Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(5): 1857-1863
Received: 13-07-2020
Accepted: 15-08-2020

Bahaderjeet Singh
Department of Plant Pathology, College of Agriculture, Guru Kashi University, Talwandi Sabo, Bathinda, Punjab, India

Mamta Beniwal
Department of Plant Pathology, College of Agriculture, Guru Kashi University, Talwandi Sabo, Bathinda, Punjab, India

Gagandeep Singh Sohi
Department of Plant Pathology, College of Agriculture, Guru Kashi University, Talwandi Sabo, Bathinda, Punjab, India

Amanpreet Singh Sran
Department of Plant Pathology, College of Agriculture, Guru Kashi University, Talwandi Sabo, Bathinda, Punjab, India

Paryotampal Kaur
Department of Plant Pathology, College of Agriculture, Guru Kashi University, Talwandi Sabo, Bathinda, Punjab, India

Corresponding Author:
Bahaderjeet Singh
Department of Plant Pathology, College of Agriculture, Guru Kashi University, Talwandi Sabo, Bathinda, Punjab, India

Studies on Alternaria leaf spot of cotton with special reference to disease management by use of chemicals and botanicals

Bahaderjeet Singh, Mamta Beniwal, Gagandeep Singh Sohi, Amanpreet Singh Sran and Paryotampal Kaur

Abstract
Cotton is one of the most important fiber cash crop of the world as well as India which belongs to the botanical family "Malvaceae". *Alternaria alternata* is an economically important pathogen on numerous crops worldwide. The experiments were conducted at Guru Kashi University, Talwandi sabo (Punjab) under the field and laboratory conditions to study the Alternaria leaf spot of cotton during season 2019-2020. The disease severity elevated in cotton crop with delay in sowing between D1, D2 and D3. American cotton varieties recorded highest disease severity than desi cotton variety in all sowing dates. Hence, American cotton varieties were more susceptible than desi cotton variety. Mancozeb show the maximum diameter of zone of inhibition at different concentrations (125, 250, 500, 1000 ppm) followed by Ridomil. In botanical management, *Allium sativum* was performed better than *Azadirachta indica.* Use of botanicals is the alternative approach for the disease management that is eco-friendly.

Keywords: Alternaria leaf spot, *Alternaria alternata,* fungicides, management, cotton

Introduction
Cotton is one of the most important commercial crop of the world as well as India which belongs to the botanical family ''Malvaceae". Cotton is referred to as "king of Fibres" and is also popularly known as "white Gold". India is the largest cotton growing country in the world with an area of around 12.24 mha. Total production of cotton in India is 36.1 million bales of 170 kg and productivity is 501 kg ha-[1]. India's share in global cotton exports is around 22% (Anonymous, 2019) [1]. India is the only country throughout the world where all the four cultivated species of cotton crop, *viz.*, *G. hirsutum*, *G. arboreum*, *G. herbaceum* and *G. barbadense* are cultivated on commercial scale. The diversity of cultivars of cotton crop and cotton agro-climatic zones in India is considerably larger as compared to other dominant cotton growing countries in the world.

Cotton is a pivotal *kharif* crop of Punjab. American cotton was grown on 2.63 lakh hectares with total production of 12.06 lakh bales and an average yield of 7.79 quintals lint per hectare (3.15 quintals lint per acre), while desi cotton occupied 4.7 thousand hectares and the total production was 16.7 thousand bales with an average lint yield of 6.03 quintals per hectare (2.44 quintal per acre) during 2018-19 (PAU *Kharif* Package of Practices 2019-20). In Punjab, the area under cultivation of cotton crop is decreasing from the last 10 years. This is due to the fact that the alternate *kharif* season crop of rice is more profitable because of cotton crop is more vulnerable to several biotic and abiotic stresses. Many biotic and abiotic factors are responsible for reduction in yield and quality deterioration of cotton in India, Diseases occupy a vital place. Cotton crop in India is known to suffer from fungal, bacterial, viral and nematode diseases have been reported from early stage to maturity. Among them, the economically most important ones are Alternaria leaf spot, bacterial blight, grey mildew, rust and vascular wilts which occur throughout the world (Kotasthane and Agrawal, 1970) [12]. Alternaria leaf blight and other leaf spotting fungus diseases of cotton pose an alarming situation (Gholve *et al.*, 2012). Vasudeva (1960) [9, 21] reported that disease was serious on three varieties of *G. hirsutum* species of cotton, the other cultivated species of *Gossypium* being resistant. A study conducted by a team of cotton experts from the government has noted that *Bt* cotton hybrids are most susceptible to diseases like bacterial blight, Alternaria leaf spot and grey mildew were the major disease on cotton identified in the central and southern regions of the country in 2004 (Ashok, 2005) [3]. Solaippan and Dason (1998) [18] found that seed cotton yield of *G. hirsutum* was greatly affected by sowing date. It was also reported to affect yield and quality of *G. arboreum* (Tuteja *et al.*, 1999) [20].

Well diffusion method was used on the solid agar media in petri dishes to control the pathogen growth by Kamble *et al*., (2000) [11]. Dighule *et al*., (2011) [8] tested six fungicides against the fungal foliar diseases of cotton. The results revealed that the fungicides Mancozeb (0.3%), Propiconazole (0.1%) and Propineb (0.3%) were significantly superior in reducing the disease intensity and increasing yield than other fungicides. Chattopadhyay *et al*., (2005) [6]; kumar (2009) [13] reported that Garlic bulb extract and Neem extract both were the better choice than fungicides for eco-friendly management of fungus diseases of oilseed crops. Keeping in view the economic value of the crop for the region and extensive damaged by leaf spot of cotton, the present study was conducted to find out the suitable management strategy against leaf spot of cotton caused by *A. alternata.*

Materials and Methods

The field experiment was conducted at Agriculture Research Farm of the Guru Kashi University, Talwandi Sabo, Bathinda (Punjab) during *kharif* crop season 2019-2020, in a split plot design with three replications. The three varieties of *G. hirsutum* (*Bt* cotton- RCH 773, RCH 650, Non *Bt*- F 2383) and one of *G. harbaceum* (FDK 124) were sown on three sowing date [15th May (D1), 3rdJune (D2) and 22ndJune (D3)] standard spacing and use recommended doses of N, P and K fertilizers in were applied in natural conditions. No plant protection measures were taken in contrast to any diseases. Observe data once in 7 days for recorded the percent disease intensity (PDI). Five randomly selected plants in each plot and were tagged for taking observations for disease component. Percent disease intensity (severity) was calculated as per 0 to 4 disease scale developed by Sheo Raj (1988) [17] (Table 1). The per cent disease index (PDI) was calculated by using the following formula that given by Wheeler (1969) [24].

$$\text{Percent disease intensity (PDI)} = \frac{\text{Sum of individual rating scale}}{\text{Total no.of leaves observed} \times \text{Maximum disease score}} \times 100$$

Table 1: Alternaria disease rating scale

Rating scale	Per cent leaf area covered (%)	Disease reaction
0	0	Immune (I)
1	0-10	Resistant (R)
2	11-20	Moderately resistant (MR)
3	21-40	Moderately susceptible (MS)
4	More than 40	Susceptible (S)

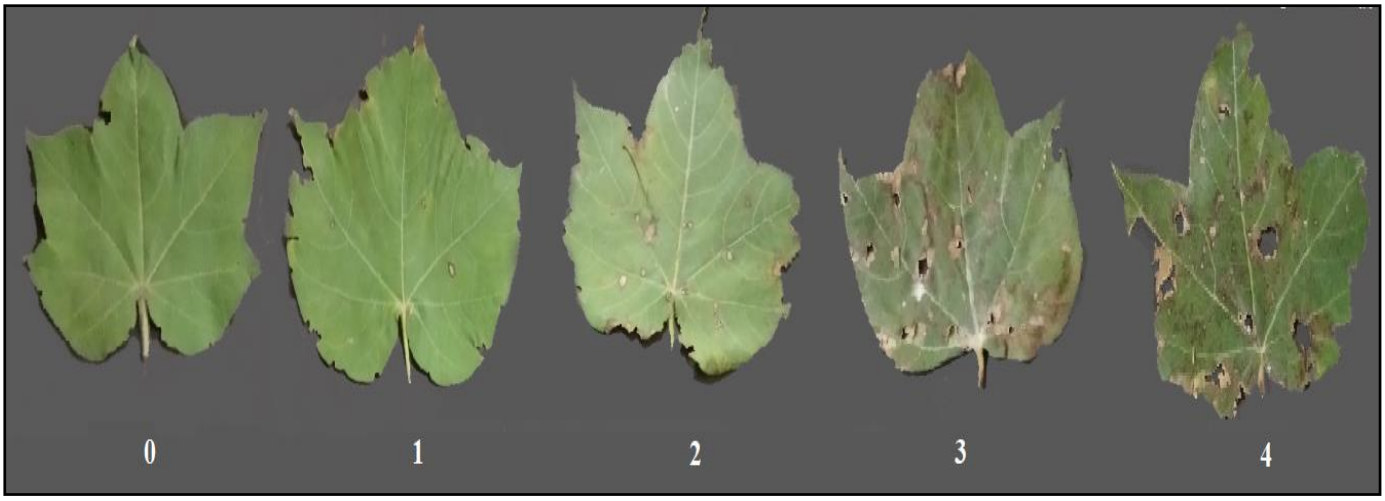

Fig 1: Disease rating scale for Alternaria leaf spot of cotton

Isolation, maintenance and identification of the pathogen

Isolation

The laboratory experiments were carried out at department of plant pathology in the University campus. Visual observations were made for manifestation of the Alternaria leaf spot of cotton typical symptoms on cotton field from the experimental area, collect infected leaves in polythene bags, brought to the laboratory and subjected to the isolation on Potato Dextrose Agar (PDA) medium. Small bits of the infected leaves of 3 to 5 mm size were cut from the diseased area along with some healthy portion, washed under running tap water followed by a wash with autoclaved distilled water and then surface was wiped off with 70% ethanol. Isolation done in sterilized Petri plates under Laminar-air-flow cabinet and incubated these plates in BOD incubator at 25±2°C temperature. Ten days after incubation, when the well developed mycelial growth of the fungus was seen on the medium, it was transferred aseptically onto PDA agar slants. Use different laboratory equipment's to isolation of pathogen *viz.,* Autoclave, Hot air oven, Hot plate, Laminar airflow Cabinet, BOD Incubator, Refrigerator, Binocular Microscope, Haemocytometer, Distillation unit, Water bath, Deep freezer, Electronic balance, Variable volume micropipettes, etc. available in the Department of Plant Pathology, College of Agriculture, were used. The common glass-wares (Borosil) *viz.,* Petri-dishes, test tubes, conical flasks, cork borer, volumetric flasks, measuring cylinder, L Shape Spreader, beakers and funnel etc.

Maintenance of the culture

The fungus was sub-culturing on the Potato Dextrose Agar (PDA) slants and allowed to grow at 25±2°C temperature in BOD incubator for 10 days. These slants were then preserved in the refrigerator at 5°C and sub-culturing once in a month. The obtained pure culture was used for further studies.

Identification of the fungus

The fungus isolated during the study was identified based on the morphology characters on media and characteristics of the colony, hyphae, conidiophores and conidia under microscopic method. The measurements of the conidiophores and conidia were recorded and compared with the standard measurements of the species given in the "CMI Descriptions of Pathogenic Fungi and Bacteria (1988) [7]".

Effect of fungicides and botanicals against *A. alternata*

Well diffusion method was use on the solid agar media in Petri dishes to control the growth of pathogen with fungicides and botanicals. The botanicals Neem oil (Azadirachtin 0.03%) and Garlic extract (1gm garlic: 10ml distilled water) were used to control the *A. alternata* in cotton. The fungicides were used in this experiment; Mancozeb 75% WP, Ridomil gold (Metalaxyl 4% + Mancozeb 64% WP) and Carbendazim 50% WP**.**

Results and Discussion

Symptomatology

The symptoms of Alternaria leaf spot of cotton was observed on different plant parts. The symptoms first appear on the upper surface of leaves in the form of small dot shape, light brown to blackish in color, circular or irregular lesions, which later on turns dark brown due to the appearance of spore masses. These spots enlarged and coalesced forming large sized spots. In the centre of such spots, light brown dot surrounded by the number of dark alternating concentric rings also appeared in some cases. Affected leaves become dry, cracking and fall off. Similar result have been reported by Watkins (1981) [23]; Gulhane and Gurjar (2011) [10]. Veins of leaves also affected. The disease also cause cankers on the stem. These results are in agreement with the findings of Padaganur (1979) [15]. The phenotypic symptoms of Alternaria leaf spot on different Species of cotton (Fig. 2) were evaluated.

The *A. alternata* pathogen infects all the four cultivated species of cotton (Srinivasan, 1994) [19]. In the *kharif* season (2019-2020), RCH 773 and RCH 650 showed the earliest occurrence of the symptoms on leaves of few plants. The appearance of *A. alternata* on leaves in the form of pinhead was first recorded in RCH 773 followed by RCH 650, F 2383 and FDK 124 in D1, D2 and D3 respectively. The RCH 773 variety showed early symptoms at 47 DAS whereas, FDK-124 showed the late symptoms at 95 DAS (Table 2).

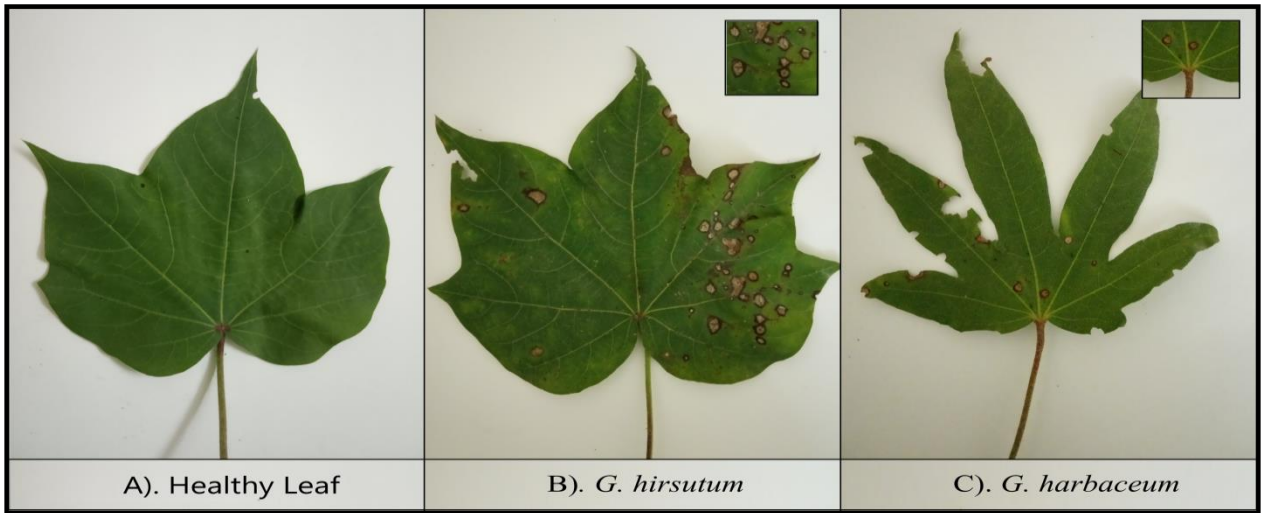

Fig 2: symptoms of Alternaria leaf spot of cotton on different species (B, C).

Table 2: The disease appearance on the varieties at different sowing dates.

Date of sowing	Varieties	Initial appearance of the disease (DAS)
D1 (15/5/2019)	V1	56 (28th week)
	V2	60 (28th week)
	V3	67 (29th week)
	V4	89 (32nd week)
D2 (3/6/2019)	V1	54(30th week)
	V2	56(30th week)
	V3	64(30th week)
	V4	85(35th week)
D3 (22/6/2019)	V1	47(32nd week)
	V2	49(32nd week)
	V3	58(33rd week)
	V4	95(36th week)

V1- RCH 773 V2- RCH 650
V3- F 2383 V 4- FDK 124

Effect of sowing date on disease severity

During *Kharif* season (2019-2020), cotton crop was sown with three different sowing date and use four different varieties to determine the disease severity of Alternaria leaf spot. The sowing date of D1 (15thMay) showed the lowest disease severity as compared to other date of sowing. The disease severity of Alternaria leaf spot of cotton in all sowing date, all the four varieties revealed increased disease severity with delay in date of sowing. The disease severity elevated in cotton crop with delay in sowing between 15thMay (D1), 3rdJune (D2), and 22ndJune (D3). Among three sowing date, the overall mean of percent disease intensity, 38.56 was recorded in D3, followed by the D2 with comparatively reduced disease intensity of 33.3 and D1 with 28.32 disease intensity. Hence, the sowing dates had no significant effect on disease severity of Alternaria leaf spot of cotton based on overall mean percent disease intensity under the third grade according to Table-1 (Sheo Raj disease rating Scale, 0-4) and one year data is not sufficient to conclude concurrent result; further experimentations are required to confirm the results. The effect of sowing dates on severity of Alternaria leaf spot of cotton is summarized in Table 3 and graphically represented in Fig. 3. Mahmood-ul-hassan *et al*., (2003) testified that during 1998-1999 under Multan (Pakistan) conditions on average two year of data, 15th may sowing date produced highest cotton yield than other sowing dates.

Effect of Cotton varieties on disease severity

Results expose that in all four cotton varieties, Alternaria leaf spot intensity varied significantly with the sowing dates and also found to be increased steadily with age of the crop (Table 3 and Fig. 3). Among the four varieties tested desi cotton variety (FDK 124) in all sowing dates showed less disease intensity, whereas RCH 773 found susceptible with maximum mean disease intensity of 31.75%, 35.78% and 43.51% in the

crop sown on D1, D2 and D3, respectively. In American cotton varieties recorded highest disease severity than desi cotton variety in all sowing dates. Hence, American cotton varieties were more susceptible than desi cotton variety (FDK 124). No variety or cultivar was observed as resistant. Therefore, emphasis need for further extensive screening of large plant breeding material to find out the newer resistant sources. Interaction effects (D×V) in respect of disease intensity (severity) at various intervals were found significant**.**

Table 3: Effect of sowing dates and cotton varieties on Alternaria leaf spot intensity (Severity) during *kharif*, 2019-2020

Sowing Dates	Varieties	Per cent disease incidence	
		Plant	
		PDI	Disease Reaction
D1 (15/05/2019)	RCH 773	31.75	MS
	RCH 650	30.18	MS
	F 2383	30.28	MS
	FDK 124	21.05	MS
Overall mean		28.32	
D2 (03/06/2019)	RCH 773	35.78	MS
	RCH 650	34.86	MS
	F 2383	33.84	MS
	FDK 124	28.72	MS
Overall mean		33.3	
D3 (22/06/2019)	RCH 773	43.51	S
	RCH 650	41.60	S
	F 2383	39.75	MS
	FDK 124	29.38	MS
Overall mean		38.56	
C.D. (P= 0.05)	D	4.35	
	V	1.64	
	D×V	3.46	

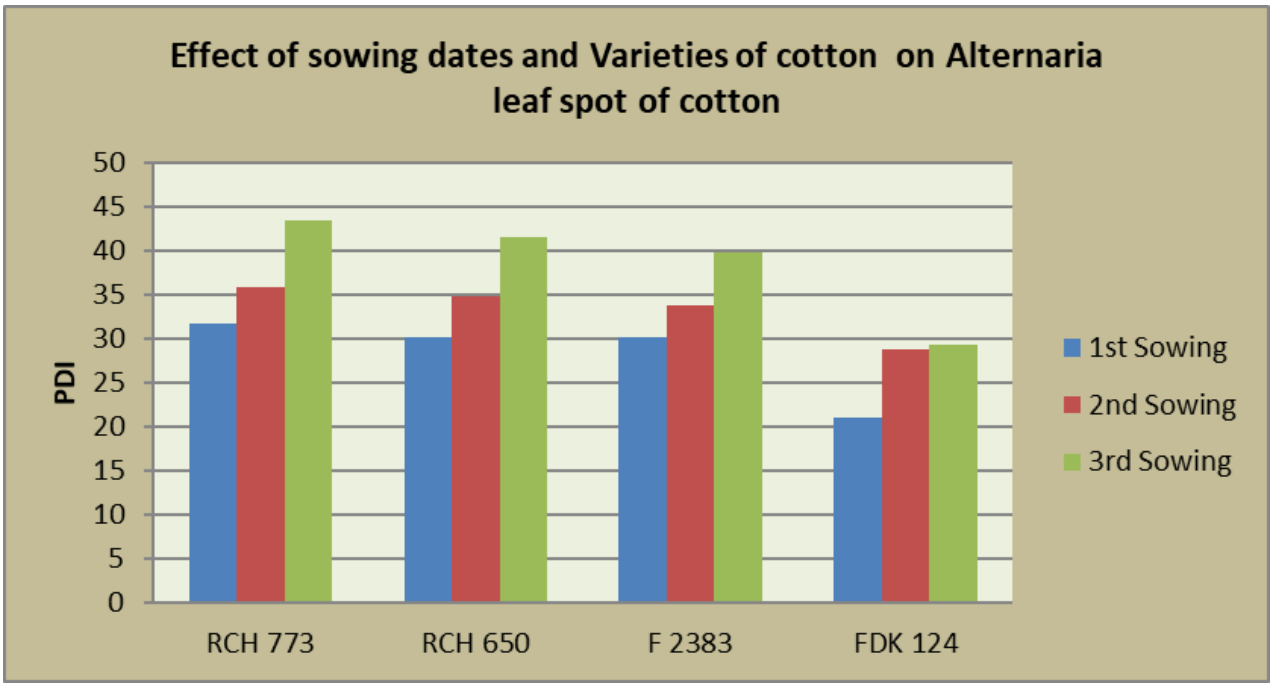

Fig 3: Effect of sowing dates and cotton varieties on Alternaria leaf spot intensity (Severity) during *kharif*, 2019-2020

Identification

The growth of the mycelial in the culture was grey to brown in colour, initially and then turned to dark black. The septate hyphae of the fungus were observed to be dark brown to black. The growth of the causal organism was very slow on the culture (PDA) medium. Dark brown to black conidia with conidiophores were observed at the time of sporulation. These results are in agreement with the findings of Srinivasan (1994) [19]. Slide culturing was done to determine the microscopic features for morphological characterization of the fungus (Fig. 4).

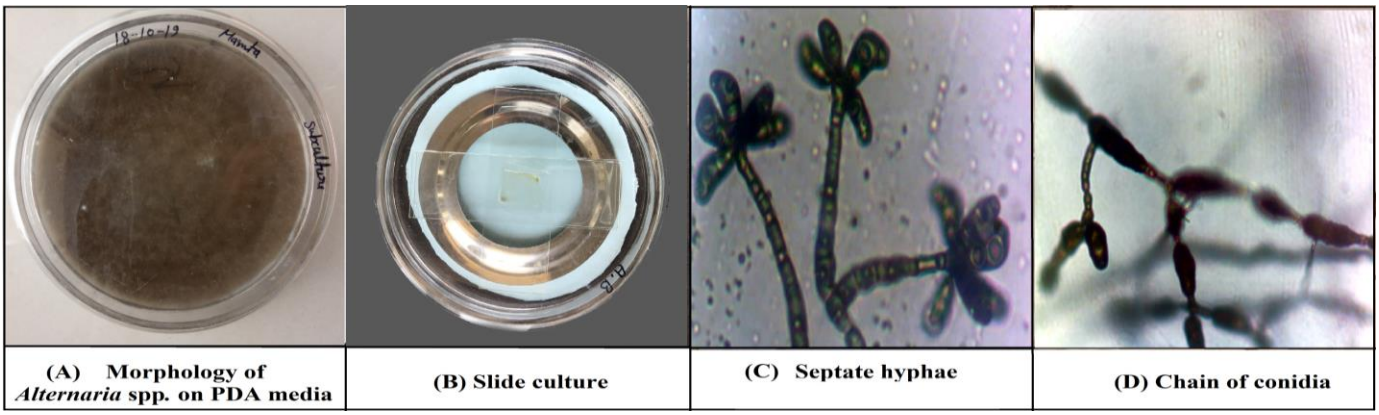

Fig 4: (A) Morphology of *Alternaria* spp. on PDA (B) Slide culture (C) Septate hyphae (D) Chain of conidia

Effect of fungicides and botanicals against *A. alternata* (Alternaria leaf spot of cotton)

Well Difussion method was used to check the antifungal activity of Fungicides and Botanicals. The fungicides namely, Mancozeb, Ridomil and Carbendazim at four different concentrations *viz.*, 125, 250, 500 and 1000 ppm and control were evaluated under laboratory conditions. In this technique make 5 mm diameter well in solid pored media (PDA) with sterile cork borer. Use 150 µl quantity of fungicides to fill in the well at all concentrations and pathogen solution was spread on the surface of the cultivated PDA with the help of sterilize glass spreader and incubate at 25±2°C temperature in BOD for 5-7 days. Measure the diameter of zone (in mm) of inhibition around the well after proper growth of *A. alternata* (After 7th days of treatment).

Mancozeb and Redomil were give significantly superior result. Minimum mycelial growh was recorded in Mancozeb followed by redomil, while maximum mycelial growth found in carbendazim. Overall mean of zone of inhibition was recorded in Mancozeb (21 mm), Ridomil (20.18 mm) and Carbendazim (0 mm). Mancozeb seems to be the most effective fungicide at all concentrations, while carbendazim was not effective against *A. alternata*. The data is summarized in Table 4 and graphically presented in Fig. 5. Suitable control (without treatment) was also maintained. Zone of inhibition pictures are show in Fig. 6.

Table 4: Zone of inhibition (diameter in mm) of fungicides against *Alternaria alternata*

S. No.	Treatments	Diameter (in mm) of zone of inhibition				Overall Mean (in mm)
		Concentration (in ppm)				
		125	250	500	1000	
1	Mancozeb	17.25	18.75	22	26	21
2	Ridomil	17	18.25	21.25	24.25	20.18
3	Carbendazim	0	0	0	0	0
4	Control	0				

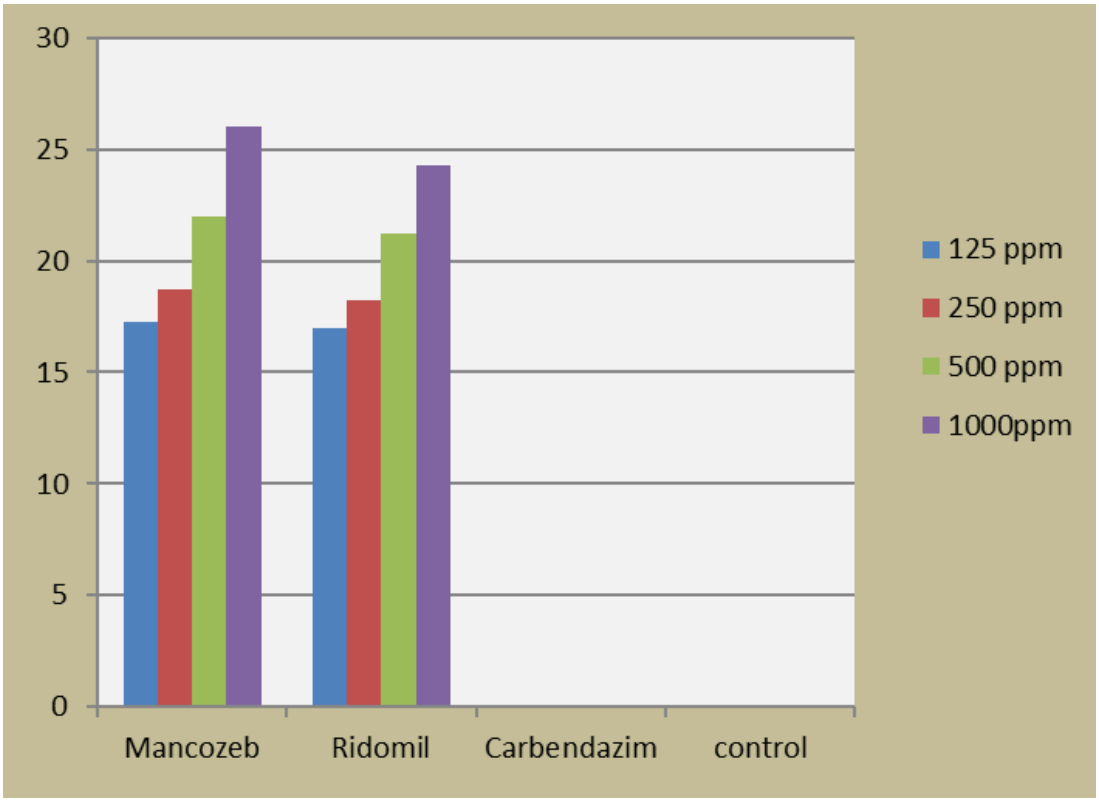

Fig 5: Zone of inhibition (diameter in mm) of fungicides against *A. alternata*

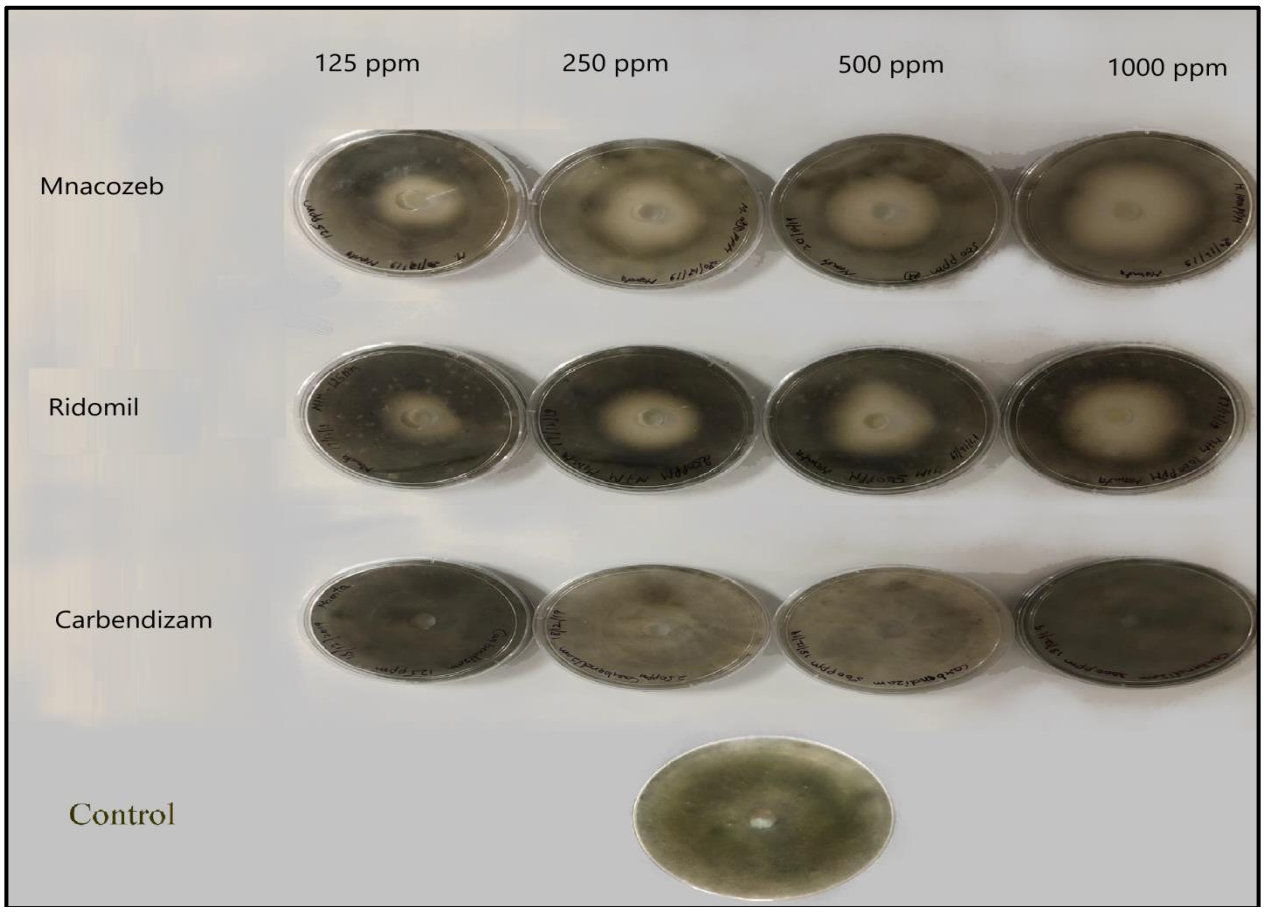

Fig 6: Zone of inhibition (diameter in mm) of fungicides against *A. alternata*

During botanical control well diffusion method was used, same as fungicide treatment. The two botanicals *viz.*, *Allium sativum* and *Azadirachta indica* were also evaluated under laboratory conditions. *A. indica* show very low antifungal activity against *A. alternata*. *A. indica* showed diameter (in mm) of zone of inhibition recorded at 125, 250, 500, 1000 and 1500ppm were 0, 2, 5, 9.5 and 11 mm, respectively. *A. sativum* was used in five different concentrations *viz.*, 25, 50, 75, 100 and 150µl. *A. sativum* show highest diameter of zone inhibition (in mm) with 25, 50, 75, 100 and 150µl were 20, 30.5, 31.25, 32.25 and 33.75 mm, respectively.

In botanical management, *A. sativum* give finest and swift result than *A. indica*. *A. indica* show very low antifungal activity against *A. alternata*. Control pictures are show in **Fig 7**. The efficacy of fungicides and botanicals in the management of Alternaria leaf spot of cotton (*A. alternata*) was also reported by others workers (Chattopadhyay *et al*., 2005; Chattannavar *et al.*, 2006; kumar, 2009; vihol *et al.*, 2009; Nigam *et al.*,2011; Bochalya *et al* 2012; Gholve *et al.*, 2012) [6, 5, 13, 22, 14, 4, 9].

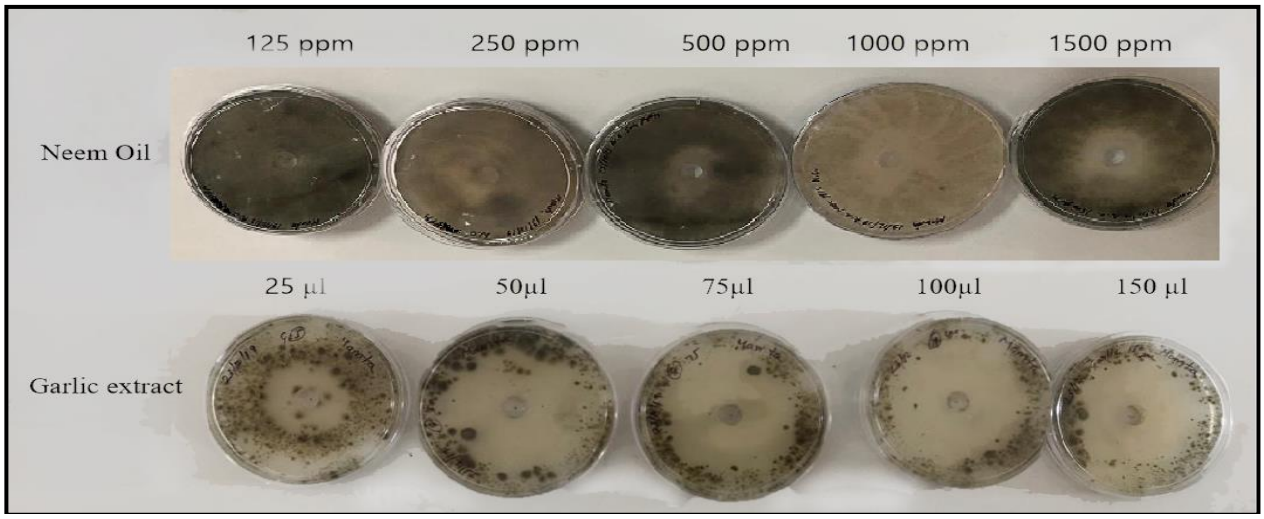

Fig 7: Zone of inhibition (diameter in mm) of botanicals against *A. alternata*

Mancozeb proved to be the most effective fungicide in controlling Alternaria leaf spot at all concentrations. Both botanicals were effective against *A. alternata* pathogen at all concentrations and found that *A. sativum* give finest and swift

result than *A. indica*. Degradation of environment due to pesticide chemicals is a worldwide problem and they have contaminated almost every component of our environment. Since, chemicals have many hazardous effects on the environment, use of botanicals is the alternative approach for management of disease and that is eco-friendly and reduces the harmful impact on environment.

Acknowledgement
The authors are thankful to Guru Kashi University, Talwandi sabo, Bathinda (Punjab) for providing basic facilities for this research work and we also thanks to Dr. Satish Kumar Sain, Senior Scientist, ICAR- Central Institute for Cotton Research, Regional Station, Sirsa-125055 (Haryana) for their useful suggestions.

References

1. All India coordinated project on cotton, Project coordinator report. Anonymous, 2019; http://www.aiccip.cicr.org.in/CD_18_19/2-PC-Report.pdf
2. Package of Practices for cultivation of Kharif crops. Punjab Agriculture University, Ludhiana. Anonymous, 2020; 37:34-49.
3. Ashok BS. *Bt* cotton prone to diseases says Indian study. An online article, 2005; www.financialexpress.com.
4. Bochalya MS, Shekhawat KS, Singh R, Chohan PK. Management of *Alternaria alternata* causing Alternaria fruit rot of brinjal under *in vitro* conditions. Biopesticides International. 2012; 8(2):131-137.
5. Chattannavar SN, Kulkarni S, Khadi BM. Chemical control of Alternaria blight of cotton. Journal of Cotton Research and Development. 2006; 20(1):125-126.
6. Chattopadhyay C, Meena PD, Godika S, Yadav MS, Meena RL, Bhunia CK. Garlic bulb extract a better choice, than chemical fungicides in managing oilseed crop diseases. Indian Journal of Mycology and Plant Pathology. 2005; 35:574-575.
7. David JC. CMI Descriptions of Pathogenic Fungi and Bacteria, CAB International Mycological Institute., Kew, Survey, England, 1988.
8. Dighule SB, Perane RR, More PE, Amle KS. Efficacy of chemical fungicides and bio-agents against major cotton fungal foliar diseases. International journal of plant protection. 2011; 4(2):263-266.
9. Gholve VM, Jogand SM, Jagpat GP, Dev U. *In vitro* evaluation of fungicides, bioagents and aqueous leaf extracts against of Alternaria leaf bright of cotton. Scientific journal of Veterinary Advance. 2012; 1(1):12-21.
10. Gulhane VA, Gurjar AA. Detection of diseases on cotton leaves and its possible diagnosis. International journal of plant protection. 2011; 5(5):590-598.
11. Kamble PU, Ramaiah M, Patil DV. Efficacy of fungicides in controlling leaf spot disease of tomato caused by *Alternaria alternata* (F.) Kessiler. Journal of Soils and Crops. 2000; 10:36-38.
12. Kotasthane SR, Agarwal SC. Efficacy of four seed protectants for the control of bacterial blight of cotton. Pest Articles and News Summaries. 1970; 16:334-335.
13. Kumar A. Ecofriendly option for the management of white rust in Indian mustard under mid hill conditions of North-Western India. Indian Phytopathology. 2009; 62(1):44-48.
14. Nigam R, Kushwan A, Shrivastava A, Shrivastva N. Bio efficacy of botanicals against A. blight of mustard caused by A. brassicae. Annals of Plant Protection Science. 2011; 19:245-246.
15. Padaganur GM. The seed borne nature of Alternaria macrospora in cotton. Madras Agriculture Journal. 1979; 66:325-326.
16. Ramegowda G. Disease scenario in Bt cotton with special reference to Alternaria leaf spot. M. Sc. (Agri.) Thesis, Uni. Agric. Sci., Dharwaad, Karnataak (India), 2007.
17. Sheo R. Grading for cotton disease, CLCR, Nagpur. Bulletin. 1988, 1-7.
18. Solaippan U, Dason AA. Influence of sowing time, intercropping and mulching on the growth and yield of rainfed cotton (*Gossypium hirsutum*). Indian Journal of Agronomy. 1998; 43:616-621.
19. Srinivasan KV. Cotton diseases. Indian Society for Cotton Improvement. Central Institute for Cotton Research, Mumbai. 1994, 157-311.
20. Tuteja OP, Singh DP, Chhabra BS. Genotypic – environment interaction on yield and quality traits of Asiatic cotton (*Gossypium arboreum*). Indian Journal of Agriculture Science. 1999; 69:220-223.
21. Vasudeva RS. Dis. of Cotton in India. A Monograph (Dastur, R. H., Asana, R. D., Sawhney, K., Sikka, S. M., Vasudeva, R. S., Khan, Q., Rao, V. P., Sethi, B. L., Eds.). 1960; 2:164-216.
22. Vihol JB, Patel KD, Jaiman RK, Patel NR. Efficacy of plant extracts, biological agents and fungicides against Alternaria blight of cumin. Journal of Mycology and Plant Pathology. 2009; 39(3):516-519.
23. Wakins GM. Compendium of cotton diseases. American Phytopathological Society Publishers, United States of America. 1981, 28-30.
24. Wheeler BEJ. An Introduction to Plant Diseases. John Wiley Publication, London. 1969, 301.