

## SEED MYCOFLORA OF SAFFLOWER AND ITS CONTROL BY USING BOTANICALS, BIOAGENTS AND FUNGICIDES-A REVIEW

\*D. Amrutha Gayathri and \*V. Madhuri

\*Department of Plant Pathology, Acharya N.G.Ranga Agricultural University, Hyderabad A.P. India.  
E mail: chinna.greens@gmail.com

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 (Zizzerini and Buonauro, 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 (Zizzerini *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. Raghuvanshi *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 *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 Ghaffar (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 Krishnappa (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 involucre 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 Maharashtra region by Relekar *et al.*, 2010 conformed 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 was 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). Sundrial (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. Shenoj *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.brassiccae* 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 brassicae* 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 *Tridax* leaf extract (5%) were least effective in controlling *Alternaria* blight in sunflower.( Amaresh *et al.*, 2002). Amaresh (2000) reported that, among plant extracts tested neem leaf extract (5%), *Ocimum canum* leaf extract (5%) and *Bougainvillea* sp. leaf extracts were found to be effective in controlling *Alternaria* blight. Sangeeth 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 extract 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 organomercurial compounds. Biological seed treatments provide economical and relatively nonpolluting delivery systems for protective materials compared to other field application systems.



Bioprotectants applied to seeds may not only protect 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 isolates* CL42, 66, 82 provided the best control. Seed treatment or spraying with spore suspensions of *Trichoderma viride* on growing plants controlled *Alternaria linicola* 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 mycelial 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 soyabean 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 @  $8.72 \times 10^7$  spores per ml and  $9.26 \times 10^7$  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 kulshreshta (2002) reported that seed treatment with *Pseudomonas fluorescens* at  $8.7$  to  $9.4 \times 10^{11}$  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 pearl millet 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). Vamnacci (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***

Basavarajiah *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 thiuron disulfide* 500 µg/ml and *triphenyl tris hydroxide* 500 µg/ml. Tetra-methyl thiuron disulfide and captaf 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 Captan 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 (1000µg/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*.

## REFERENCES

- Abhijith, R., Vrijendra, S and Nandini, N. (2010). Antifungal efficacy of some plant extracts for inhibition of *Alternaria carthami*. Journal of Natural Products and Resources. 1(3):384-386.
- Ali, T.E.s., Nasir, M.A and Shakir, A.S. (1992). *In vitro* evaluation of certain neem products as mould inhibitors against post-harvest fruit rotting fungi of tomato. Pakistan Journal of Phytopathology. 4 (1-2): 58-61.
- Amaresh, Y.S., Nargud, V.B and Somasekhar, B. (2002). Use of botanicals and fungitoxicants against *Alternaria helianthi* the causal agent of sunflower leaf blight. Indian Journal of Plant Protection. 30 (1): 55-58.
- Amaresh, Y.S. (2000). Epidemiology and management of *Alternaria* leaf blight and rust of Sunflower. Ph.D. Thesis, University of Agricultural Sciences, Dharwad, p.321.
- Annapurna, J., Iyengar, D.S., Rao, S.N and Bale Rao, V.T. (1989). Antimicrobial activity of leaf extracts of *Anona squamosa*. Pesticides. 23 :43.
- Awadhiya, G.K. (1992). Seed borne pathogenic mycoflora of safflower. Crop Research 5(2): 344-347.
- Ayyavoo, R and Shanmugam, N. (1982). Control of *Alternaria* leaf spot of safflower. Pesticides
- Borkar, S.G and Shinde, D.(1989). Detection of externally seed borne *Alternaria carthami* on safflower seeds. Agricultural Science Digest. 9 (3):120-122.
- Chowdhury, S. (1944). An *Alternaria* disease of safflower. Journal of Botanical Science. 23: 59.
- Chattopadhyay, C . (2001). Yield loss attributable to *Alternaria* blight of safflower (*Carthamus tinctorius* L) and some effective control measures. Journal of Mycology and Plant Pathology. 31(3):298-302.
- Centre for Monitoring Indian Economy (CMIE) Pvt. Ltd. Mumbai. April, (2009 -10).
- Centre for Monitoring Indian Economy (CMIE) Pvt. Ltd. Mumbai. April, (2010 -11).
- Cook, R.J and Baker, K.F. (1983). The nature and practice of biological control of plant pathogens. American Phytopathological Society St. Paul Minnesota, Academic Press, pp. 359.
- Gaikwad, S.J and Behere, G.T. (2001). Biocontrol of wilt of safflower caused by *Fusarium oxysporum* f. sp. *Carthami*. Fifth International Safflower Conference, Williston, N.D., U. S. A., July 23-27.
- Gawade, S.B., Padule, D.N., Mandhare, V.K., Game, B.C and Suryawanshi, A.V. (2009). Efficacy of bioagents on seed mycoflora, seed germination, seedling vigour index and field emergence in Deshi cotton (*Gossypium aerborium*). Journal of Plant Disease Sciences. 4(2): 176-179.
- Harichand and Surendra singh. (2004). Effect of plant extracts of *Alternaria* blight of mustard *Alternaria brassicae*. Indian Journal of Plant Protection. 32(2):143-144.
- Irwin, J.A.G. (1976). *Alternaria carthami*, a seed borne pathogen of safflower. Australian Journal of Experimented Agriculture and Animal Husbandry. 16: 921-925.

- Kolase, S.V., Deokar, C.D and Sawant, D.M. (2000). Effect of plant extract on the incidence of safflower wilt. *Sesame and Safflower News Letter*. No-15.
- Kazmi, A.R., Niaz, I and Jilani, G. (1993). Evaluation of some plant extracts for antifungal properties. *Pakistan Journal of Phytopathology*. 5(1-2): 93-97.
- Krishna, K., Akbar, M and Kalpana sastry, R. (1998). *In vitro* evaluation of fungicides against *Alternaria carthami* Choudury incitant of leaf spot of safflower. *Indian Journal of Plant Protection*. 26 (2):181-182.
- Lal, H.C., Upadhyay, J.P and Ojha, K.L. (1998). Management of *Alternaria* leaf blight of pigeon pea through plant extracts and chemicals of plant origin. *Journal of Applied Biology*. 8(1): 80-85.
- Lalit Mahatma, Singh, S.D and Lodha, P.C. (2001). Pathogenic seed mycoflora of Sesame (*Sesamum indicum* L.). *Journal of Mycology and Plant Pathology*. 31(3):377-379.
- Lakshman Prasad and Karuna Vishnunavat. (2004). Detection of *Alternaria brassicae*, *Alternaria brassicola* and their transmission through seed to seedling and to plants in Califlower. *Seed research*. 32 (1): 84-88.
- Mina D. Koche, Kothikar, R.B., Anvikar, D.G and Shilpa, U. (2009). Effect of seed dressing fungicides and bioagent on survival of seed borne fungi and shelf life of soya bean. *Crop Research*. 38 (1,2 ,3):215-218.
- Murumkar, D.R., Indi, D.V., Gud, M.A and Shinde, S.K. (2008). Field evaluation of some newer fungicides against leaf spot of safflower caused by *Alternaria carthami*. Seventh International conference, Wagga Wagga, NSW, Australia, November 3-6.
- Mesta, R.K., Benagi, V.I., Srikanth Kulkarni and Shankergoud, I. (2009). *In vitro* evaluation of fungicides and plant extracts against *Alternaria helianthi* causing blight of sunflower. *Karnataka Journal of Agriculture Science*. 22(1):111-114.
- Nagaraja, O and Krishnappa, M. (2011). Detection of seed borne fungi and safflower (*Carthamus tinctorius* L.) seed quality. *Seed research*. 39 (2): 176-182.
- Nagaraja, O., Krishnappa, M., Sathisha, A.M. (2009). Seed mycoflora associated with castor, *Ricinus communis* L. and their effect on germination. *Journal of Oil Seeds Research*. 26(2):177-180.
- Narendra Singh and Verma, O.P. (2010). Management of *Alternaria* blight by *Alternaria alternata* in *Adusa (Adhatoda vasica)*. *Indian Journal of Agriculture Sciences*. 80(7): 631-635.
- Neergaard, P., (1977). *Seed Pathology*, Vol I and II, MacMillan Press, London, U.K, p.1187.
- Padaganur, G.M and Anil kumar, J.B. (1976). Seed mycoflora of safflower and its control. *Pesticides*. 10 (7):39-41.
- Paul, Y.S. (1989). Seed borne mycoflora of soybean and its control in Himachal Pradesh. *Journal of Mycology and Plant Pathology*. 19(3):253-257.
- Patni, C.S., Kolte, S.J and Awasthi, R.P.(2005). Efficacy of botanicals against *Alternaria* blight (*Alternaria brassicae*) of mustard. *Indian Phytopathology*. 58 (4) : 426-430.
- Petri, G.A. (1974). Fungi associated with seed with seeds of rape, turnip rape, flax and safflower in western Canada. *Canadian Plant Disease Survey*. 54:155-65.
- Prasad, R.D., Navaneetha, T., Chandra Girish, M.S and Manasa, Ch. (2009). Seed borne nature *Alternaria carthami* in safflower. *Journal of Oil Seeds Research*. 26:492-493.
- Prasad, K.V.V. 1985. Seed borne *Alternaria carthami* in safflower and its control. *Indian Phytopathology*. 38:602.
- Prasanna kumar, M.K., Nargund, V.B and Khan, A.N.A. (2006). Laboratory evaluation of fungicides and Botanicals against *Alternaria alternata* causing post harvest disease in mango. *Mysore Journal of Agriculture Science*. 40(1):21-26.
- Pushpavathi, B., Rajender, J and Narayan reddy, P. (2012). Detection of seed mycoflora of safflower. *Journal of Oil Seeds Research*. 29: 364-367.
- Rajagopalan, T and Shanmugam, N. (1983). Studies on the best range and seed borne nature of *Alternaria carthami* incitant of leaf spot of safflower. *Madras Agricultural Journal*. 70 (12):832.
- Raghuwanshi, K.S and Deokar, C.D.(2002). Studies seed borne mycoflora of safflower. *Sesame and safflower news letter*. No-17
- Rajeswari, B., Keshavulu, K and Krishna rao, V. (2012). Management of seed mycoflora of safflower. *Journal of Oil Seeds Research*. 29: 332-335.
- Ramegowda, G., Naik, M.K., Patil, M.B and Arun, S. (2007). Evaluation of fungicides, botanicals and bioagents against leaf spot caused by *Alternaria macrospora* in cotton. *Indian Journal of Plant Protection*. 35 (2) : 316-319.
- Relekar, N.N., Khalikar, P.V and Nikam, P.S. (2010). Survey and surveillance of *Alternaria* blight of safflower caused by *Alternaria carthami* in Marathwada region. *Journal of Plant Disease Sciences*. 5(1): 195-197.

- Sanjeeth kumar, Upadya, J.P and Sanjeev kumar. (2005). Evaluation of plant extracts for control of *Alternaria* leaf spot of *Vicia faba*. *Annals of Plant Protection Science*. 14(1):151-156.
- Siddaramaiah, A.H., Desai, S.A., Bhat, R.P and Hedge, R.K. (1980). Eradication of *Alternaria carthami* chowdhury a seed borne pathogen of safflower. *Pesticides*. 22-23.
- Singh, S.N., Agarwal, S.C and Khare, M.N. (1987). Seed borne pathogenic mycoflora of safflower, their significance and control. *Seed research*. 15:190-191.
- Singh Kiran, Singh, A.K. and Singh, R.P., (2005), Detection of seed mycoflora of chickpea(*Cicer arietinum*). *Ann. Pl. Protec. Sci*. 13 (1): 1-4.
- Shenoi, M.M., Murthi, K.K., Sreenivas, S.S and Wajid, S.M.A. (1998). *In vitro* evaluation of botanicals for mycotoxic properties against *Alternaria alternata* causing brown spot disease of tobacco. 24(2): 77-81.
- Shivpuri, A., Sharma, O.P and Jhamaria, S.L. (1997). Fungitoxic properties of plant extracts against pathogenic fungi. *Journal of Mycology and Plant Pathology*. 27(1):29-31.
- Shreetha, S.K., Mathur, S.B and Munk, L. (2000). *Alternaria brassicae* in seeds of rapeseed and mustard, its location in seeds, transmission from seeds to seedlings and control. *Seed Science and Technology*. 28: 75-84.
- Sundriyal, R.C.(1997). Fungitoxic properties of flower extracts of some wild plants of Garhwal Himalaya. *Advances in Plant Sciences*. 4(2):230-234.
- Thiribhuvanamala, G and Narasimhan, V. (1998). Efficacy of plant extracts on seed borne pathogens of sunflower. *Madras Agricultural Journal*. 85(5-6):227-230.
- Usha, D., Santha Lakshmi Prasad, M., Naresh, N., Sujatha, K and Prasad, R.D. (2012). Evaluation of botanicals against *Alternaria carthami* L. in in vitro conditions. *Journal of Oil Seeds Research*. 29 : 443-446.
- Valand, G.B., Gaikwad, N.Y and Patel, A.J. (1983). Seed borne fungi of some Sesame varieties. *Indian Journal of Mycology and Plant Pathology*. 13(3): 363-365.