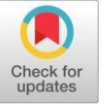


Research Article

Open Access

Host Range Studies of *Alternaria* Species on Sunflower and Efficacy Studies of Fungicides Against *A. Alternata* Under Both *in-Vitro* and *in-Vivo* Conditions



Divyashree^{1,2*}, C. P. Manjula¹, Rahul L. Joshi³, C. H. Sai Bhavana¹, Jayashree Anandakumar² and J. Harish²

¹All India Coordinated Research Project on sunflower and Directorate of Research, UAS, GKVK, Bangalore-560065, India.

²Department of Plant Pathology, University of Agricultural Sciences, GKVK, Bangalore, Karnataka-560065, India.

³Department of Plant Pathology, NMCA, Navsari Agricultural University, Navsari, Gujarat-396450, India.

ABSTRACT

Alternaria blight is the major profound disease of sunflowers induced by divergent species of *Alternaria*. Among numerous species of phytopathogenic fungi the genus *Alternaria* Nees ex Fr. is distributed extensively throughout the world. It is extremely virulent in infection in all economically important plants, including grains, pulses, oilseeds, spices, vegetables, and ornamentals. Current studies aim to explore the widened host range of *Alternaria* species, influenced by strain variability and environmental factors, leading to yield losses and compromised crop quality. Additionally, these studies conducted to understand expanded host range of sunflower *Alternaria* species on various crops and weeds within the Asteraceae family. The host range study of *Alternaria* spp. recovered from sunflowers belonged to six *Alternaria* species collected from different commercial sunflower growing regions of Karnataka. *A. alternata*, *A. tenuissima*, and *A. helianthi* exhibit a broad host range, infecting various plants of the Asteraceae family, including African marigold, safflower, China aster, and Parthenium. Conversely, *A. gossypina*, *A. burnsii*, and *A. solani* specifically infect sunflowers and do not affect other host species. The results of the host range test indicate that *Alternaria* spp. infecting sunflower can demonstrate pathogenicity towards other hosts as well, especially under favorable conditions. *In vitro* testing of novel fungicide compounds against the pathogen revealed that Fluopyram 17.7% + Tebuconazole 17.7% 400 SC, applied at concentrations of 100, 250, 500, 750, and 1000 ppm, effectively prohibited the mycelial growth of the pathogen. Conversely, the most favorable outcomes in the field were observed with seed treatment using Fluxapyroxad FS at 1.5 g/kg seed, resulting in the minimal disease severity (7.36% PDI) and highest seed yield (2153 Kg/ha). This was followed by a foliar spray application of Fluopyram 17.7% + Tebuconazole 17.7% 400 SC at 1 ml/L upon disease onset.

Keywords: Sunflower, *Alternaria* spp, Symptoms, Asteraceae, Host range, New fungicides, Management

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is the second most important oilseed crop in demand after groundnut in India. Karnataka is the leading sunflower-producing state in India, with 64 per-cent of total area and 52 per-cent of production [1]. However, productivity (597 kg ha⁻¹) is less compared to the national average of 752 kg ha⁻¹ [2].

Sunflower leaf blight, instigated by *Alternaria* spp., ranks among the most devastating and prevalent diseases globally, including India [3-7]. The pathogenic fungi can infect the sunflower's head, stems and leaves, causing a wide range of light to dark lesions, blighting, leaf drop, and even death of the plant. Under severe conditions (warm temperatures and high relative humidity), the disease potentially causes premature defoliation [8]. The genus *Alternaria* holds a prominent position and is critically important because it causes widespread diseases in different host species. According to reports, nine different species of *Alternaria* are responsible for the leaf blight on sunflowers worldwide [9].

The pathogen could either exhibit host specificity or induce diseases in other host species. The host spectrum studies of all *Alternaria* species on other members of the Asteraceae family help in identifying alternate hosts and developing suitable management strategies during the off and on-season sunflower cropping. Recent research findings indicate that *A. alternata* is the predominant and particularly aggressive causal agent of leaf blight/leaf spot in sunflowers [10]. As an air-borne or seed-borne pathogen, they cause moderate to severe damage to foliage, yield potential, and quality of the produce. Hence, the application of fungicide in the management of disease during the cropping season gives efficient disease control and less crop damage. The present investigations on management studies using new combi product fungicides and a host range of pathogens involve a lucrative method for elaborate control of *Alternaria* pathogen in the sunflower field.

METHODS AND MATERIALS

Host range of the *Alternaria* species

Host range and pathogenic potentiality of the predominant *Alternaria* spp. (6) from diseased sunflower plants, isolated from surveyed regions was carried out in a net house using host plants from Asteraceae family viz., marigold, safflower, aster and important weeds (Parthenium, Billygoat and Telegraph weed) commonly found in sunflower fields. Healthy seeds of different host plants were planted in pots filled with sterilized soil.

*Corresponding Author: Divyashree

DOI: <https://doi.org/10.21276/AATCCReview.2024.12.02.184>

© 2024 by the authors. The license of AATCC Review. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Species of *Alternaria* isolated from sunflowers grown in southern Karnataka were inoculated onto 20–35 day-old host plants with a homogenized suspension of 12 day-old pathogen culture (1×10^6 spores/ml). These host plants were predisposed to 95 per-cent relative humidity by covering with transparent polyethylene bags for 24 hours before and after inoculation in greenhouse conditions and then 3 days it was kept under normal conditions. Observations on infection symptoms were recorded after 5 days of inoculation and reaffirmation was made using microscopic and morphological characteristics. The pathogen was artificially inoculated on the bottom and middle leaves of 20-35 days-old plants with a homogenous spore suspension.

2. *In vitro* evaluation of new fungicide molecules against *A. alternata*

The efficacy of 5 combi products Fluopyram 17.7 % + Tebuconazole 17.7 % 400 SC, Azoxystrobin 18.2 % + Difenconazole 11.4 % SC, Boscalid 25.2 % + Pyraclostrobin 12.8 % WG, Dimethomorph 12 % + Pyraclostrobin 6.7 %, Picoxystrobin 7.05 % + Propiconazole 11.71 % SC and one systemic fungicide (Myclobutanil 10 % WP) were analysed against *A. alternata* at different concentrations on PDA using poisoned food technique [11] at different concentrations (100, 250, 500, 750 and 1000 ppm). Sterilized PDA was cooled to 50 °C and an appropriate quantity of stock solution of fungicides was added to the media.

The 15 to 20 mL of poisoned PDA was poured into 90 mm Petri plates and allowed to solidify. 5 mm disc of the actively growing pathogen (*A. alternata*) culture of 12 days old fungal culture was shifted aseptically to the center of each Petriplate having the poisoned medium. Due to the high prevalence and dominant presence of *A. alternata* species identified in our recent study in southern Karnataka, alongside the observation that many fungicides share a common mode of action rather than targeting species-specific mutations, we have chosen to evaluate these specific pathogenic species to gauge their susceptibility to new fungicidal compounds.

Control was maintained with non poisoned PDA and a culture disc was placed in it. Inoculated plates were placed in incubators set at 27 ± 1 °C for 21 days. The fungal colony's radial growth was assessed by measuring its diameter in two perpendicular directions, and the average diameter was computed. The percentage of growth inhibition compared to the control was estimated using the following formula [12].

$$I (\%) = \frac{C-T}{C} \times 100$$

Where,

I = Percent inhibition,

C = Growth in control

T = Growth in treatment

3. Field evaluation of new molecules against *Alternaria* blight

A field trial was undertaken to evaluate the comparative effectiveness of new fungicide molecules tested against *Alternaria* blight of sunflower under *in-vitro* in fields during *Kharif* 2022 on KBSH 44 at Zonal Agriculture Research Station, Gandhi Krishi Vignana Kendra, Bengaluru (Fig S. 1). The field was arranged in Randomized Complete Block Design (RCBD) with 7 treatments and 3 replications which included seed treatment and foliar spray combinations (Table S1).

The first fungicidal spray was undertaken on the appearance of

disease (12/1/22) and 10 days after the first spray (23/1/22) second spray was carried out. In control no chemical was sprayed. Percent disease index (PDI) was documented with the help of a disease scale [13] before the spray, and 12 days after each spray, and yield was calculated separately. The data were analysed statistically, using arc sin transformation for PDI calculated using the key assessment and RCBD calculation for analysis [14].

RESULTS

Host range of the pathogen

The host range studies of distinct *Alternaria* species on sunflowers were conducted using plants of the asteraceae family usually the weeds which could serve as a alternate host in noncropping season. First symptoms appeared at distinct days for each inoculum (UASB-1, UASB-2, UASB-3, HCA-3, CS-2, and CS-3) (Table 1).

The African marigold, Safflower, China aster, Billygoat weed, Telegraph weed, and Parthenium exhibited more susceptibility with specific symptoms development by *A. alternata* (UASB-1 isolates). Whereas, African marigold, Safflower, China aster, and Parthenium revealed moderate susceptibility with less symptoms development upon inoculation by *A. tenuissima* (UASB-2 isolates) and *A. helianthi* (UASB-3). Billygoat and Telegraph weeds did not exhibit development of symptoms for *A. tenuissima* and *A. helianthi* (Table 2 and Fig. 2). The remaining three *Alternaria* species - *A. burnsii* (CS-3), *A. solani* (HCA-3) and *A. gossypina* (CS-2) did not infect or develop any infection on any of the host species studied.

However, the results obtained in this study unveiled that a few species of the pathogen is not host-specific and can infect other plants also. The severity of the symptoms was observed to be increased in African marigold, Safflower China aster and Parthenium but no further development of the symptoms was seen in Billygoat and Telegraph weeds.

5. *In vitro* evaluation of new fungicide molecules against *A. alternata*

The efficacy of five combi products *viz.*, Fluopyram 17.7 % + Tebuconazole 17.7 % 400 SC, Azoxystrobin 18.2 % + Difenconazole 11.4 % SC, Boscalid 25.2 % + Pyraclostrobin 12.8 % WG, Dimethomorph 12 % + Pyraclostrobin 6.7 %, Picoxystrobin 7.05 % + Propiconazole 11.71 % SC and one systemic fungicide Fluxapyroxad 33 % W/V evaluated against *A. alternata* at five varied concentrations (100 ppm, 250 ppm, 500 ppm, 750 ppm, and 1000 ppm) under *in vitro* by the poisoned food technique revealed the mean mycelial growth suppression ranging from 36.59 to 100 percent in total (Table 3; Fig. 2 & Fig. 3). Highest mycelial growth inhibition (100 %) was observed in Fluopyram 17.7 % + Tebuconazole 17.7 % 400 SC under all concentrations assayed and it was on par to Picoxystrobin 7.05 % + Propiconazole 11.71% SC (89.40 %). The least mycelial growth inhibition (54.07 %) was recorded in Dimethomorph 12 % + Pyraclostrobin 6.7 %.

A significant difference has been perceived concerning concentrations assayed, 1000 ppm recorded the highest percent of growth inhibition (100 %) while lowest prohibition was noticed in 100 ppm (35.56 %), maximum mycelial growth inhibition (100 %) was recorded with Fluopyram 17.7 % + Tebuconazole 17.7 % 400 SC products at all concentrations out, Azoxystrobin 18.2 % + Difenconazole 11.4 % SC recorded least growth inhibition at 100 ppm (35.56 %).

6. Field evaluation of new fungicides against *A. alternata* infecting sunflower

The seed treatment with Fluxapyroxad FS @ 1.5 g/kg of seeds succeeded by foliar sprays with different combined fungicides was carried out to study their effect on *Alternaria* leaf spot disease. Seed treatment with Fluxapyroxad FS @ 1.5 g/kg seed subsequent by foliar spray with Fluopyram 17.7 % + Tebuconazole 17.7 % 400 SC @ 1 ml/L recorded lowest incidence of *Alternaria* leaf spot disease (7.36 %) followed by, T2 - Seed treatment with Fluxapyroxad FS @ 1.5 g/kg seed and foliar spray with Azoxystrobin 18.2 % + Difenconazole 11.4 % SC @ 1ml /L (11.23 %).

SA significant difference was not noticed in yield components among the different treatments. But the highest yield (2153 kg/ha) was recorded in T1-Seed treatment with Fluxapyroxad FS @ 1.5g/kg seed followed by foliar spray with Fluopyram 17.7 % + Tebuconazole 17.7 % 400 SC @ 1 ml/L recording the lowest incidence of *Alternaria* leaf spot disease (Fig. 4a & 4b).

DISCUSSION

Host-specificity assay is an a vital constituent present in all biological source of inoculum control programs and host range of any pathogen is a criteria that reveals its virulence, aggressiveness and host inclination of *Alternaria* spp. Which is true for a pathogens that were isolated from survey of sunflower growing fields in Karnataka. The studies revealed that African marigold, China aster and Safflower were found to be infected by *A. alternata*, *A. tenuissima*, and *A. helianthi* with distinct symptoms, but not by the other three newly recorded species on sunflower (*A. solani*, *A. burnsii* and *A. gossypina*) found in Karnataka. Whereas, *A. alternata* was the only pathogenic species that infected on Parthenium, Billygoat and Telegraph weeds which exhibited moderate to susceptible symptoms. This can be due to crop species inherent capacity to resist the invading pathogen, i.e. non-host resistance.

The findings of host range study were similar to [15-17] studies on *A. helianthi*, *A. tenuissima* and *A. alternata*. Where in the research demonstrated that three species from each study have a wide range of hosts and are also capable of infecting non-host plants, which include weeds (*Xanthium strumarium*) and plants (barnyard millet, finger millet, foxtail millet ,and rice) from different families. Sometimes the pathogen is naturally exposed to plants other than the target and infected [18].

The virulence pattern of *Alternaria* isolates from various geographic regions can also change its infection ability on the same and related plants. [19] analyzed the virulence of *A. solani* and *A. alternata* on tomato and potato crops revealed that, in every trial, *A. solani* isolates displayed high virulence, whereas *A. alternata* isolates exhibited minimal or no symptoms post-inoculation. The studies suggested that the plant pathogen virulence also influenced by various factors like environmental conditions and cropping systems [20-21].

Furthermore, it has been discovered that the pathogenicity of *A. cinerariae* is not strictly confined, as previously assumed, to the Senecio genus. This preference for specific host tribes is noteworthy because many *Alternaria* species typically have limited host ranges, often restricted to an individual genus or family. For instance, *A. alstroemeriae* E.G. Simmons & C.F. Hill affects *Alstroemeria* plants [22], while *A. brassicae* targets plants within the Brassicaceae family [23]. The host selectivity, pathogenicity, and virulence of *A. alternata* and other species are the result fro, the production of host-specific toxins [24]. *Alternaria* is a ubiquitous genus in many ecosystems,

encompasses saprophytic, pathogenic and even endophytic species, making it a diverse source of secondary metabolites. The synthesis of both host-specific toxins (HSTs) and non-host-specific toxins (NHSTs) emerges as a critical factor driving its survival and compatibility for non-host infectivity of these *Alternaria* species [25]. Non-host-specific toxins induce mild phytotoxic effects across a wide range of plant species and are considered an additional factor contributing to disease, complementing penetration mechanisms and enzymatic processes. While they typically function as virulence factors, exacerbating the severity of disease symptoms, there are instances where they are not essential for initiating disease, as they can also be toxic to plant species beyond the pathogen's host range. They might simply prepare the host for the onset of the disease [26].

Overall, *Alternaria* species typically act as foliar pathogens, gradually damaging host tissues by diminishing their photosynthetic capability. Infection prompts the development of necrotic lesions, occasionally exhibiting a target-like pattern due to growth disruptions induced by adverse environmental situations. The fungus inhabits the center of the lesion, encircled by an uninvaded chlorotic halo, a typical observed symptoms in the disease progression of necrotrophic pathogens. This zone forms due to the distribution of fungal metabolites, such as toxins, which serve as a symptomatic factor in certain infection processes [27-28]. Correspondingly, cross-pathogenicity of *F. oxysporum* was recently highlighted on Solanaceae and Cucurbitaceae crops, possibly facilitated by horizontal gene transfer of pathogenicity from other formae speciales of *F. oxysporum* [29].

The present studies provided information on the pathogenic infectivity of *A. alternata* on *P. hysterophorus* a common weed found in the sunflower growing field, which act as collateral host during offseason for pathogen survival.

Additionally, several investigations unveiled that many Asteracea plants are infected with several *Alternaria* species. [30-35]. The study deciphers the cross infectivity of polyphagous and virulent strain of *A. alternata* and *A. tenuissima* isolated from sunflower, infecting on other plants of same family. However, *A. helianthi*'s host specificity was not limited to sunflowers; it may also infect and produce symptoms on other crops in the same family (Marigold, Safflower, China aster).

The *in vitro* testing of fungicides gives valuable data on their ability to effectively combat a pathogen in the shortest period of time, and as a result, The subsequent field testing gives good management of disease, 5 combi products evaluated at five different concentration (100, 250, 500, 750 and 1000 ppm) revealed, Fluopyram 17.7 % + Tebuconazole 17.7 % at 100, 250, 500, 750, 1000 ppm (100 %) were more effective exhibiting cent per prohibition of mycelial growth than other fungicides. *In vitro* evaluation of three commercial fungicides from distinct categories on *Fusarium proliferatum* revealed that Fluopyram 20 % + Tebuconazole 20 % and Tebuconazole 50 % + Trifloxystrobin 50 % demonstrated significant efficacy in reducing the mycelial growth with EC50 values <2 ppm [36].

The field assessment of the new fungicide molecules recorded the lowest incidence of *Alternaria* leaf spot and the highest yield in Seed treatment with Fluxapyroxad FS @ 1.5 g/kg seed followed by foliar spray with Fluopyram 17.7% + Tebuconazole 17.7% 400 SC @ 1 ml/L (7.36 % PDI & 2153 Kg/ha) followed by, Seed treatment with Fluxapyroxad FS @ 1.5 g/kg seed followed by foliar sprays with Azoxystrobin 18.2 % + Difenconazole 11.4 % SC @ 1ml /L (11.23 % PDI & 2113 Kg/ha).

The study updates the use of combi products in field management during initiation of the disease for further prevention of disease spread and severity in the field.

[37] reported that lowest leaf spot severity of sunflower was recorded in seed treatment with Carbendazim 25 % + Iprodione 25 % @ 0.2 ppercentand two sprays of Propiconazole @ 0.1 per cent at 30 and 45 DAS resulting in the lowest incidence (18.33 and 33.3 per cent respectively) and highest disease reduction (73.9 and 53.6 percent respectively). Field evaluation of three fungicides on leaf spot and fruit rot of Pomegranate in replicated field revealed that Fluopyram 17.7 % + Tebuconazole 17.7 % and Fluxapyroxad 250 G/L + Pyraclostrobin 250 G/L reduced disease by 88.4 and 66.6 percent respectively In comparison to the untreated control [38]. These reports are following the observations on controlling of *Alternaria* pathogen under field conditions in sunflowers.

Future studies on host range studies of *Alternaria* species in sunflower can explore integrated management strategies, including fungicide application. This research will contribute to developing effective management practices to mitigate yield losses and maintain crop quality in sunflower cultivation.

CONCLUSION

The host range studies revealed that some of the pathogen species lack host specificity and have the ability to infect other plants of Asteraceae family. *A. alternata*, *A. tenuissima*, and *A. helianthi* showed symptoms of infection on African marigold, China aster, Safflower and Parthenium. However, Billygoat and Telegraph weed were mildly susceptible to *A. alternata* infection. The remaining three uncovered *Alternaria* species – *A. solani*, *A. burnsii*, *A. gossypina* and *A. gossypina* failed to invade or create any infection on these host species studied. Thus, *A. burnsii*, *A. solani* and *A. gossypina* are species that are host specific and non-host resistance may be exhibited by these host species for the pathogen. The study revealed the sources of potential inoculum and also their survival during off season on non-host species, which also advances our understanding of the host and non-host boundaries for *Alternaria* species infection.

The *in vitro* (100 % prohibition of mycelial growth) and *in vivo* (lowest incidence of *Alternaria* leaf spot and highest yield) evaluation studies recorded in Fluopyram 17.7 % + Tebuconazole 17.7 % 400 SC as effective fungicide and to be recommended for field condition compared to other new combination fungicide molecules. Hence, the same fungicide was included for the control of leaf spot disease on sunflowers under field circumstances.

Fresh insights into host range and cross-infectivity of these diversified pathogenic species will be beneficial for choosing appropriate strategies to manage the disease using the new-generation fungicide molecules.

Supplementary data

Supplementary data is available in the table and Figure

Acknowledgments

We are thankful to Y. M. Somashekara, S. D. Nehru, CoA, UAS, GKVK, Bengaluru-65, for providing facilities to conduct the experiments of the student's thesis work as well as for the research project.

Funding

All India Coordinated Research Project on sunflower and Directorate of Research, UAS, GKVK, Bangalore -560065.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have approved to influence the work reported in this paper.

Ethical statement

All the experimental procedures involving only on plant species were conducted following the University of Agricultural Science, Bangalore institutional guidelines. There are no human and animal subjects/trials conducted in this article and informed consent is not applicable.

Disclosure statement

The authors declare that there are no financial/commercial conflicts of interest.

Author contributions

Divyashree (Conceptualization [supporting], Data curation [lead], Formal analysis [lead], Investigation [lead], Visualization [lead], Writing –original draft [lead]), C. P. Manjula (Supervision [supporting], Validation [equal], Writing –review & editing [equal]), Rahul L. Joshi and (Supervision [supporting], Validation [supporting], C. H. Sai Bhavana (conceptualization [supporting], Supervision [supporting], Formal analysis [supporting], Jayashree Anandakumar (Conceptualization, Data curation, Writing –review & editing- supporting) and J. Harish (Writing –original draft [supporting], Data curation [supporting]).

Table 1: Days required for the symptom's appearance on various host species by inoculation of *Alternaria* species/isolates

Sl. No.	Common name (Scientific name)	Reaction						Days to initial symptoms					
		UASB-1	UASB-2	UASB-3	HCA-3	CS-2	CS-3	UASB-1	UASB-2	UASB-3	HCA-3	CS-2	CS-3
1	African marigold (<i>Tagetes erecta</i>)	++	+	+	-	-	-	3	5	5	-	-	-
2	Safflower (<i>Carthamus tinctorius</i>)	++	+	++	-	-	-	8	9	3	-	-	-
3	China Aster (<i>Callistephus chinensis</i>)	++	+	+	-	-	-	8	5	6	-	-	-
4	Billygoat weed (<i>Ageratum conyzoides</i>)	+	-	-	-	-	-	15	-	-	-	-	-
5	Telegraph weed (<i>Heterotheca grandiflora</i>)	+	-	-	-	-	-	-	-	-	-	-	-
6	Parthenium / Santa Maria (<i>Parthenium hysterophorus</i>)	++	+	+	-	-	-	3	5	-	-	-	-

Note: - Not infected, + Infected, ++ Severe infection

Table 2: Symptoms recorded by each *Alternaria* species/isolates on various host plants

Sl.No	Host plants	Symptoms observed on various host plants upon infection by <i>Alternaria</i> species		
		<i>A. alternata</i>	<i>A. tenuissima</i>	<i>A. helianthi</i>
1.	African marigold	Initial symptoms are small, round, yellow, brown, or black spots, frequently with concentric rings. First emerge on lower leaf, specifically on the leaf edge. It also infects flower buds with black necrotic symptoms.	Small, irregular, light to dark purplish-coloured circles with a yellow halo that later turned dark brown.	Very little infection on leaves with light necrotic lesions.
2.	Safflower	Young leaves of inoculated plants recorded lesions that appear in colour from brown to black and have noticeable yellow halos. Subsequently dark specks grow into bigger deep lesions that are oval to diamond-shaped	Minute symptoms were noticed during early infection with light necrotic lesion later there was no additional symptom development.	Symptoms appear as circular, dark brown to black lesions with concentric rings that resemble a target pattern with light grey centre.
3.	China Aster	Patches on foliage are light brown to dark, 1 to 2 mm in diam, and had concentric rings in the early stages.	Brown lesions first appeared, circular to irregular. Later, they did not enlarge and were seen restricted only on to the leaf margins.	Infection begins in the leaf margin as tiny lesions that later develops into a severe dark brown blighted areas.
4.	Billygoat weed	No apparent spot-like pattern on leaves, symptoms began to develop towards the margin as a blighted pattern with no specific spot like pattern.	No symptoms.	No symptoms.
5.	Telegraph weed	Development of typical leaf spot is seen under favourable environmental conditions, spots continue to grow in size and turn from red to dark brown.	No symptoms.	No symptoms.
6.	Parthenium	Irregular dark brown to black lesions on the leaves that later grew larger in size and spread to other parts of the leaves.	Tiny light brown spots with yellow halo.	The symptoms on leaves were as spots of light brown, erratic, and spherical shape which dispersed throughout the leaves.

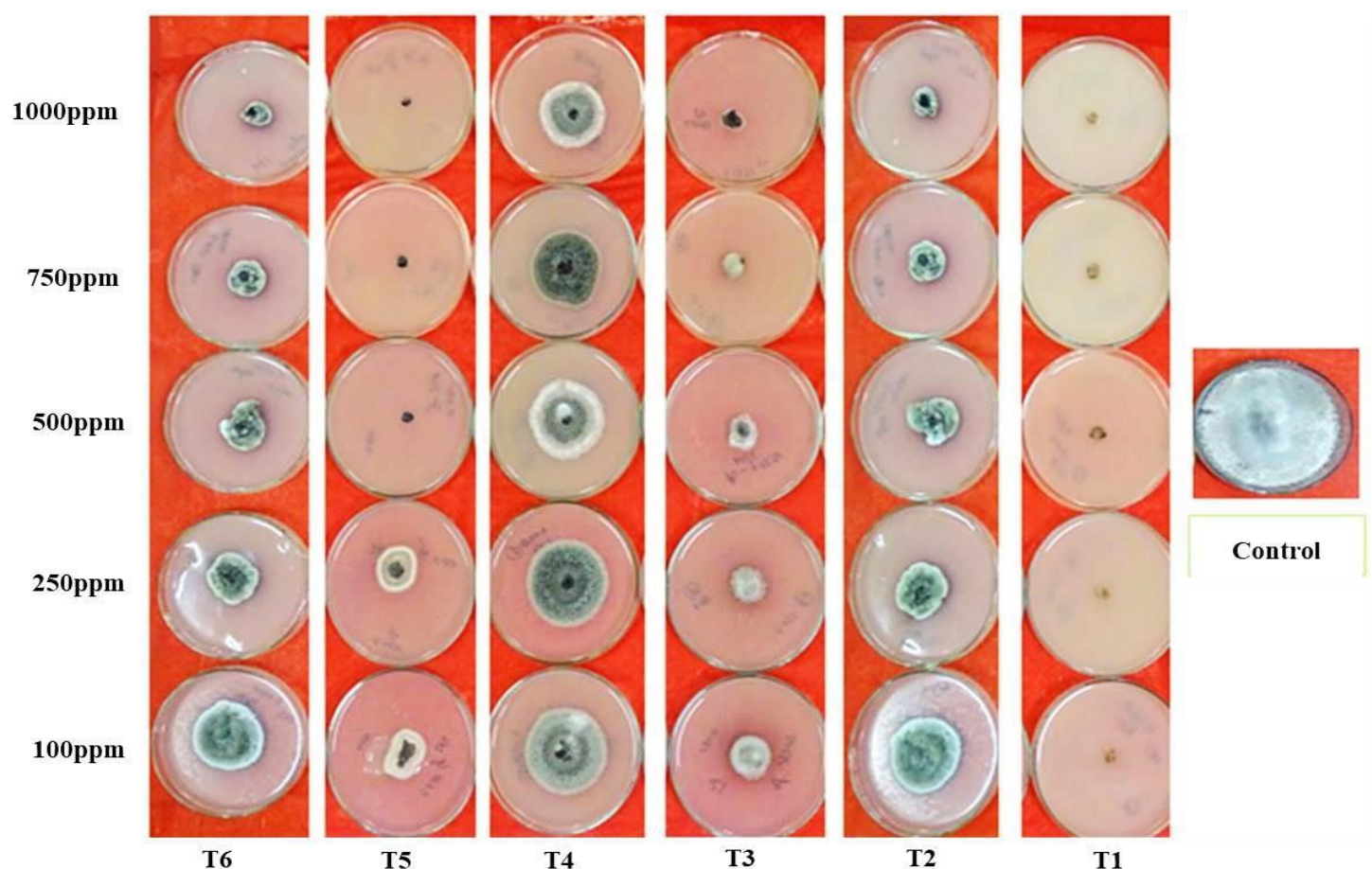
Table 3: In vitro evaluation of new fungicides against *A. alternata* infecting sunflower

Tr. No.	Fungicides	Per cent inhibition over control**					Mean
		Concentration (ppm)					
		100	250	500	750	1000	
T ₁	Fluopyram 17.7%+ Tebuconazole 17.7% (400 SC)	100 (89.96)	100 (89.96)	100 (89.96)	100 (89.96)	100 (89.96)	100 (89.96)
T ₂	Azoxystrobin 18.2% + Difenconazole 11.4% SC	35.56 (36.59)	52.96 (46.68)	70.37 (57.00)	75.19 (60.10)	90.74 (72.26)	64.964 (54.52)
T ₃	Boscalid 25.2% + Pyraclostrobin 12.8% WG	73.7 (59.13)	75.56 (60.34)	87.41 (69.19)	91.48 (73)	95.19 (77.29)	84.668 (67.79)
T ₄	Dimethomorph 12% + Pyraclostrobin 6.7%	42.59 (40.72)	46.67 (43.07)	51.11 (45.62)	59.63 (50.53)	70.37 (57.00)	54.074 (47.38)
T ₅	Picoxystrobin 7.05%+ Propiconazole 11.71% SC	69.63 (56.54)	77.41 (61.60)	100 (89.96)	100 (89.96)	100 (89.96)	89.408 (77.60)
T ₆	Myclobutanil 10% WP	44.07 (41.58)	65.56 (54.04)	64.44 (53.37)	79.26 (62.88)	87.41 (69.19)	68.148 (56.21)
Mean		60.925 (54.08)	69.693 (59.20)	78.83 (67.51)	84.26 (71.0)	90.61 (75.94)	76.87 (65.58)
Source		S.Em ±			C.D. (P=0.01)		
Fungicide (F)		0.27			1.00		
Concentration (C)		0.24			0.91		
F×C		0.60			2.24		

Note * Mean of three replications; Figures in parenthesis are arc sine transformed values

Table 4: Field evaluation of new fungicides against *A. alternata* infecting sunflower

Trt. No.	Treatment	<i>Alternaria</i> leaf spot (%Disease severity)	Seed yield (Kg/ha)	B:C ratio
T ₁	Seed treatment with Fluxapyroxad FS @ 1.5 g/kg seed followed by foliar spray with Fluopyram 17.7 %+ Tebuconazole 17.7 % (400 SC) @ 1 ml/L	7.36 (15.74)	2153	1: 1.74
T ₂	Seed treatment with Fluxapyroxad FS @ 1.5 g/kg seed followed by foliar sprays with Azoxystrobin 18.2 % + Difenconazole 11.4 % SC @1ml /L	11.23 (19.58)	2113	1:1.73
T ₃	Seed treatment with Fluxapyroxad FS @ 1.5 g/kg seed followed by foliar sprays with Boscalid 25.2 % + Pyraclostrobin 12.8 % WG @ 1g/L	12.29 (20.52)	2010	1:1.70
T ₄	Seed treatment with Fluxapyroxad FS @ 1.5 g/kg seed followed by foliar spray with Dimethomorph 12 % + Pyraclostrobin 6.7 % @1.5 g/ L	18.75 (25.66)	2197	1:1.68
T ₅	Seed treatment with Fluxapyroxad FS @ 1.5 g/kg seed followed by foliar spray with Picoxystrobin 7.05 %+ Propiconazole 11.71 % SC @ 2 g/L	14.68 (22.53)	1973	1:1.71
T ₆	Seed treatment with Fluxapyroxad FS @ 1.5 g/kg seed followed by foliar sprays with Myclobutanil 10 % WP @ 0.5 g /L	20.42 (26.86)	1865	1:1.68
T ₇	Control	23.98 (29.32)	1688	-
	CV (%)	7.24	0.338	
	SEM+/-	0.649	3.904	
	CD@ 5%	2.023	12.62	

**Fig. 2: Effect of new fungicide molecules on the inhibition of radial growth of *A. alternata***

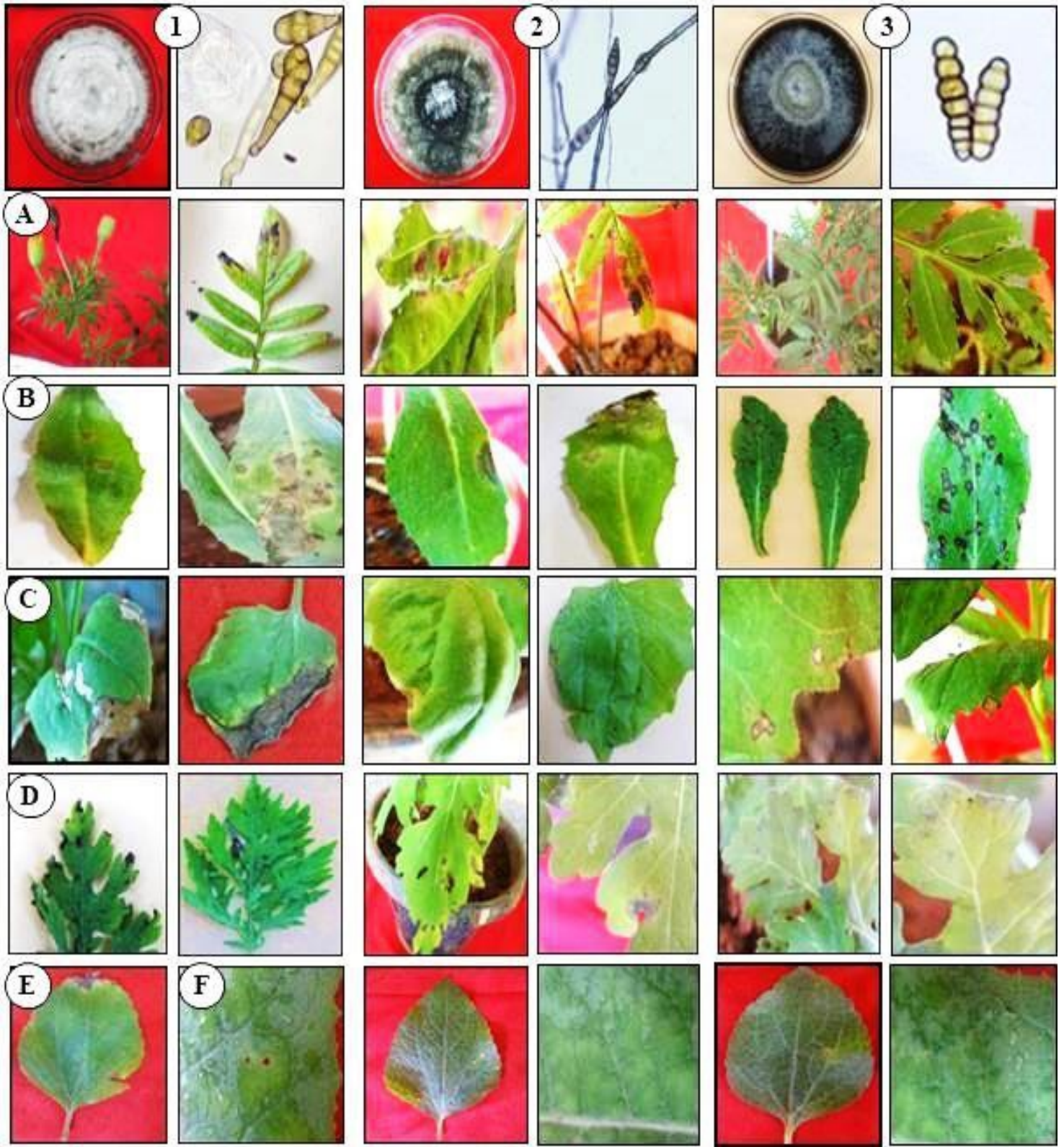


Fig. 1: Symptoms on various host plants by *Alternaria* species

Symptoms developed by Species such as *A. alternata* (1), *A. tenuissima* (2) and *A. helianthi* on crops such as Marigold(A), Safflower(B) and Aster(C) weeds include (Parthenium), Billygoat(E) and Telegraph weed.

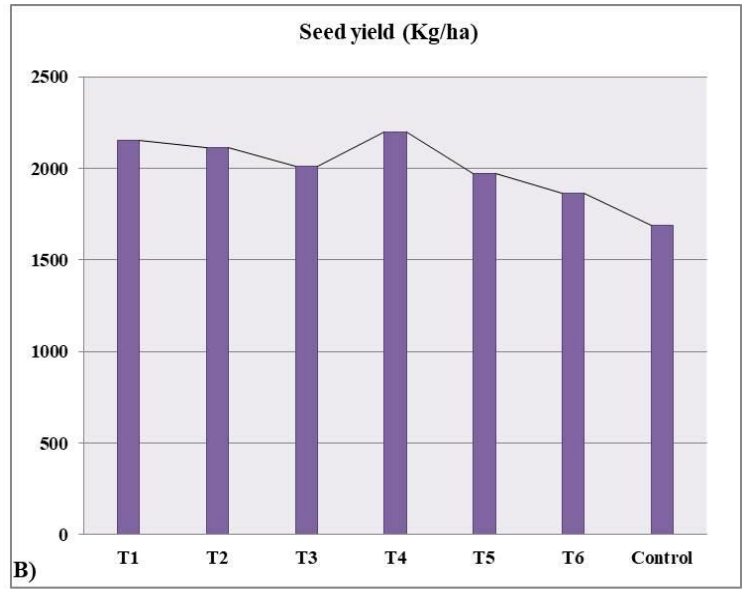
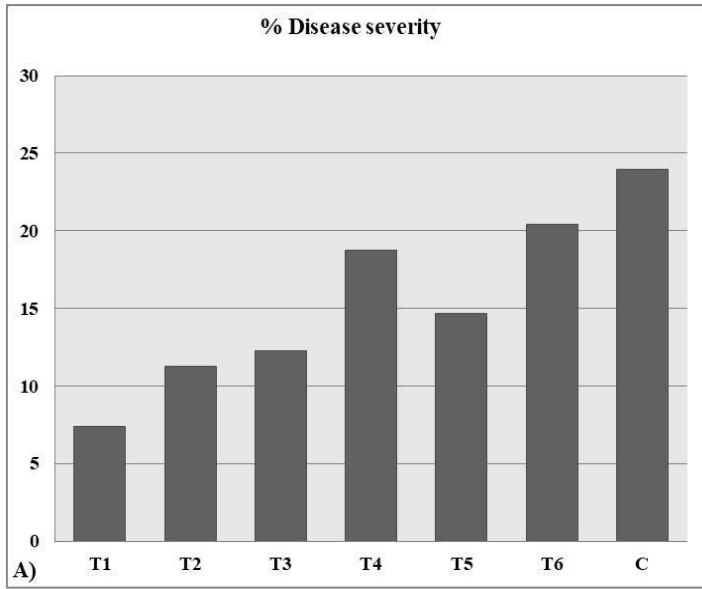


Fig 4a: Effect of different new fungicides on disease severity of sunflower leaf blight

Fig 4b: Effect of different new fungicides on seed yield of sunflower

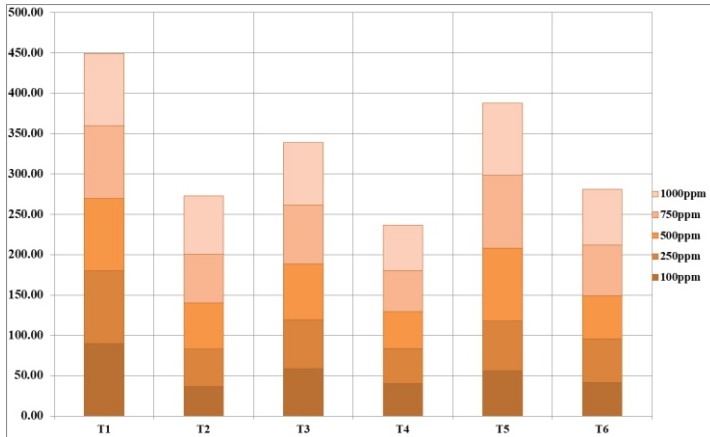


Fig 3: Effect of new fungicides on inhibition of radial growth of *A. alternata*



Supplementary data (Figure)

Fig S1.: Effect of new fungicides on *Alternaria* spot incidence in field condition

Supplementary data (Tables)

Table S.1: The details of fungicides evaluated in field condition

Sl. No	Treatment	Details
1	T ₁	Seed treatment with Fluxapyroxad FS @ 1.5 g/kg seed followed by foliar spray with Fluopyram 17.7 % + Tebuconazole 17.7 % 400 SC @ 1 ml/L
2	T ₂	Seed treatment with Fluxapyroxad FS @ 1.5 g/kg seed followed by foliar sprays with Azoxystrobin 18.2 % + Difenconazole 11.4 % SC @ 1ml /L
3	T ₃	Seed treatment with Fluxapyroxad FS @ 1.5 g/kg seed followed by foliar sprays with Boscalid 25.2 % + Pyraclostrobin 12.8 % WG @ 1 g/L
4	T ₄	Seed treatment with Fluxapyroxad FS @ 1.5g/kg seed followed by foliar spray with Dimethomorph 12 % + Pyraclostrobin 6.7 % @1.5 g/L
5	T ₅	Seed treatment with Fluxapyroxad FS @ 1.5 g/kg seed followed by foliar spray with Picoxystrobin 7.05 % + Propiconazole 11.71 % SC @ 2 g/L
6	T ₆	Seed treatment with Fluxapyroxad FS @ 1.5 g/kg seed followed by foliar sprays with Myclobutanil 10 % WP @ 0.5 g /L
7	T ₇	Control

REFERENCES

1. Anonymous. (2020). Ministry of Agriculture, Govt. of India. www.indiastat.com.
2. Anonymous. (2016). Package of Practices, UAS, Raihcur, 159.
3. Hansford, C.G. (1943). Contributions towards the fungus flora of Uganda, fungi imperfecti, In: Proc. Linnean Soc. London, 154th Session 67:34-67.
4. Carson, M.L. (1985a), Epidemiology and yield loss associated with *Alternaria* blight of sunflower. *Phytopathology*. 75:1151-1155.
5. Tubaki, K.; Nishihara, N. (1969). *Alternaria helianthi* (Hansf.) comb. Nov. *Trans Br Mycological Society*, 53:147-149.
6. Allen, S.J.; Brown, J.F.; Kochman, J.K.; (1983). Effect of temperature, dew period and light on the growth and development of *Alternaria helianthi*. *Phytopathology*, 73:893-896.
7. Kong, G.A.; Kochman, J.K.; Brown, J.F. (1995). A greenhouse assay to screen sunflower for resistance to *Alternaria helianthi*. *Annual Applied Biology*, 127:463-478.
8. Prathuangwong, S.; Kao, S.W.; Sommartya, T.; Sinchaisri, P. (1991). Role of four *Alternaria* spp. causing leaf and stem blight of sunflower in Thailand and their chemical controls. *Kasetsart Journal of Social Science*, 25:112-124.
9. Wang, T.; Zhao, J.; Sun, P.; Wu, X. (2014). Characterization of *Alternaria* species associated with leaf blight of sunflower in China. *European Journal of Plant Pathology*, 140:301-315.
10. Kgatle, M.G.; Truter, M.; Ramusi, T.M.; Flett, B.; Aveling, T.A.S. (2019). *Alternaria alternata*, the causal agent of leaf blight of sunflower in South Africa. *European Journal of Plant Pathology*, 151:677-688.
11. Nene, Y. L.; P. N. Thapliyal. (1973). "Fungicide in plant diseases control 2nd edition," 325.
12. Vincent, J.M. (1947). Distribution of fungal hyphae in the presence of certain inhibitors. *Nature* 159:850.
13. Mayee, C.D.; Datar, V.V. (1986). *Phytopathometry*. Tech. Bull.-1, Marathwad Agric Univ Parbhani 251.
14. Mckinney, H. (1923). Influence of soil temperature and moisture on infection of wheat seedlings by helmin. *Journal of Agricultural Research*, 26:195.
15. Quimby, P.C. (1989). Response of common cocklebur (*Xanthium strumarium*) to *Alternaria helianthi*. *Weed technology*, 3:177-181.
16. Roopa, R.S.; Yadahalli, K.B.; Kavyashree, M.C. (2014). Evaluation of natural plant extracts, antagonists and fungicides against early blight caused by *A. Solani* *in vitro*. *Bioscan* 9:1309-1312.
17. Hariprasad, K.; Nagaraja, A.; Patil, S. (2018). Host range of *Alternaria tenuissima* incitant of Kodo blight. *Journal Mycopathological Research*, 56:153-155.
18. Watson, A. K., Delfosse, E.S. (1984). Host specificity of plant pathogens in biological weed control. In *Proceedings of the VI International Symposium on Biological Control of Weeds*, 577-586.
19. Stammler, G., Bohme, F., Philippi, J., Miessner, S. and Tegge, V. (2014). Pathogenicity of *Alternaria* species on potato and tomato. PPO Special Report 16: 85-96.
20. Laloi, G.; Montarry, J.; Guibert, M.; Andrivon, D.; Michot, D.; Le, May; C. (2016). Aggressiveness changes over time in populations of *Didymella pinodes* over winter and spring pea cropping seasons. *Applied Environmental Microbiology*, 82:4330-4339.
21. Suffert, F.; Goyeau, H.; Sache, I.; Carpentier, F.; Gelisse, S.; Morais, D.; Delestre, G. (2018). Epidemiological trade-off between intra- and interannual scales in the evolution of aggressiveness in a local plant pathogen population. *Evolutionary Application*, 11:768-780.
22. Nishikawa, J.; Nakashima, C. (2013). Taxonomic characterization and experimental host ranges of four newly recorded species of *Alternaria* from Japan. *Journal of Phytopathology*, 161: 604e616.
23. Bains, P.S.; Tewari, J.P. (1987). Purification, chemical characterization and host-specificity of the toxin produced by *Alternaria brassicae*. *Physiological and Molecular Plant Pathology*, 30:259271.
24. Scheffer, R.P.; Livingston, R.S. (1984). Host-selective toxins and their role in plant diseases. *Science*. 223:17-21.
25. Wang, H.; Guo, Y.; Luo, Z.; Gao, L.; Li, R.; Zhang, Y.; Kalaji, H.M.; Qiang, S.; Chen, S. (2022). Recent advances in *Alternaria* phytotoxins: A review of their occurrence, structure, bioactivity, and biosynthesis. *Journal of Fungus*, 8:168.
26. Ballio, A. (1991). Non-host-selective fungal phytotoxins-biochemical aspects of their mode of action. *Experientia*, 47:783-790
27. Agarwal, A.; Garg, G.K.; Devi, S.; Mishra, D.P.; Singh, U.S. (1997). Ultrastructural changes in Brassica leaves caused by *Alternaria brassicae* and destruxin B. *Journal of Plant Biochemical Biotechnology*, 6:25-28.
28. Tewari, J.P. (1983). Cellular alterations in the blackspot of rapeseed caused by *Alternaria brassicae*. *Phytopathology*, 73:831.
29. Lopez-Orona, C.A.; Hernandez-Verdugo, S.; Velarde-Felix, S.; Garzon-Tiznado, J.A.; Sy, O.; Retes-Manjarrez. J.E. (2019). Cross pathogenicity of *Fusarium oxysporum* isolated from peppers. *European Journal Plant Pathology*, 154:1111-1123.

30. Chowdhury, S. (1944). An *Alternaria* disease of safflower. *Journal of Indian Botanical Science*, 23:59-65.
31. Mallikarjunaiah, R.R.; Rao, V.G. (1972). *Alternaria* Blight of Garden Asters. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/ Journal of Plant Disease Protection*, 702-709.
32. Tomioka, K.; Sato, T.; Koganezawa, H. (2000). Marigold leaf spot caused by *Alternaria tagetica* new to Japan. *Ethiopian Journal of Agricultural Science*, 66:294-298.
33. Park, K.S.; Lee, S.G. (2003). Leaf Spot of safflower (*Carthamus thinctorius*) caused by *Alternaria carthami* and *A. alternata*. *Research on Plant Disease*, 9:159-161.
34. Li, Y.; Shen, J.; Pan, B.H.; Guo, M.X.; Wang, Q.X.; Ouyang, C.B.; Yan, D.D.; Cao, A.C. (2014). First report of leaf spot caused by *Alternaria alternata* on marigold (*Tagetes erecta*) in Beijing, China. *Plant Dis* 98:1153-1153
35. Lee, N.H.; Shin, J.H.; Kim, H.Y.; Kim, S.H.; Kim, K.S. (2021). Isolation and Evaluation of Fungicides for the Control of *Alternaria alternata* Causing *Alternaria* Leaf Spot on Aster scaber and *Ligularia fischer*. *Journal of Agricultural Life Environmental Science*, 33:93-102.
36. Paton, L.G.; Marrero, M.; Llamas, D.P. (2017). *In vitro* and field efficacy of three fungicides against *Fusarium* bulb rot of garlic. *European Journal of Plant Pathology*, 148:321-328.
37. Karuna, K, Jagadish KS, Geetha KN, Shadakshari YG (2012) Evaluation of efficacy of chemical fungicides and a plant product for the management of *Alternaria* blight of sunflower. *Indian Phytopath* 65:305- 306
38. Xavier, K.V.; Kc, A. N.; Vallad, G. E. (2020). Fungicide application timing essential for the management of leaf spot and fruit rot on pomegranate in Florida. *Plant Disease*. 10:1629-1637.