



Efficacy of fungicides and plant extracts against *Alternaria alternata* causing leaf blight of chandrasur (*Lepidium sativum*)

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ABSTRACT

Alternaria species are causing diseases over broad range of agro-horticultural important crops with economic impact. The present experiment was conducted on leaf blight disease of chandrasur (*Lepidium sativum*) and the pathogen identified as *Alternaria alternata*. The fungus produces profuse dark brown to blackish mycelium on potato dextrose agar and transverse as well as longitudinal septate conidia. Attempts were also made to investigate the suppressive potential of fungicides and plant extracts. *In vitro* evaluation of fungicides, viz. Mancozeb, Metalaxyl-MZ, Copper oxy-chloride, Copper hydroxyl-chloride, Carbendazim, Azoxystrobin, Tebuconazol, Nativo and leaf extracts of *A. indica*, *P. pinnata* and *M. alliacea* was performed following the “poisoned food technique”. Among the tested fungicides Tebuconazole + Trifloxystrobin and Tebuconazol (0.1%) were found most effective to inhibit the mycelium growth up to 89% and neem leaf extract (5.00%) was quite superior over the other plant extracts. Assessment of infected seed samples registered 17.40% and 42.00% reduction in oil content and seed weight, respectively, under the severe epiphytotics.

Key words: Conidia, Epiphytotics, Mycelium

Lepidium sativum, commonly known as chandrasur or asaliya, is an important glabrous annual herb, belongs to the oil plants family *Brassicaceae*. The crop is believed to be originated in high elevated regions of Ethiopia and Eritrea and grown in tropical and sub-tropical countries of the globe. In India, asaliya crop grown during winter for seed production, particularly in western part of country such as in Rajasthan, Gujarat, Madhya Pradesh, etc. In Ayurveda, chandrasur is an important plant and seeds, leaves and roots are medicinally and economically important. The seeds have been used for treating various human ailments such as diarrhoea, respiratory problem, cough, bleeding piles and to enhance sexual desire (Sarkar *et al.* 2014). Mucilage of seeds allays irritation of mucous membrane of intestine, whereas, seeds paste used in external application for lumber pain and rheumatism (Joshi 2018). Seeds contain about 20–25% oil which possess alpha linolenic acid (32–34.0%) as major fatty acid and significant amount of polyunsaturated fatty acids (46.8%) and monounsaturated fatty acids (37.6%) (Prajapati *et al.* 2014).

Diseases affect the quality as well as quantity of any crop produces, chandrasur is also invaded by several fungal pathogens. *Hyaloperonospora parasitica* causes downy mildew (Mandal *et al.* 2008) and *Alternaria alternata* causes devastating leaf blight disease (Reddy 2017). Brown necrotic spots symptoms on the leaf margins were observed which further expending towards the leaf midrib. *Alternaria* species are mainly saprophytes and commonly found in soil or on decaying plant debris. In the present study the causal agent of the leaf blight disease was determined and the post infection changes were recorded. Attempts were also made to evaluate the suppressive potential of fungicides and plant extracts. Various seed health and yield parameters were also investigated. This study will aid in the understanding and devising the disease management strategies for growers.

MATERIALS AND METHODS

Experimental site and sample collection: The study was carried out during 2016–17 at the ICAR-Directorate of Medicinal and Aromatic Plants Research, Boriavi, Anand, Gujarat (India). The experimental site located at 22 ° 35' N and 72 ° 55' E at an altitude of about 45.1 m amsl. The samples of infected leaf and stem were collected from the experimental field for the said study.

Isolation and morphological study of the pathogen: The leaf and stem tissues of the plant exhibiting typical symptoms of leaf blight were selected to retrieved the pure

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culture of the pathogen. Small piece of the leaf and stem was cut and surface sterilized with the sodium hypochlorite solution (NaOCl) of the 4.0% concentration for 3 min and followed by three washing with the sterile distilled water. The water was removed with the help of sterile blotting paper and thereafter the tissues were transferred on the sterile PDA plates and kept on incubation at 25 + 2°C in BOD incubator. The fungal mycelium growth observed after 72 h of incubation was transferred on the other PDA plate for pure culture. The morphological and *in vitro* efficacy of the fungicides was performed from the seven days old culture of the fungus.

Preparation of plant extracts: Plant leaf extracts used for this experiment were prepared indigenously. Fresh leaves of neem (*Azadirachta indica*), karanj (*Pongamia pinnata*) and lahsunbel (*Mansoa alliacea*) were collected from the ICAR-DMAPR, Boriavi farm, Anand. One kilogram of leaves of each plant were homogenized in mixture grinder and fermented separately in the 1 l of cow urine in utensils for 7 days. After seven days the solution were filtered through the muslin cloth and considered as 100% of concentration. The botanical extracts and fungicides were added into melted PDA aseptically at the time of plate preparation for *in vitro* study.

In vitro evaluation of fungicides and plant extracts: The experiment was conducted for measurement of mycelium growth inhibition following the Poisoned food technique (Sharvelle 1961). The standardized concentration of the fungicides and botanical extracts were mixed in the sterilized PDA and amended PDA poured in the sterilized petri plates. The petri plates of solidified PDA inoculated with a 6mm mycelium disk cut with the sterile corn borer from the 7 days old fungus culture. The inoculated plates were placed at 25+2°C in BOD incubator for incubation. Each treatment was replicated thrice and the growth of fungus colony was measured after 7 days of inoculation. The percent inhibition of the mycelium growth over the control was calculated as;

$$PI = \frac{C - T}{C} \times 100$$

where PI, Per cent inhibition; C, Mycelium growth of the fungus in absent of treatment; T, Mycelium growth of the fungus with treatment.

Soxhlet oil extraction: To measure the oil content in chandrasur, three replications of seeds from each infected and healthy seed lot were used for oil extraction. The standard soxhlet solid-liquid extraction method was followed for the oil extraction of chandrasur seeds. When the soxhlet extraction was completed, the solvent was removed using the rotary evaporator (Quan *et al.* 2004).

RESULTS AND DISCUSSION

Symptoms and morphology of pathogen: The symptoms of leaf blight disease initially appeared as brown to dark brown minute dots on chandrasur leaves margins. These spots gradually enlarged and covered the entire leaf lamina. In severity of the disease, spots coalesce and eventually

leaf defoliated. On the stem, branches and pods, necrotic lesions with dark brown to black spots on outer margins. The entire pods of the infected plants covered with the black powdery mass of conidia of the fungus and producing powdery appearance on the crops. The infected seed shrunk and discolored, were recovered from the infected plants (Fig 1) as also reported by Wahg *et al.* (2013). *Alternaria* species produces the host selective toxins (HST) and severity of the disease correlated with the HST production (Nishimura and Kohmoto 1983, Ito *et al.* 2004), which play an important role in the pathogenesis. *Alternaria* spp. causes foliar disease, having economic impact on variety of host plants includes cereals, vegetables, fruits, ornamental and forest trees. Additionally, the genetic and morphological variability of the genus *Alternaria*, make it more compatible to survive in diverse environmental conditions (Logrieco *et al.* 2009). *Alternaria alternata* causing the leaf blight disease on chandrasur in India and on *Lepidium draba* which is an invasive weed, the disease caused by the *A. brassicae* in North America (Caesar and Lartey 2009). The pure culture of pathogen was retrieved from the freshly developed water soaked lesion of stem of crop plants and fungal colony effuses grayish to blackish tinge having brown to dark brown septate mycelium on PDA. The conidia of the pathogen in host tissue were brown to dark brown and consists 2–3 transverse and 3–7 longitudinal septa. Size of the mature conidia ranged from 9–12.5 × 24–35 µm. These morphological features supported by earlier work on different crops that *A. alternata* produced grayish colored mycelium on PDA and conidiophores were brown to dark brown colored. The conidia were morphologically characterized by 2–9 transverse and 0–2 longitudinal septation, and arised in chain as by other workers (Simmons 1995, Devappa and Kumar 2016, Reddy 2017). The diverse species of genus *Alternaria* are the destructive pathogen, having different mode of parasitism as are either endophyte on leaving host or saprophyte on organic substrate or a post-harvest pathogen. The pathogen perpetuates on the diseased seeds or infected plant debris (Mehta *et al.* 2002). The pathogen species have broad host range on different plant species and families (Dang *et al.* 2015, Woudenberg *et al.* 2015, Saharan *et al.* 2016). The pathogen can be easily identified based on morphology of the conidia and pattern of conidia formation. The conidia formed separately or in chain, ovoid to obclavate, pale brown to dark brown, multi celled to muriform and beak is mostly present.

In vitro efficacy of fungicides and plant extracts: The fungicides and plant extracts tested *in vitro* against *A. alternata*, variably reduced the mycelial growth of the fungus (Table 1). Among the tested fungicides, systemic fungicides tebuconazole and tebuconazole+trifloxystrobin were found to be most efficient with the 89% inhibition of mycelium growth in comparison to the contact fungicides. The other systemic fungicides, azoxystrobin and carbendazim failed to inhibit the growth, where 4.67 and 4.55 cm mycelium growth recorded, respectively, as compared to 6.24 cm in check. Cuprous hydroxide showed 80.43% inhibition

Table 1 *In vitro* efficacy of fungicides and plant leaf extracts on the mycelium growth of *A. alternata*

Treatment	Conc. (%)	Conc. of used AI*(%)	Mycelium growth (cm)	Inhibition over control (%)
Azoxystrobin	0.1	2.35	4.67	25.17
Tebuconazole+ Trifloxystrobin	0.1	5.0+2.5	0.67	89.31
Tebuconazole	0.1	2.59	0.63	89.84
Mancozeb	0.25	18.75	1.50	75.94
Carbendazim	0.25	12.50	4.55	27.00
Metalaxyl-M	0.25	1.0+16.00	1.97	68.46
Copper oxychloride	0.25	12.50	1.66	73.43
Cuprous hydroxide	0.25	13.45	1.22	80.43
<i>A. indica</i> (LE)*	5.0	500.0	2.52	59.60
<i>P. pinnata</i> (LE)*	5.0	500.0	2.97	52.43
<i>M. alliacea</i> (LE)*	5.0	500.0	3.03	51.36
Check	00	00	6.24	00
SEm ±			0.069	
CD at 5%			0.203	
CV %			4.57	

*LE indicating leaf extracts; *AI denotes active ingredient

followed by the sulphur fungicide Mancozeb (Dithane M 45) which inhibited the mycelium growth by 75.94% over the check. Among the plant leaf extracts of three plants, viz. *Azadirachta indica*, *Pongamia pinnata* and *Mansoa alliacea* were tested at the 5% concentration. The mycelium growth of the pathogen recorded 2.52, 2.97 and 3.03 cm in *A. indica*, *P. pinnata* and *M. alliacea* treatments respectively, and inhibited the mycelium growth by 59.60, 52.43 and 51.36% over the control. The results supported by the previous studies conducted on *A. alternata* the lowest disease severity was reported in the mancozeb foliar spray against alternaria blight disease of Indian mustard (Meena *et al.* 2010). Plants extracts are eco-friendly safe to apply in the medicinal and aromatic plant for disease and pest management.

The neem (*A. indica*) leaf extract were reported effective against the *Alternaria porri* pathogens at different concentration causing purple blotch disease in onion (Meena 2012) alternaria blight of Indian mustard (Meena *et al.* 2004, Bairwa *et al.* 2015) and explored the different neem based product against the *A. tritricina* (Singh *et al.* 2003). The neem leaf extract was also found quite effective against the *A. alternata* of chandrasur in this study under *in vitro* conditions to inhibit the mycelium growth. The pathogen also affects the structure and morphology of chandrasur seeds and changed from ovule shape brick red color to shriek brown colored. The results showed that leaf blight disease of chandrasur cause significant seed and oil yield losses as observed in the other crops, especially in the crucifers (Saharan *et al.* 2016) and change the biochemical constituents of infected host (Meena *et al.* 2017).

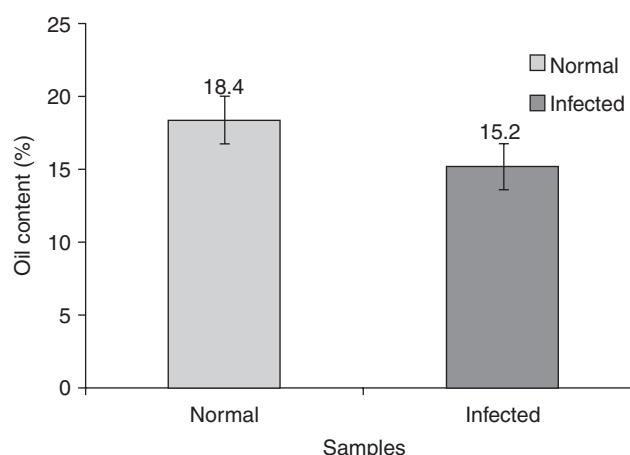


Fig 1 Oil content in the chandrasur seeds was measured.

Quantitative and qualitative losses: Chandrasur grown as minor crop in western part of the country and considered a medicinal as well as industrial importance crop. The green leaves used for salad and also possess some medicinal properties. The seeds are used as milch animals feed to improve the milk production.

Both qualitative and quantitative losses were recorded on the pathogen infected plants. The herbage yield drastically affected by the infection of the pathogen due to necrosis and fall out of leaves. The seeds which were initially healthy having smooth oval shaped and reddish brown to dark brick coloration. As a result of infection the healthy seeds converted to rough, shriveled and brown to blackish color (Fig 1E). Additionally, *A. alternata* also affect the mucilage content of the seeds. Test weight of the seeds recovered from the disease free (healthy) plants was 1.871 g, while 1.083 g of the seeds collected from infected chandrasur plants. Besides seed yield, oil content of 100 g healthy seeds was 18.4 g and that of the infected seeds 15.2 g in chandrasur. Thus, the yield loss and oil content reduction was recorded up to 42% and 17.40%, respectively, oil content in the chandrasur seeds. The crop produced reasonably good yield even as a catch crop with minimum fertilizers and irrigation. But biotic stresses particularly, leaf blight disease is limiting factor in commercial cultivation of the chandrasur and significantly affects the seed yield (Melkania 1980).

It was observed that leaf blight of chandrasur is a devastating disease causes qualitative as well as quantitative losses to the crop. It has also been revealed that new generation systemic fungicides tebuconazol and tebuconazole + trifloxystrobin (Nativo) were found most efficient to inhibit the mycelium growth. The study may lead in implementation of management strategies and mitigate the losses.

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