Leaf spot of safflower caused by *Alternaria carthami* Chowdhury is common in all the safflower growing regions of the world. The disease was reported from India by Chowdhury (1944) and subsequently from erstwhile USSR (Nelen and Vasileva, 1960), United States (United States Department of Agriculture, 1961), Ethiopia and Kenya (Ellis and Holliday, 1970), Africa (Weiss, 1971), Australia (Irwin, 1975), Pakistan (Stovold, 1979) and Italy (Zazzerini and Buonaurio, 1981). Zimmer et al., (1963) assessed that more than 15 per cent yield loss of safflower is caused by *Alternaria* spp. in the USA. In India, it is the major destructive disease of safflower and estimated to be causing 25-60 per cent yield loss every year (Krishna Prasad and Basuchaudhury, 1988). Preliminary surveys on the intensity of *Alternaria* leaf blight in northern India revealed 27-90 per cent yield loss when the disease appeared at early stages of crop growth (Krishna Prasad and Basuchaudhury, 1991). Siddaramaiah et al., (1963) and Mahabaleswarappa (1981) reported the severity of leaf blight of safflower in the state of Karnataka. Severe leaf blight leading to blighting of leaves was documented by Mohanty et al., (1981) in Orissa. Patil and Jadav (1985) and Indi et al., (1988) reported the economic losses caused due to *Alternaria carthami* in Maharashtra region. Singh et al., (1991) and Awadhiya (1992) noticed the occurrence of *Alternaria* leaf blight leading to considerable economic losses in Madhya Pradesh.

**Seed Mycoflora of Safflower**

Padaganur and Anil kumar (1976) studied seed mycoflora of safflower and observed *Curvularia* sp, *Alternaria* sp, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium* sp on different seed lots of two varieties. Rajagopalan and Shanmugam (1983) isolated *Alternaria carthami* from surface sterilized safflower seed and observed that the pathogen is externally seed borne and seldom carried internally. In addition to *Alternaria alternata*, *Alternaria carthami* was also carried as dormant mycelium in the pericarp of safflower (Zazzerini et al. 1985). Prasad (1985) tested 35 varieties of safflower by standard blotter method for the detection of *Alternaria carthami*. The fungus was found to be associated with 27 cultivars to the extent of 4-42 per cent resulting in pre and post emergence seedling mortality. Raghuwanshi et al., (2002) studied seed mycoflora of safflower cultivars and found that the seed germination and vigour was adversely affected by *Alternaria, Fusarium, Aspergillus* sp. in cultivars viz., A-1, Manjira, APRR-3, CO-1, Bhima, HUS-305, A-300, S-144, K-1, JSF-1, NRS-209 and Gima. Singh et al. (1987) studied seed mycoflora associated with 13 varieties of safflower and recorded eleven fungal species associated with seeds. Among the species detected, the occurrence of *Alternaria* spp and *Rhizoctonia* spp was found to be high with 40 and 30 per cent respectively. Borkar and Shinde (1989) reported that externally seed borne nature of *Alternaria carthami* in safflower not only reduced the seed quality by causing seed rot but also seedling decay, pre and post emergence mortality of seedlings. The results revealed that externally seed borne infection of *Alternaria carthami* was about 48 to 100 % in safflower seeds. Awadhiya (1991) studied seed mycoflora associated with fifty varieties of safflower and recorded the occurrence of *Alternaria, Fusarium, and Macrophomina* sp. and the results indicated that the occurrence of *Alternaria carthami* was found to be predominant (100%). Prasad et al., (2008) studied seed borne nature of *Alternaria carthami* in safflower by using component plating technique. Maximum infection of *Alternaria carthami* was occurred on seed coat (76.6%) followed by endosperm (38.3%) and embryo (20.4%). Pushpavathi et al., (2012) analyzed the seed mycoflora of 12 safflower cultivars by using standard blotter, agar plate and seed washing methods showed the association of 10 fungal species among them *Alternaria carthami* (2-54%) was detected by all the three methods. Rajeswari et al., (2012) studied seed mycoflora associated with safflower seed samples. They reported the occurrence of *Alternaria carthami, Alternaria alternata, Macrophomina phaseolina, Fusarium oxysporum, Aspergillus flavus, Aspergillus niger, Curvularia lunata* and *Rhizopus* sp.
Detection Methods

Several methods have been developed to detect the seed borne microflora and these have been reviewed by Neergaard (1977). The emphasis has been given to those methods, which are simple, easy, economic, sensitive, reproducible and efficient. Valand et al. (1983) used modified blotter i.e., the blotter paper was treated with Sodium hypochlorite (0.02%) solution to minimize saprophytic fungi like Rhizopus in the detection of seed borne fungi of some sesame varieties. Raut (1985) reported that pre treatment of sunflower seeds with 2% sodium hypochlorite solution for 5 min reduced the counts of Alternaria helianthi by more than 30%. Paul (1989) analyzed soybean seeds by standard blotter method, agar plate method and deep freeze method to detect internal and external seed borne mycoflora. A total of 26 fungal species were found associated with soybean seeds. Dawar and Ghaflar (1990) reported that blotter method was more effective in detection of seed borne fungi of sunflower than by agar plate method. Durga Prasad and Kulshrestha (1996) used modified blotter method i.e., deep freeze method for detection of Alternaria helianthi in sunflower seed. Lalit Mahatma et al., (2001) reported that agar plate method was found slightly superior over modified standard blotter paper method for isolation of seed mycoflora of Sesame. Ramesh and Avitha (2005) reported that by blotter technique more fungi were isolated as compared to agar plate method. But in contrary some workers observed that both the methods were equally valuable and supplementary to each other (Kumhar et al., 2005). Nagaraja (2009) reported standard blotter method was found to be superior over potato dextrose agar method, water agar method and 2,4-D method for detection of seed mycoflora associated with castor. Nagaraja and Krishnapppa (2011) reported that standard blotter method was found to be superior over potato dextrose agar method, water agar method and 2,4-D method for detection of Alternaria carthami in safflower seeds. Pushpavathi et al., (2012) reported that standard blotter method was best for the detection of seed mycoflora associated with safflower seed followed by agar plate method.

MORPHOLOGICAL CHARACTERISTICS OF THE TEST PATHOGEN (Alternaria carthami)

Vegetative hyphae of Alternaria carthami were septate, inter and intra cellular, when young sub- hyaline, narrow and sparsely septate but when mature the border is dark coloured and more frequently septate. Conidiophores, stout, erect, rigid, unbranched, septate and slightly constricted at the septa, arising singly or in clusters. Conidia light brown and translucent, muriform, formed at the tips of the conidiophores singly or in chains, 3 to 11 celled, longitudinal septa few, usually possessing a long beak (Chowdhury, 1944).

Symptomatology

Chowdhury (1944) observed the Symptoms expressed by Alternaria carthami on safflower and reported the appearance of disease before flowering. It manifested on all aerial parts of the plant especially leaves. Initially, minute brown to dark brown spots, 1 to 2 mm in diameter with concentric rings on leaves were produced. The diameter of the spots increased gradually to one centimeter. Later, the spots coalesced and formed large lesions. The centre of the spot was light brown and was surrounded by a number of dark rings alternating with light ones. In the advanced stages shot holes appeared and the leaf blade broke in an irregular manner. Spots on the stem and petioles were elongated. Affected floral buds failed to open, shriveled and dried up. Pre-emergence and post emergence death of seedlings has been reported by Irwin (1976) which was considered as a seed borne infection. Seed discoloration was found in infected seeds. Krishna Prasad and Basuchaudhury (1991) also observed the symptoms produced by Alternaria carthami on safflower and the disease first appeared on leaves as small light brown to dark brown spots, 1-2 mm in diameter, which gradually spread to upper leaves and the diameter of spots increased to 1 cm. a brown dot was found in the centre of the spot. On the stem, symptoms appeared as elongated dark brown to black spots. Later cracks developed on the stem. In floral parts, the symptoms were first observed at the base of the involucral bracteoles which ultimately spread to other parts of the capitulum. The initial symptoms incited by Alternaria carthami appeared from seedling stage and continue till maturity of the crop. Symptoms of Alternaria leaf spot due to Alternaria alternata and Alternaria zinnia was reported.

Pathogenicity Studies

Shrestha et al., (2000) reported seed borne nature of Alternaria brassicae. Presence of Alternaria brassicae in the seeds of rapeseed was confirmed by observing symptoms in seedlings raised from surface sterilized seeds collected from infected plants. Lakshman Prasad et al., (2004) detected seed borne pathogens by using dry seed examination, washing test and incubation methods in cauliflower. He proved pathogenicity of Alternaria brassicae by observing chlorotic symptoms in seedlings raised from seed of infected plants. A pot culture experiment conducted in Maharashatra region by Relekar et al., 2010 confirmed the pathogenicity of Alternaria carthami when the leaves of safflower were inoculated with 12 days old culture of test pathogen by observing the disease symptoms after 4-5 days of inoculation.
Efficacy of Certain Botanicals against Test Pathogen (*Alternaria carthami*)

Biological screening of higher plants has shown that many of these plants contain highly potent inhibitors of plant pathogens. Some of these inhibitors provide complete protection against the diseases and in many cases the antipathogenic activity was obtained with crude extracts. The nature of inhibitors characterized from higher plants was found to be different. Annapurna *et al.* (1989) found that aqueous leaf and fruit extracts of neem were found to be effective in inhibiting the growth of *Alternaria padwickii* in rice seeds. Neem oil was also effective in checking the growth of *Alternaria alternata* causing post harvest rotting of tomato (Ali *et al.*, 1992). Sundriyal (1991) reported that floral extracts of *Lantana camara* inhibited spore germination and germ tube growth of *A. solani* in vitro while Conidial germination was completely inhibited after five hours exposure. Shenoi *et al.* (1998) reported that *Pongamia glabra* extract was effective against *Alternaria alternata* causal agent of tobacco. Spore germination, mycelial growth and sporulation of *A. helianthi* were inhibited by *Pongamia glabra* extracts in vitro (Thiribhuvanamala and Narasimhan. 1998). Extracts of turmeric rhizome was found to be inhibitory to the growth of *Alternaria alternata* (Khzmi *et al.*, 1993). Lal *et al.* (1998) tested various plant extracts against *Alternaria alternata* and observed that extracts of *Achyranthus* sp was found to be most effective in inhibiting the mycelial growth by 61.9 per cent followed by *Azadiracta indica*. Chattopadhyay (2001) evaluated six plant extracts against *Alternaria carthami* causal agent of leaf spot and blight of safflower. Bulb extract of *Allium sativum* was the best among the tested botanicals by causing 79.6% reduction in mycelia growth of *Alternaria carthami* followed by *A. indica* (75.3%). Harichand and Surrender Singh (2004) reported that bulb extract of *Allium sativum* was effective against *A. brassicaceae* while *Calotropis procera* was less effective. Bulb extract of *Allium sativum* recorded the highest inhibition of *Alternaria alternata*. (Chaudary *et al.*, 2003). Patni *et al.*, (2005) evaluated effect of leaf extracts of six medicinal plants against *Alternaria brassicaceae* causal agent of leaf blight of mustard and found that Eucalyptus followed by Calotropis extracts were promising in inhibiting the growth and sporulation of the test pathogen. Prasanna kumar *et al.* (2006) tested various plant extracts against *Alternaria alternata* *Ocimum* leaf extract was found to be the best in inhibiting the mycelial growth of *Alternaria alternata* to an extent of 77.62 per cent followed by neem leaf extract (45.30). neem seed kernel extract (5%), *Ocimum* leaf extract (5%), and *Tridex* leaf extract (5%) were least effective in controlling *Alternaria alternata*. (Chaudary *et al.*, 2003). Amareth (2000) reported that, among plant extracts tested neem leaf extract (5%), *Ocimum canum* leaf extract (5%) and *Bougainvilea* sp. leaf extracts were found to be effective in controlling *Alternaria* blight. Sangeth kumar *et al.*, (2005) observed that *Azadirachta indica* recorded the highest inhibition of growth of *Alternaria alternata* causal organism of *Vicia faba*. Narendra Singh and Verma (2010) reported the effectiveness of garlic clove extract in checking growth and conidial germination of *Alternaria alternata*. Raja (2000) reported a high reduction in mycelial growth of *Alternaria solani* with extract of garlic followed by neem and prosopis. Mesta *et al.*, (2005) reported the efficacy of certain plant extracts and results indicated that neem leaf extract was found to be effective in controlling *Alternaria helianthi* with 38.49 per cent inhibition of spore germination and 43.90 per cent inhibition of mycelial growth. Ramegowda *et al.* (2007) evaluated seven botanicals on *Alternaria macrospora*, causing leaf spot of Bt cotton. Among botanicals, garlic and onion bulb extracts at 7.5, 5.0, 2.5 per cent concentrations were effective in inhibiting the mycelial growth of *Alternaria macrospora*. Alpa *et al.*, (2010) revealed that neem extract showed 93.7% inhibition of the seed mycoflora thereby enhancing the seed germination as compared to Ricinus plant extract (87.5%) and *T. viride* (62.5%). Jyothi singh and Kerkhi (2010) noted that the efficacy of *Trichoderma harzianum* (36.0%), *T. viride* (25%), neem leaf extract (21.8%) and linseed leaf extract (14.2%) in reducing disease intensity of *Alternaria* blight of linseed. Jyothi singh and Kerkhi (2010) noted that the efficacy of *Trichoderma harzianum* (36.0%), *T. viride* (25%), neem leaf extract (21.8%) and linseed leaf extract (14.2%) in reducing disease intensity of *Alternaria* blight of linseed. Asit Dubey *et al.*, (2010) revealed that Neem leaf was most effective in controlling the *Alternaria* blight (66.8%) followed by extracts of garlic and onion in Malabar nut. Abhijit *et al.*, (2010) reported antifungal efficacy of some plant extracts for inhibition of *Alternaria carthami* following poisoned food technique and results showed that garlic bulb extract was more effective in inhibiting the test pathogen by 56.4%. Usha *et al.*, (2012) evaluated various plant extracts against *Alternaria carthami* *Calotropis* leaf extract was found to be the best in inhibiting the mycelial growth of *Alternaria carthami* to an extent of 28.6 per cent followed by *Carthamus* and *Prosopis* (25.70). Neem oil was less effective against *Alternaria carthami* as compared to bioagents and fungicide seed treatments (Rajeswari *et al.*, 2012).

**Efficacy of Certain Bioagents against Test Pathogen (*Alternaria carthami*)**

The uses and expectations of biological seed treatments are greater today due to the impact of environmental regulations that have either banned or restricted the use of older seed dressing fungicides such as organomercural compounds. Biological seed treatments provide economical and relatively nonpolluting delivery systems for protective materials compared to other field application systems.
Bioproteoctants applied to seeds may not only protects seeds but also may colonize and protect roots and increase the plant growth. (Taylor and Harman, 1990). Palazon et al., (1988) reported that Trichoderma viride was strongly antagonistic to fruit rot pathogens Alternaria tenuis and Botrytis cinerea in vitro. Similarly Sesan (1990) showed that Trichoderma viride a strong antagonist against Sclerotinia sclerotiorum and Alternaria radicina on stored carrot in vitro. Leifert et al., (1992) reported that Serratia and Pseudomonas showed in vitro antagonism against Botrytis cinerea and Alternaria brassicola, they were also tested in vivo and Pseudomonas isolated CL42, 66, 82 provided the best control. Seed treatment or spraying with spore suspensions of Trichoderma viride on growing plants controlled Alternaria liniola on linseed (Mercer et al.,1993). Deshmukh et al., (1994) showed that Trichoderma viride seed treatments were not as effective as the fungicides treatments and reduced seed borne fungi of jowar by only 25-30%. Sawant et al., (1999) reported that of all the Trichoderma viride treatments, seed treatment combined with soil applications was most effective and recorded the lowest incidence of early blight of tomato caused by Alternaria solani seed treatment with Trichoderma viride completely eliminated the seed borne pathogens of Red gram. Babu et al. (2000) tested fungal antagonists in vitro against growth of A.solani and in tomato plants against the leaf blight disease. The results showed that T. harzianum followed by T. viride were significantly effective in inhibiting mycelia growth of A.solani. Seed treatment with Trichoderma viride eliminated seed borne infection of pigeon pea by A.alternata (Fr.) Keissler, Rhizoctonia bataticola (Taub.)Butler. Rhizoctonia solani Khun and Curvularia lunata (Wakker) Boed with significant increase in seed germination, vigor index and fresh weight of seedling over untreated control (Pradeepkumar et al.,2000). Seed treatment with Trichoderma viride eliminated seed borne infection of soyabeen by Alternaria, Aspergillus, Curvularia, Rhizoctonia, Fusarium and Rhizopus with significant increase in seed vigor index, shoot-root length of seedling over untreated control.(Mina D. Koche, 2009). Gaikwad and Behere (2001) evaluated seed treatment with Trichoderma harzianum and Aspergillus fumigatus as biocontrol agents @ 8.72x10^7 spores per ml and 9.26x10^7 spore per ml against Fusarium oxysporum f.sp. carthami which reduced the disease incidence to 100 per cent on susceptible safflower cultivar (Cv. Tara) under glass house conditions. Prasad and kulshreshtha (2002) reported that seed treatment with Pseudomonas fluorescens at 8.7 to 9.4 X 10^11 cfu/ml isolated IV gave the greatest seedling emergence 92-100% and lowest incidence of Alternaria blight infested seedlings in sunflowers caused by Alternaria helianthi. Singh et al., (2003) evaluated the fungicides and biocontrol agents against seed mycoflora of pearlmillet includes Alternaria alternata, Aspergillus flavus, Trichoderma harzianum and Pseudomonas fluorescens were proved to be effective. Prasad (2003) studied the efficacy of Trichoderma spp. against Fusarium oxysporum f.sp. carthami , the incitant of safflower wilt under glass house conditions. T. viride as soil application recorded less disease (26.6) as compared to seed treatment (46.6%). Fungicide carbendazim treatment recorded 80.0 per cent disease incidence as compared to pathogen check 93.3 per cent. Raju et al. (2003) studied the effect of biocontrol agents against Fusarium oxysporum f.sp. carthami causing wilt of safflower and reported that seed treatment with Thiram + T. harzianum + T. viride completely inhibited the disease which is on par with seed treatment with T. viride, T. harzianum and carbendazim. T. harzianum when sprayed on plants against Alternaria blight of mustard, the disease severity was found to be only 33.59% Patni et al., (2005). Thorat et al., (2005) studied the inhibitory effect of Trichoderma spp. against Alternaria solani and reported that T. harzianum was more effective recording 60% inhibition followed by T. viride. Ghosh et al., (2002) reported that T. viride and T. harzianum effectively inhibited the growth of A. alternata on gerbera. Ramegowda et al. (2007) evaluated T. viride and T. harzianum indigenous and exogenous strains in vitro. Maximum inhibition (62.3%) was noticed in Trichoderma viride (E) followed by Trichoderma harzianum (I). Vannacci (1991) reported that among all antagonists Trichoderma harzianum gave the best control against seed borne Alternaria raphani in Radish. The seed treatment of Pseudomonas fluorescens + Trichoderma viride was effective in reducing seed mycoflora i.e Fusarium oxysporum, Fusarium moniliforme, Aspergillus flavus, Aspergillus niger and Alternaria macrospora by 87, 100, 79, 76 and 100 per cent, respectively over untreated control. (Gawade et al., (2009). Rajeswari et al., (2012) studied seed mycoflora associated with safflower seed samples and efficacy of seed treatments with bioagents and fungicides against seed mycoflora of safflower were evaluated. Results indicated that seed treatments with bioagents (6 g/kg), fungicides and botanicals (10 ml/kg) enhanced seedling quality and were found to be effective in reduction of total seed mycoflora and seedling mortality.

**Efficacy of Certain Fungicides against test pathogen Alternaria carthami**

Basavaraajah et al., (1979) evaluated in vitro efficacy of eight fungicides against Alternaria carthami and complete inhibition of fungal growth was observed in case of tetra-methyl thionur disulfide 500 µg/ml and triphenyl tris hydroxide 500 µg/ml. Tetra-methyl thiuron disulfide and captof were highly effective against Alternaria carthami at higher concentrations of 2000 µg/ml (Siddaramaiah et al., 1979). Siddaramaiah et al. (1980) tested nine fungicides for eradication of Alternaria carthami from heavily infected safflower seeds, the fungus was completely eradicated in Capton and RH 2161 treated plates (Zero per cent).
Ayyavoo and Shanmugam (1982) evaluated five fungicides against *Alternaria carthami* and it was observed that the treatment Dithane Z-78 (0.1%) was able to reduce the incidence of the disease (16%) significantly over others. Carbendazim and *Thiophanate methyl* failed to check the growth of *Alternaria carthami* in *in vitro* even at higher concentrations but showed good performance under field conditions suggested that the two fungi act against the pathogen by increasing the phenolic compounds which are fungitoxic. (Deshmukh and Karve, 1983). Quadric and deshpande (1985) studied the influence of five different fungicides on *Alternaria* blight of safflower and found that carbendazim has lowest disease intensity ratings. Pieta and Pastucha (1993) found that thiram and carbendazim gave best control of *Phoma exigua* and *Alternaria alternata* which were predominant seed borne pathogen of soyabean. Dipping the chilli fruits in carbendazim solution (10000µg/ml) for 10 min effectively controlled the seed borne pathogens including *Alternaria alternata* (Datar, 1996). Shivankar et al. (2000) reported that the carbendazim treatment at 0.1% recorded the highest germination shoot length (9.37) in the *Alternaria alternata* infected wheat seeds. Datar (1996) studied the effect of fungicide against onion purple blotch caused by *Alternaria porri* and reported that all the fungicides including carbendazim proved effective. Srinivas et al. (1997) reported that carbendazim was the most effective control against blight of sunflower caused by *Alternaria alternata*. Krishna et al., (1998) evaluated six fungicides at seven different concentrations in *in vitro* for their efficacy against *Alternaria carthami* using poison food technique. The results indicated that the most and least effective fungicides found effective were aureofungin and carbendazim respectively. Murumkar et al. (2008) evaluated certain newer fungicides against *Alternaria carthami* causes leaf spot of safflower and the results indicated that spraying with carbendazim 50 WP (0.1%) immediately after disease appearance followed by need based sprays it was found to be effective in the management of *Alternaria carthami*.

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