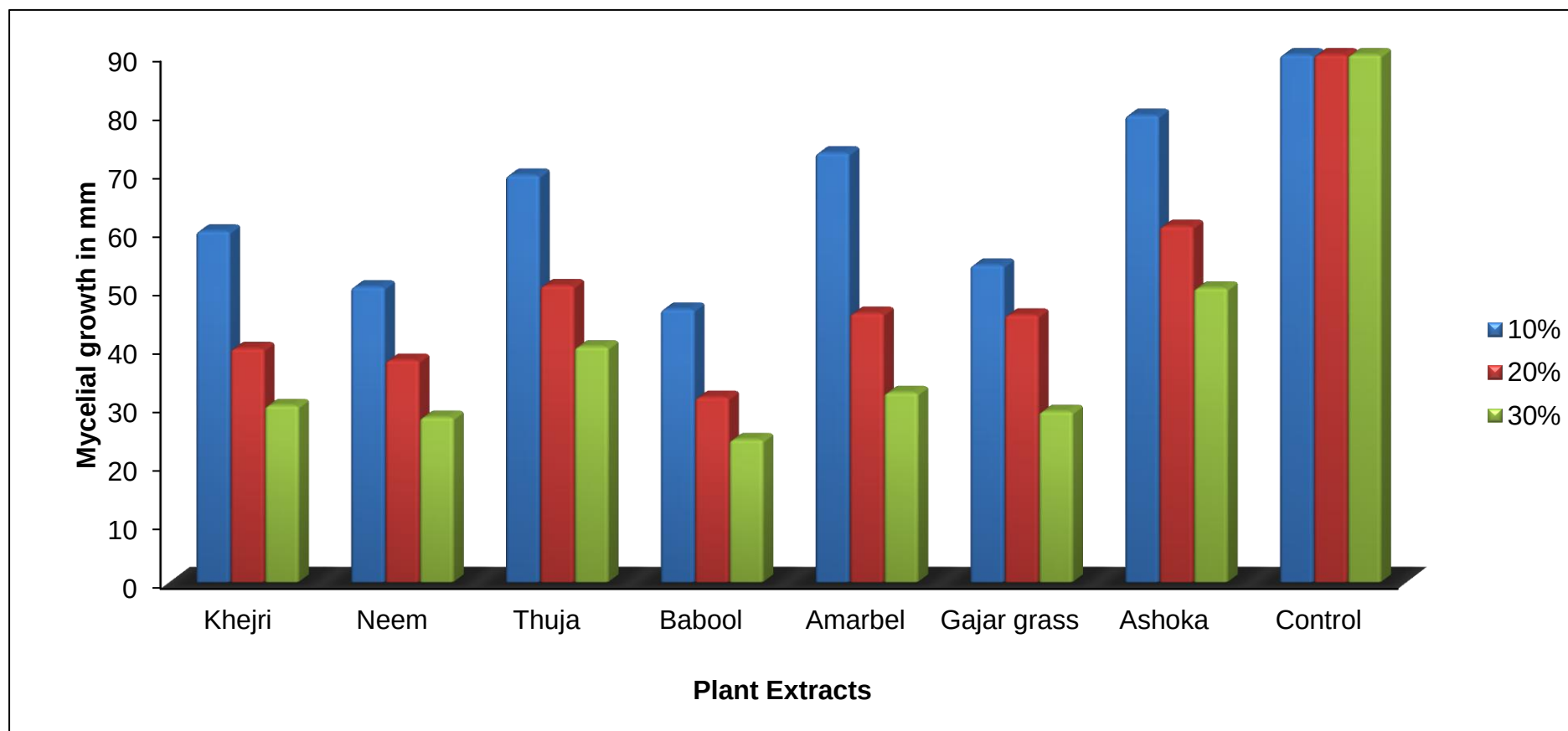


Fig. 4: Effect of plant extracts on mycelial growth of *A. sesami*

Table 8B: Efficacy of plant extracts on sporulation and spore germination per cent of *A. sesami*

S.No.	Plants	Sporulation			Spore germination per cent		
		10%	20%	30%	10%	20%	30%
1	Neem	++	++	+	24.0	18	11.5
2	Khejri	+++	++	++	26.5	29.5	20.0
3	Thuja	+++	++	+	20.0	9.5	7.0
4	Babool	++	++	+	28.0	13.5	8.5
5	Gajar grass	++	+	+	19.0	7.5	4.0
6	Amarbel	+++	++	+	27.0	17.5	12.0
7	Ashoka	+++	+++	++	22.0	20.0	11.0
8	Control	++++	-	-	80.0	80.0	80.0

*Mean of three replications

- Nil, +poor, ++Moderate, +++ Good, ++++ Abundant

4.10 Effect of fungicides on the mycelial growth, sporulation and spore germination of *Alternaria sesami*:

To evaluate the effect of different fungicides on mycelial growth, sporulation and spore germination per cent of *Alternaria sesami*, this experiment was conducted. Seven fungicides Mancozeb, Hezxaconazole, Chlorothalonil, Azoxystrobin, Propiconazole, Tebuconazole, and Difenoconazole were tested by poisoned food technique at three concentrations viz. 250, 500 and 1000 pm.

All the fungicides significantly inhibited the growth of at all the concentrations tested and radial growth ranged from 0.0 to 51.7 mm in treatments as compared to control (90.0mm). Among all the fungicides evaluated, Propiconazole (34.1 mm) was found to be most effective followed by Difenoconazole (36.1 mm) and Hexconazole (40 mm) in inhibition of mean radial growth at 250 ppm. Propiconazole (17.0 mm), Difenoconazole (17.8 mm) and Tebuconazole (21.5 mm) most effective at 500 ppm. Propiconazole and Difenoconazole completely inhibited radial growth at 1000 ppm. However, mycelial growth decreased as the concentration increases.

All the fungicides significantly inhibited the sporulation as well as spore germination also. *A. sesami* showed nil to good sporulation at different concentrations viz. 250, 500 and 1000 ppm. Propiconazole, Hexaconazole, Tebuconazole, Difenoconazole and Mancozeb were found to be most effective in inhibition of sporulation at tested concentrations. Fungicides also influenced spore germination. Among all the fungicides, spore germination per cent was the minimum in Propiconazole (9.5) and in Difenoconazole (12.5) at 250 ppm. Whereas in Propiconazole (5.5) and Difenoconazole (8.0) minimum spore germination per cent was found at 500 ppm. No spore germination was observed in Propiconazole and Difenconazole at 1000 ppm.

Table 9A: Efficacy of fungicides on the mycelial growth and per cent inhibition of *A. sesami*

S.No.	Fungicides	Mean radial growth(mm)*			Per cent growth inhibition at 1000 ppm
		Concentration (ppm)			
		250	500	1000	
1	Mancozeb	50.3	25.0	16.1	82.0
2	Hexaconazole	40.0	21.7	11.5	87.2
3	Chlorothalonil	51.7	32.1	22.5	75.0
4	Azoxystrobin	47.8	30.1	20.5	77.2
5	Propiconazole	34.1	17.0	0.0	100
6	Tebuconazole	44.8	21.5	13.3	85.2
7	Difenoconazole	36.1	17.8	0.0	100
8	Control	90.0	90.0	90.0	
S. Em±		0.64	0.57	0.42	
C. D. (P = 0.05%)		1.95	1.72	1.29	

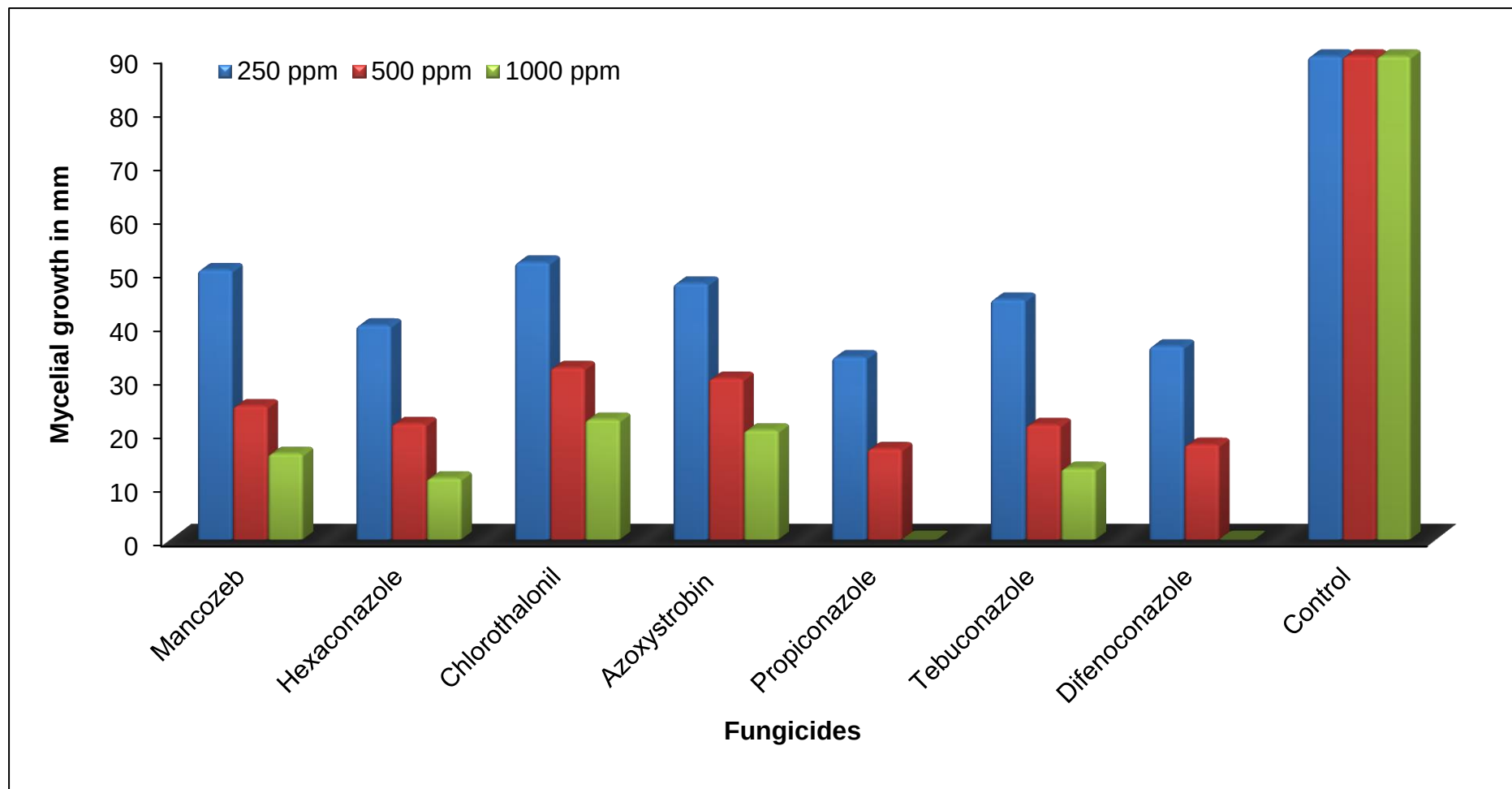


Fig. 5: Effect of fungicides on mycelial growth of *A. sesami*

Table 9B: Efficacy of fungicides on the sporulation and spore germination per cent of *A. sesami*

S.No.	Fungicides	Sporulation			Spore germination per cent		
		250	500	1000	250	500	1000
1	Mancozeb	++	+	–	20.0	11.5	5.0
2	Azoxystrobin	+++	++	+	23.0	16.0	10.0
3	Chlorothalonil	+++	++	+	28.5	19.0	12.0
4	Hexaconazole	++	+	–	18.5	13.0	4.6
5	Propiconazole	++	+	–	9.5	5.5	0.0
6	Tebuconazole	+++	++	–	21.5	16.0	2.0
7	Difenoconazole	++	+	–	12.5	80.0	0.0
8	Control	++++	–	–	82.0		-

*Mean of three replications

- Nil, + Poor, ++Moderate, +++ Good, ++++Abundant

4.11 Screening of germplasm lines

A field experiment was conducted to find out resistant source against *A. sesami*. 200 germplasm including susceptible check (Phule til-1) were screened against Alternaria blight under natural field conditions. The disease index varied from 7 to 72 per cent. None of the germplasm was found to be completely free (0 PDI) from the disease. However, 4 germplasm viz. IC-96222 (8.7 PDI), NIC-8400 (9.3PDI), ES-3707 (8.0 PDI) and IC-204500(7.0 PDI) exhibited less than 10 per cent disease index and considered as resistant (1-10 PDI). Whereas, 13 germplasm VOSI-8458, IS-101, SI-371, NIC-8181, NIC-16104, ES-2186-2, IC-204648, IC-14160-1, IC-96128, IS-607-1-84, NIC13598, MT-6761 and GRT-83124 were found to be moderately resistant (11-25 PDI). 166 germplasm were moderately susceptible (26-50 PDI), whereas,16 germplasm viz. KIS-3571, SI-931, SI-1671, NIC-16146, NIC-8261-1, ICA-14105, IS-649, IC-131607, EC-33507, IS-321, GRT-8618-1, IS-667-1-84, S-336, KIS-398, EC-665 and SI-1225 were found to be susceptible (51-70 PDI). Disease index in susceptible check (Phule Til-1) was 72.0 per cent.

Table 10A: Per cent Disease index (PDI) of *Alternaria sesami* on germplasm lines

S.No.	Germplasm	PDI	S.No.	Germplasm	PDI	S.No.	Germplasm	PDI	S.No.	Germplasm	PDI	S.No.	Germplasm	PDI
1	KIS-3571	56.7	41	SI-46-1	40.0	81	IC-204137	40.0	121	DSK-1	30.0	161	SI-3237	50.0
2	VOSI-5846	35.0	42	SI-205	30.0	82	SI-1033	60.0	122	IC-14120-1	40.0	162	IC-131607	60.0
3	VOSI-8458	23.3	43	SI-3296	46.7	83	NIC-8401	60.0	123	ES-48	43.3	163	SI-3231	40.0
4	EC-14121	50.0	44	SI-7212	45.0	84	SI-3279	40.0	124	NIC-8165	45.0	164	EC-33507	55.0
5	EC-334952	40.0	45	NIC-16325	36.7	85	NIC-7866	50.0	125	ES-29	35.0	165	IS-321	53.3
6	NIC-8202	35.0	46	N1C-8350	50.0	86	NIC-16122	30.0	126	S-0439	50.0	166	EC-334989	40.0
7	IS-445	40.0	47	EC-303427	40.0	87	KMR-13	45.0	127	NIC-8202	30.0	167	ICA-14146-A	40.0
8	IS-101	20.0	48	NIC-16387	30.0	88	NIC-16146	60.0	128	ICA-14105	53.3	168	IS-665	30.0
9	IS-136	50.0	49	NIC-16359	32.5	89	NIC-17257	50.0	129	IS-649	55.0	169	EC-334280	30.0
10	IC-132408	40.0	50	NIC-8262-1	33.3	90	T-13-3-1	40.0	130	SI-1818	45.0	170	IS-475	33.0
11	IS-200	30.0	51	NIC-16358	40.0	91	NIC-8261-1	53.3	131	ES-249	43.3	171	GRT-83124	50.0
12	IS-92—2	50.0	52	NIC-10646-1	50.0	92	NIC-16220	40.0	132	ES-2186-2	23.3	172	GRT-83124	20.0
13	IS-146	40.0	53	KMR-6	40.0	93	IC-96222	8.7	133	TS-261	35.0	173	GRT-8618-1	55.0
14	SI-1032	45.0	54	IC-204979	45.0	94	NIC-8252-1	30.0	134	IC-204648	25.0	174	IC-306651	36.7
15	IS-113	40.0	55	KMR-11	30.0	95	NIC-8584	50.0	135	NIC-16407	35.0	175	IS-667-1-84	55.0
16	SI-931	60.0	56	IC-1634-3	40.0	96	BUC-8357	40.0	136	ES-3707	8.0	176	S-0505	40.0
17	SI-3265-5	40.0	57	NIC-8214	50.0	97	NIC-8340	35.0	137	IS-245-1	40.0	177	S-336	60.0
18	SI-44	40.0	58	IS-184-1	35.0	98	NIC-16115	50.0	138	IS-190	45.0	178	KIS-362	36.7
19	SI-205-1	43.3	59	KM-13	40.0	99	NIC-16393	50.0	139	IS-3082	40.0	179	SI-1248-B	20.0
20	SI-199-2-84	43.3	60	SI-3281	35.0	100	NIC-8400	9.3	140	RJS-78	50.0	180	IS-425-C	45.0
21	SI-983	43.3	61	SI-205	36.7	101	NIC-8371	40.0	141	GRT-8329	45.0	181	EC-334983	30.0
22	IS-118	50.0	62	SI-329	40.0	102	NIC-8181	25.0	142	NIC-16073	45.0	182	SI-1679	45.0
23	IS-107	40.0	63	SI-1143	33.3	103	NIC-16223	35.0	143	IC-204500	7.0	183	SI-76-1	45.0
24	SI-982	35.0	64	SI-1147	55.0	104	NIC-17849	30.0	144	NIC-16221	35.0	184	KIS-398	55.0
25	NIC-8322	35.0	65	SI-1074-1	40.0	105	NIC-8432	40.0	145	MT-67-18	33.0	185	KIC-1634-B	50.0
26	RJS-44	30.0	66	SI-42	36.7	106	NIC-16204	45.0	146	B-203	45.0	186	EC-334985-1	43.3
27	IS-207	35.0	67	SI-1116	46.7	107	NIC-8286	45.0	147	IC-14160-1	25.0	187	IS-461-1-84	45.0
28	IS-208	40.0	68	SI-7650	33.3	108	NIC-16340	40.0	148	S-0337	36.7	188	EC-334999	35.0
29	SI-371	20.0	69	SI-1114	43.3	109	NIC-16414	30.0	149	KMR-17	36.7	189	EC-665	60.0
30	SI-1671	53.3	70	SI-25	40.0	110	EC-334985	30.0	150	IC-96128	25.0	190	SI-1225	56.7
31	SI-85	43.3	71	SI-3264-2	40.0	111	NIC-8254	45.0	151	S-0228	36.7	191	EC-334993	45.0
32	SI-17	35.0	72	SI-212	35.0	112	NIC-16415	40.0	152	ES-110-C	40.0	192	SI-2008	40.0
33	IS-684	40.0	73	SI-1169	35.0	113	NIC-8268	40.0	153	IS-607-1-84	20.0	193	NIC-8288	46.7
34	SI-870-1	43.5	74	SI-772	35.0	114	NIC-8394	33.3	154	NIC-16236	30.0	194	SI7192	30.0
35	SI-339	46.7	75	SI-775	45.0	115	ES-120-1-84-A	33.3	155	SI-1926	40.0	195	SI-3263	50.0
36	SI-554	50.0	76	SI-43	35.0	116	NIC-8488	43.3	156	NIC13598	23.3	196	SI-712	50.0
37	SI-553	40.0	77	SI-3315-5	35.0	117	NIC-16096	33.0	157	IS-308-A	50.0	197	SI-29973	40.0
38	NIC-17508	35.0	78	SI-3291	30.0	118	NIC-7897	33.0	158	MT-6761	20.0	198	SI-1146	50.0
39	SI-1585	40.0	79	SI-780	46.7	119	NIC-16104	23.3	159	IS-37	40.0	199	EC-335004	50.0
40	SI-75	36.7	80	SI-11	50.0	120	NIC-7834	30.0	160	78-301	26.7	200	Phule Til- 1	72.0

Table 10B: Response of germplasm against *A. sesami*

S.No.	Response	Germplasm	No. of lines
1	Immune (0 PDI)	None	0
2	Resistant (1-10 PDI)	IC-96222, NIC-8400, ES-3707 and IC-204500	4
3	Moderately resistant (11-25 PDI)	VOSI-8458, IS-101, SI-371, NIC-8181, NIC-16104, ES-2186-2, IC-204648, IC-14160-1, IC-96128, IS-607-1-84, NIC13598, MT-6761 and GRT-83124,	13
4	Moderately susceptible (26-50 PDI)	VOSI-5846, EC-14121, EC-334952, NIC-8202, IS-445, IS-136, IC-132408, IS-200, IS-92—2, IS-146, SI-1032, IS-113, SI-3265-5, SI-44, SI-205-1, SI-199-2-84, SI-983, IS-118, IS-107, SI-982, NIC-8322, RJS-44, IS-207, IS-208, SI-85, SI-17, IS-684, SI-870-1, SI-339, SI-554, SI-553, NIC-17508, SI-1585, SI-75, SI-46-1, SI-205, SI-3296, SI-7212, NIC-16325, NIC-8350, EC-303427, NIC-16387, NIC-16359, NIC-8262-1, NIC-16358, NIC-10646-1, KMR-6, IC-204979, KMR-11, IC-1634-3, NIC-8214, IS-184-1, KM-13, SI-3281, SI-205, SI-329, SI-1143, SI-1147, SI-1074-1, SI-42, SI-1116, SI-7650, SI-1114, SI-25, SI-3264-2, SI-212, SI-1169, SI-772, SI-775, SI-43, SI-3315-5, SI-3291, SI-780, SI-11, IC-204137, SI-1033, NIC-8401, SI-3279, NIC-7866, NIC-16122, KMR-13, NIC-17257, T-13-3-1, NIC-16220, NIC-8252-1, NIC-8584, BUC-8357, NIC-8340, NIC-16115, NIC-16393, NIC-8371, NIC-16223, NIC-17849, NIC-8432, NIC-16204, NIC-8286, NIC-16340, NIC-16414, EC-334985, NIC-8254, NIC-16415, NIC-8268, NIC-8394, ES-120-1-84-A, NIC-8488, NIC-16096, NIC-7897, NIC-7834, DSK-1, IC-14120-1, ES-48, NIC-8165, ES-29, S-0439, NIC-8202, SI-1818, ES-249, TS-261, NIC-16407, IS-245-1, IS-190, IS-3082, RJS-78, GRT-8329, NIC-16073, NIC-16221, MT-67-18, B-203, S-0337, KMR-17, S-0228, ES-110-C, NIC-16236, SI-1926, NIC13598, IS-37, 78-301, SI-3237, SI-3231, EC-334989, EC-334989, ICA-14146-A, ICA-14146-A, EC-334280, IS-475, GRT-83124, IC-306651, S-0505, KIS-362, SI-1248-B, IS-425-C, EC-334983, SI-1679, SI-76-1, KIC-1634-B, EC-334985-1, IS-461-1-84, EC-334999, EC-334993, SI-2008, NIC-8288, SI7192, SI-3263, SI-712, SI-29973, SI-1146 and EC-335004.	166
5	Susceptible (51-70 PDI)	KIS-3571, SI-931, SI-1671, NIC-16146, NIC-8261-1, ICA-14105, IS-649, IC-131607, EC-33507, IS-321, GRT-8618-1, IS-667-1-84, S-336, KIS-398, EC-665 and SI-1225	16
6	Highly Susceptible (71-100 PDI)	Phule Til-1	1

DISCUSSION

Sesame is an important oil seed crop of this country and thought to have originated in Africa (Brar and Ahuja 1979; Ram et al. 1990). It is widely grown in tropical and subtropical regions. Its production is often concentrated in marginal and sub marginal lands (Ashri 1998). Most of the sesame seeds are used for oil extraction and the rest are used for edible purposes (El Khier et al. 2008). Sesame is grown primarily for its oil-rich seeds. Before seeds were appreciated for their ability to add nutty flavour or garnish foods, they were primarily used for oil and wine (Ghandi 2009).

Sesame suffers from many diseases during the growth period. Alternaria blight is one of the serious disease which causes the considerable qualitative and quantitative yield losses in the crop. In case of severe infection of Alternaria blight, severe yield losses occur. In this content present investigation was undertaken on “Studies on Alternaria blight of sesame”. The objective of this study was to understand the symptomatology, survival of pathogen on seed, role of media and pH on the growth and sporulation of *A. sesami* and to study the different approaches for the management of *A. sesami in vitro* and to find out the source of resistance in sesame against the pathogen.

The disease symptoms were mainly manifested on the leaf blade as brown, round to irregular spots varying from 1 mm to 2 cm in diameter. Initially water soaked appeared which later turned greyish to dark brown with concentric zonations. In severe infections several spots coalesced and defoliation occurs. The dark brown water soaked lesions were also seen on the stem, midrib and on the veins of the capsules. Small brown spots appeared on the capsules leading to formation of shrivelled and deformed seeds which reduced the quantity of yield as well as its quality. The lesions enlarged and black mass of conidia is visible on the brown, necrotic tissue surface. On stem, infection of the fungus resulted in lesions of various sized at different heights. The margins of the lesion were dark brown surrounding lighter brown or grey area. On pods symptoms appeared as brown to dark

brown irregular spots and velvet mass of conidia were observed on the pods of sesame plant. Similar symptoms of *A. sesami* have been reported by many workers (Malaguti et al. 1972; Davide 1975; Sahani and Mahapatra 2014).

On the basis of studies of morphological characteristics during present investigation, the following characters are in complete conformity with finding of Neergaard (1945).

1. Conidia formed singly
2. The spores have long filamentous beak
3. Spores colour yellowish brown to dark brown
4. Presence of polymorphic spores

The conidia are straight or slightly curved, obclavate, yellowish brown to dark brown in colour and measure $32-115 \times 18-32 \mu\text{m}$. The conidia have 4-12 transverse septa and 1-4 longitudinal septa. The conidia have long beak which may be simple or branched and from 25 to $210 \times 2-4 \mu\text{m}$ in size. The morphological description of the fungus agrees with the description given by Kvashnina (1928), Kawamura (1931), Mohanty and Behera (1958), Leppik and Sowell (1964), Ellis (1970), Rajpurohit (1981), Savitha et al. (2013) for *Alternaria sesami*. Neergaard (1945) classified the genus *Alternaria* in to three groups namely-

1. Longicatenatae (long chain forming)
2. Brevicatenatae (short chain forming)
3. Non catenatae (Non chain forming)

On the basis of spore morphology the *Alternaria sesami* falls under the section non catenatae. Since the pathogen produces conidia singly with a long filiform beak. Very rarely a chain of two spores may be observed. The above morphological characters and pathogenic behaviour on sesame plants, the fungus was to be recognised as *Alternaria sesami* (Kaw.) Mohanty and Behera.

Under artificial inoculation conditions visible symptoms were observed after 5 days of inoculation. Although the symptoms were similar to the observation made in fields however, the size of the spots was smaller under

artificial inoculation. The re-isolation from infected plant parts yielded similar fungus, hence proved pathogenicity of *Alternaria sesami*. Similarly pathogenicity test of *Altrenaria sesami* was confirmed by (Mc Donald 1964 and Yu et al. 1982).

In the present investigation it was found that *A. sesami* was associated with all the seed samples collected from different places. However, per cent seed infection varied from 2 to 19. Esewi (1984) also found that seed infection of *A. sesami* was 16.5 to 63.5% in Aydm, Turkey. Similarly Kushi (1977) noticed that *Alternaria sesami* was associated with 10 seed samples, out of 26 samples of sesame seeds. Blotter method was found to be more efficient than agar plate method and ragdoll method. The results were in accordance with the findings of Kushi and Khare (1979) who found that standard blotter method was most effective.

Survival of *A. sesami* was recorded from November (after harvest) to the end of June (sowing time) i.e. up to 7.5 months in sesame seeds and plant debris under refrigerator (low temperature) and laboratory conditions (room temperature). In another studies on *A. brassicae* causing blight of in mustard, it has been reported to survive for 10 to 11 months in plant debris buried in field conditions (Tripathi and Kaushik 1984). However Shrestha et al. (2003) have reported that the pathogen survived in the seed lots at least one year at 5 and 10°C and act as source of primary inoculum. *A. alternata* causing blight of broad bean remained viable in diseased plant debris from 11 to 13 months in laboratory and from 10 to 12 months in soil, respectively. It remained viable from infected seeds upto 15 months in laboratory (Dubey and Patel 2000). Similarly *A. sesami* casing blight of sesame could be survived in seed and crop debris under field conditions upto 11 months (Naik et al. 2007).

Every living being requires a food for its growth and reproduction and fungi are not an exception to it. Fungi secure the food and energy from the substrate upon which they live in the nature. In order to culture the fungi in the laboratory it is necessary to furnish those essentials elements and compounds in the medium which are required for the growth and other life process. Media studies have shown the ability of the *Alternaria sesami* to grow and sporulate on a variety of media which suggests the capability of the

pathogen to utilize different forms of nutrients for its growth and sporulation. These studies have further indicated that PDA and Oat meal agar gave good growth and better sporulation than synthetic ones. This could be attributed to the complex nature of the natural media which contained various sugars, amino acids, growth factors etc. in comparison to synthetic media which had only one source of carbon and nitrogen each. Berry (1960) and Siddaramaiah (1981) also reported that the fungus grows nicely on PDA medium. He further observed that isolates of *Alternaria sesami* were found to differ markedly in cultural characteristics and virulence. The isolates differ in appearance (colony character) and growth rate too, good growth of *Alternaria ricini*, *A. cymopisdis* and six different species of *Alternaria* on PDA and Oat meal agar (Pawar and Patel 1957, Rangaswami and Rao 1957 and Rangaswami and Sambandan 1960 respectively). Hubballi et al. 2010 also reported that PDA medium was good for the growth of *Alternaria alternata*.

Hydrogen ion concentration governs the metabolic activities of growing organisms both in nature and artificial cultures. In this case the pH of the soil and of the host plays an important part because then reaction of the substrate is largely responsible for influencing growth and sporulation of the pathogen. Investigations carried out by the author have revealed wide adaptability of the fungus because it was found to be capable of growing on a wide range of pH (4.5 – 7.5). However maximum growth observed at 6.5. Although good growth was observed on a pH range between 6.0 to 6.5. Abundant sporulation was observed on a narrow range of pH between 6.0 to 6.5. This indicated that the fungus resumes good mycelial growth and sporulation at acidic pH range of 6.0 to 6.5. Saeed et al. (1995) reported that pH 6 to 6.5 is suitable for the growth and sporulation of *A. alternata*. Similar findings were also reported by Jash et al. (2003) that pH 6 to 6.5 is the suitable for the growth and sporulation of *A. zinniae pape* causing leaf and flower blight of marigold. Singh et al. (2009) found that maximum radial growth of *Alternaria alternata* was observed at the pH 7.0. Hassain and Khutan (1997) reported that maximum growth of *A. porri* was observed at the pH 6.0 to 7.0. However, Samuel and Govindaswamy (1972) reported that pH 5 was best for mycelial growth and pH 7 for sporulation of *A. sesami*.

Preparations derived from higher plants are known to possess disease controlling properties. Frequently, it is reported that many plants possess antifungal properties against *Venturia inequalis* (Gulliver 1947), *Phytophthora nicotianae* fsp *parasitica* (Yadav et al. 1998). Hence there is urgent need to develop alternate method of managing the plant diseases using plant based derivatives which are eco-friendly, cost-effective, bio-degradable and easily accessible along with many additional advantages to suit the organic based agriculture. The antifungal potential of plant extracts results from the presence of secondary metabolites in leaf extracts. In the present investigation, efficacy of seven plant extracts was evaluated against *A. sesami* at three concentrations 10, 20 and 30 per cent. Among the seven plant extracts maximum per cent growth inhibition was recorded on the leaf extracts amended medium of the Babool followed by Neem and Gajar grass. The maximum inhibition of radial growth was observed at 30 per cent concentration. In the leaf extract of Gajar grass, alkaloid, flavonoid, tannin, glycoside, saponin, steroid, terpenoid, phenol and anthraquinone are major secondary metabolites which probably resulted in inhibition of mycelial growth as well as inhibition of spore germination. Among all the secondary metabolites major bioactive ingredients present in the leaves of the Gajar grass are as camphor, parthenin, parthenolide and alpha-pinene. Similar ingredients were extracted by Singh and Shrivastava (2013); Roy and Shaik (2013). In neem leaf extract, presence of some natural products called triterpenoids (Limonoids) are the major bioactive ingredients in the form of nimbidin, gedunin, cyclic trisulphide, cyclic tetrasulphide which shows antifungal properties have already been reported by Kumar et al. (2010). The inhibitory effect of Neem and Prosopis on mycelial growth of *A. tenuissima* was reported by Raja (2010). Poor sporulation was observed in Neem, Khejri, Babool, Thuja and Amarbel leaf extracts amended media at 30 per cent concentration. Whereas maximum inhibition of spore germination was observed in Gajar grass leaf extracts followed by Thuja and Babool leaf extracts. Similarly Patni et al. (2005) recorded effect of leaf extract of *Parthenium hysterophorus* on sporulation and spore germination of *Alternaria brassicae*. Antifungal activities of plant extracts on *Alternaria* sp. were also

reported by Seetharaman et al.2001; Raghvendra et al. 2009;Sharma and Pandey 2011; Meena 2012.

Use of fungicides is an alternative method of controlling the diseases of crop in the absence of resistant cultivars. Hence, fungicides would continue to be one of the major tool of integrated disease management (IDM). Evaluation of different fungicides *in vitro* is a handy tool to screen a large number and thus can serve as guide for field testing. The data presented (in Table 9A) shows that all the fungicides significantly inhibited the growth of fungus at all the concentrations. Radial growth ranged from 0.0 to 51.7 mm in treatments as compared to control (90.00mm). Among all the fungicides evaluated Propiconazole and Difenoconazole were found to be most effective followed by Hexaconazole and Tebuconazole in inhibition of mean radial growth of *A. sesami* at 1000 ppm. However mycelial growth decreased as the concentration increased. Similarly same results were also reported by Sharma and Pandey (2011) on *Alternaria burnsii* and Singh et al. (2006) on *Alternaria alternata*.

All the fungicides significantly inhibited the sporulation as well as spore germination. Among all the fungicides evaluated Hexaconazole, Propiconazole, Difenoconazole and Mancozeb completely inhibited the sporulation. Among all the fungicides Propiconazole (0.0%) and Difenoconazole (0.0%) were found most effective followed by Hexaconazole (4.6%) and Mancozeb (5.0%) spore germination at 1000 ppm concentration. Similar results were also reported by Kumar et al.2005 on *A. alternata* and Taware et al. (2014) on *Alternaria carthami*.

Use of resistant varieties is an easy, efficient and economical method of disease control. The resistant varieties not only ensure protection against diseases, but also save money spent on other measures of control. During present investigation 200 germplasm including susceptible check (Phule Til-1) were screened. No germplasm was found to be immune (0 PDI) against *A. sesami*. However, 4 germplasm exhibited resistant reaction (1-10 PDI), whereas, 13 showed moderately resistant reaction (11-25 PDI). 166 germplasm exhibited moderately susceptible reaction (26-50 PDI) and 16 exhibited susceptible reaction (51-70 PDI) against *A. sesami*. Phule Til-1

(susceptible check) recorded 5 grade which showed highly susceptible (71-100 PDI). Earlier worker also screened sesame germplasm / varieties against *Alternaria sesami* viz. Barboza et al. (1966), Jayaramah and Siddaramaiah (1981) and Sharmarao et al. (2003). Nema et al. (2009) screened 68 varieties of sesame and found that 13 varieties viz. N-8, JLT-7, JLT-26, Purva-1, Co-1, Paiyur-1, Thilthara, Thilarani, B-27, Rajeshwari, Krishna and Thilothama exhibited resistance reaction.

SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

Sesame plays an important role in the oilseed economy of semi-arid tropics of the world. India contributes the highest acreage. The low productivity of sesame in India is attributed to poor crop management with reference to number of biotic and abiotic stresses. The crop is mainly grown under rain fed and input starved, marginal and sub-marginal conditions without protection measures. Out of several biotic stresses, Alternaria blight caused by *Alternaria sesami* affects severely at all the growth stages of the crop growth during *Kharif*; nevertheless the plants were observed to be most susceptible at 8-10 week age. Looking to the seriousness of the disease investigations on certain aspects of the disease were carried out. These were to examine the symptoms produced by the pathogen on different plant parts, to isolate, identify and test the pathogenicity of the fungus from the disease samples, to study survival of the pathogen, to work out role of physical parameter on mycelial growth and sporulation of *A. sesami*, to study the different approaches for the management of *A. sesami in vitro* and to find out the source of resistance in sesame against the pathogen and results are summarised here under;

The disease manifests mainly on the leaf blade as brown, round to irregular spots varying from 1 mm to 2 cm in diameter. In severe infections several spots coalesce together involving a major portion of the leaf blade and affected leaves dry and usually drop off. The fungus was isolated from the infected leaves and pathogenicity was proved by Koch's postulates.

The fungus characterised by simple, erect, more or less flexuos, yellowish brown conidiophores, arising singly or in small fascicles, simple or branched, smooth-walled, pale to light brown in colour, 0-3 septate with a single, small and swollen, terminal, pigmented conidiogenous site at the apex, up to 92 µm long and 2 - 6 µm wide and each bearing conidia singly. Conidia were straight or slightly curved, obclavate, yellowish brown to dark brown in

colour and measure $32-115 \times 18-32 \mu\text{m}$ with a long hyaline beak measuring $25-210 \times 2-4 \mu\text{m}$.

A. sesami was detected in all the seed samples with range from 2 to 19 per cent seed infection.

Alternaria sesami survived more than 7.5 months under both refrigerator and laboratory conditions.

Maximum growth and sporulation of *A. sesami* was found on Potato dextrose agar and Oat meal agar media. Whereas, the fungi sporulated poorly on Czepeck's-Dox agar medium. Among the seven pH tested, maximum radial growth and sporulation was recorded at pH 6.5.

The leaf extract of Khejri, Neem, Thuja, Babool, Gajar grass, Amarbel and Ashoka were evaluated at 10, 20 and 30% concentrations for their antifungal efficacy against *A. sesami* using poisoned food technique. Leaf extracts amended media of Babool, Neem and Gajar grass were found most effective in inhibition of mycelial growth. Leaf extracts of Gajar grass and Babool were found to be most effective in inhibition of sporulation and spore germination.

Seven fungicides were evaluated against *A. sesami* by "poisoned food technique" at three concentrations. Propiconazole and Difenoconazole, Hexaconazole and Tebuconazole were most effective in inhibition of the mycelial growth. Propiconazole and Difenoconazole were found to be most effective for inhibition of sporulation and spore germination.

Conclusions:

The study was successfully carried out in meeting the objectives

1. *Alternaria sesami* is a seed borne pathogen. So before sowing seed must be treated with protective fungicides and diseased free seed should be used for sowing purpose in order to reduce primary inoculum of the disease.
2. *Alternaria sesami* survived more than 7.5 months under refrigerator and laboratory conditions.

3. Potato dextrose agar and Oat meal agar media should be preferred for isolation and further mycelial studies.
4. The pathogen *A. sesami* preferred acidic conditions for mycelial growth and sporulation.
5. Inhibition of mycelial growth, sporulation and spore germination percentage of *A. sesami* was the minimum in Babool leaf extract followed by Neem leaf extract.
6. Propiconazole and Difenoconazole were most effective for inhibition of mycelial growth, sporulation and spore germination.

Suggestions for further work:

Much useful information has been generated on various aspects of *Alternaria* blight of sesame to minimize yield losses. This investigation has also thrown some light on morphological and cultural characters of *Alternaria sesami*. Yet some more work on *A. sesami* should be conducted keeping in view the following aspects:

1. Collection of number of isolates from different localities and study of morphological, cultural, pathogenic and molecular variability will provide information for identifying the existence of races if any.
2. Evaluation of more number of plant extracts, botanicals and bio-agents will provide an opportunity to find out alternate of chemicals.
3. Isolation, identification and commercialization of bioactive ingredient having antifungal properties, present in plant extracts should be carried out for eco-friendly management of diseases.
4. Screening of more number of sesame genotypes by artificial inoculation as well as under controlled conditions will provide an opportunity for identifying good resistance genotype since presently available varieties do not show complete resistance.
5. Epidemiological studies will also provide opportunities to overcome the disease.

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