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Article *in* Indian Phytopathology · March 2019

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RESEARCH ARTICLE

Sensitivity of different fungicides against isolates of *Alternariaster helianthi* (Hansf) Tubaki and Nishihara, leaf blight in sunflower

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Received: 11 September 2018 / Revised: 19 November 2018 / Accepted: 21 December 2018

Abstract
The potentiality of six fungicides viz., propiconazole, mancozeb, difenoconazole, hexaconazole, combination fungicides, iprodione + carbendazim, carbendazim + mancozeb was evaluated against 220 isolates of *Alternariaster helianthi* an incitant of leaf blight of sunflower. Blight affected leaf samples were collected, isolated and cultured on sunflower leaf extract medium specific for *A. helianthi.* Isolates were collected from seven different sunflower growing states of India viz, Andhra Pradesh, Bihar, Karnataka, Maharashtra, Tamil Nadu, Telangana and West Bengal to maintain the wide sample range and to include major disease prevalent areas. The average inhibition of fungal growth recorded with the test fungicides ranged from 34.9 (mancozeb) to 66.2% (propiconazole). Among them, propiconazole inhibited the mycelia growth more than 50% irrespective of the isolates tested followed by combination fungicide of carbendazim + mancozeb. Out of all, four fungicides showed more than 50% inhibition of mycelia growth/fresh weight (propiconazole > comb fungicide carbendazim + mancozeb > comb fungicide ipriodine + carbendazim > difenoconazole > hexaconozole > mancozeb). Cluster analysis and principle component analysis revealed that highly sensitive isolates were clearly scattered from others and these were collected from the state of Karnataka viz., *Ah*-39, *Ah*-85, *Ah*-87, from Bihar *Ah*-153, *Ah*-154, *Ah*-159 and from Andhra Pradesh *Ah*-61, *Ah*-62, *Ah*-63, *Ah*-64, *Ah*-10, *Ah*-145, *Ah*-146. Propiconazole and combination fungicide of carbendazim + mancozeb were the efficient group observed according to ANOVA carried out with the average fresh weight data of the 220 isolates against fungicides. Spraying of propiconazole and combi fungicide carbendazim + mancozeb were found effective for management of leaf blight of sunflower under pot culture conditions. This study reinforces the potential of the azole group act as antifungal agent against *A. helianthi.*

Keywords *Alternariaster helianthi* · Fungicides · Sunflower · Mycelial growth · Glass house studies · Sensitivity

Introduction

Sunflower is one of the important oilseed crops in India and occupies fourth place among oilseed crops in terms of acreage and production. Leaf blight caused by *Alternariaster helianthi* (Hansf). Tubaki and Nishihara is one of the major constraints in the profitable cultivation of sunflower in India. It has both pre and post harvest impact and has been found to cause 30–80% losses in seed yield and 17–33% reduction in oil content (Deokar et al. 2014). Extensive damage of sunflower crop was recorded in many parts of Andhra Pradesh with 57% yield loss (Srinivas et al. 1998). It is one of the most destructive and wide spread disease which causes 57–80% of yield loss under severe epiphytotic conditions (Hiremath et al. 1990; Mayee 1994; Mayee and Wankhede 1997; Balasubrahmanyam and Kolte 1980, Shankergoud et al. 2006). There are various weather factors that influences the disease severity and host can be affected at all stages of crop growth. Typical symptoms include chlorotic and black necrotic lesions on seedlings, leaves, stems responsible for low production and productivity of sunflower.

There is no known resistant variety/hybrid available against this disease. The sunflower cultivar found resistant at one place becomes susceptible at other place. This indicates the existence of races of the pathogen and also its evolution along with the introduction of new cultivars in major sunflower growing areas of the country. Diversity analysis of isolates of *A. helianthi* gives the information on variation in aggressiveness of the pathogen from different

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Published online: 06 March 2019

sunflower growing areas of the country. It has become inevitable to opt for fungicidal spray for management of the disease (Amaresh 2000). While common cultural practices may help to reduce the spread of the disease and the commonest practice against pathogenic *A*lternaria sp. mainly relies on the use of fungicides. Often many commonly used fungicides fail to give satisfactory control of leaf blight under field conditions, particularly during the rainy season (Saksena et al. 1979; Mukewar and Gera 1980). Therefore, it is essential to find out a potent fungicide, which would be effective in controlling the disease. In the present study, both individual and combination fungicides have been evaluated in in vitro conditions against 220 isolates of *A. helianthi*. Based on the inhibition of mycelial growth of the isolates by different fungicides, these were tested against leaf blight of sunflower under pot culture conditions.

Materials and methods

The fungicides were selected based on the literature of commonly used fungicide against leaf blight of sunflower. The fungicides evaluated in this study are four individual fungicides viz., propiconazole (tilt) 25% EC, difenoconazole (score) 25% EC, hexaconazole (contaf) 5% EC, Mancozeb (indofil M-45) 75% WP and two combination fungicides iprodione + carbendazim (quintal), carbendazim + mancozeb (SAAF).

Isolation of different isolates of *A. helianthi*

The sunflower leaves showing typical symptoms of leaf blight were collected from various places of seven different major sunflower growing states of India viz., Karnataka, Maharashtra, Tamil Nadu, Andhra Pradesh, Bihar, West Bengal and Telangana and designated as different isolates of *A. helianthi Ah*-1 to *Ah*-220. Identification of *A. helianthi* isolates was confirmed based on the morphology of conidia as given by Simmons (2007). All the isolates were purified by monospore isolation and maintained in the plates of host specific sunflower leaf extract medium (SLEM). Fresh subcultures were made and used for in vitro testing of fungicide sensitivity. Pathogencity of isolates was proved by whole plant assay method under glass house conditions (Santha Lakshmi Prasad et al. 2008).

In vitro evaluation of fungicides against *A. helianthi*

The relative efficacy of six fungicides were tested against 220 isolates of *A. helianthi* under laboratory conditions by poisoned food technique (Nene and Thapliyal 1993) with sunflower leaf extract broth at Division of Crop Protection, ICAR-Indian Institute of Oilseed Research, Hyderabad, Telangana State. 100 ml of sunflower leaf extract broth was poured in 250 ml conical flasks and autoclaved at 15 lb pressure for 15 min. All the fungicides mentioned in Table 1 were used at 50 ppm concentration. After cooling the medium, the required quantity of each fungicide was incorporated into each flask, except control. Three replications were maintained for each fungicide. The flasks were then inoculated with 7 mm mycelial discs cut from actively growing *A. helianthi* culture. The broth without incorporation of the fungicide served as control. The flasks were incubated in shaking incubator maintaining the temperature of 25 ± 2 °C for 7 days. After incubation period, the medium containing the mycelial growth of *A. helianthi* fungus was filtered through previously weighed Whatman filter paper No. 41. The mycelial mat on filter paper was oven dried at 60 °C for 24 h and weighed. The dry/fresh mycelial weight was calculated by subtracting weight of previously weighed filter paper from weight of filter paper with mycelial mat. Percent inhibition of mycelia growth was calculated using the formula of Vincent (1947):

$$\text{Percent inhibition} = \frac{C - T \times 100}{C},$$

where, C = weight of fungal colony in control (mg), and T = weight of fungal colony in treatment (mg). The experiment was carried out for all 220 isolates, with six fungicides.

Evaluation of fungicides under greenhouse conditions

The efficacy of fungicides was tested under greenhouse conditions against leaf blight by whole plant assay method (Santha Lakshmi Prasad et al. 2008). Sunflower seedlings were raised in pots containing red soil, sand, FYM in the ratio of 3:2:1 up to 25 days. Spore suspension was prepared from 9 days old *A. helianthi* culture grown on SLEM and adjusted to 1×10^6 spores/ml. Spore suspension was sprayed on sunflower

Table 1 In vitro efficacy of fungicides on mycelia growth and percent inhibition of *A. helianthi*

Fungicide	Mean growth (fresh weight in g)	% inhibition over control
Propiconazole	.36[a]	66.16
Difenoconazole	.50[b]	52.10
Hexaconazole	.54[b]	48.71
Mancozeb	.68[c]	34.93
Iprodione + Carbendazim	.49[b]	52.98
Carbendazim + Mancozeb	.38[a]	63.34
Control	1.05[d]	–
C.D (P = .05)	.026	

Fresh weight followed by common letter (a/b/c/d) are not significantly different at $P < 0.05$

plants with an atomizer and Tween-20 was added to the spore suspension which acts as a sticker. The control plants were maintained by spraying water only. Plants sprayed with spore suspension alone served as pathogen check. After spraying, all plants were covered with polyethylene cover to provide congenial humid conditions for development of disease for 1–2 days. The disease symptoms were initiated on the inoculated foliage 3 days after inoculation as small specks and specific fungicide for each treatment was sprayed 5 days after inoculation on each plant. Leaf blight data was recorded 5 days later the fungicide spray from each plant and scored for disease severity by following 0–9 scale of Mayee and Datar (1986) as given below: 0—no symptoms on the leaf; 1—small circular, scattered, brown spots covering 1% of the leaf area; 3—spots enlarging, dark brown in color covering 1–10% of the leaf area and infection on lower most leaves of the plant; 5—spots enlarging, dark brown in color covering 11–25% of leaf area and infection on half of the plant; 7—spots dark brown coalescing, occupying 26–50% of leaf area and covering 1/3 of the plant; 9—spots uniformly dark brown, coalescing, covering 50% or more leaf area, severe infection of all leaves and the head infected to a greater degree.

Percent disease index (PDI) was calculated by using the formula given by Wheeler (1969):

$$\text{PDI} = \frac{\text{Sum of numerical disease ratings}}{\text{No. of plants/leaves observed}} \times \frac{100}{\text{maximum disease rating value}}.$$

From this, percent disease index was computed as per standard formula. The experiment was repeated twice with three replications and ten plants per replication.

Statistical analysis

The percent inhibition of mycelial growth was calculated on fresh weight (g) basis. ANOVA has been carried out for the data of fresh weight measured from all the isolates against the fungicides analyzed under study. Sensitivity of the isolates collected from different states/regions was done using k-means cluster analysis with SAS 9.3 version for the six fungicides separately grouping 220 isolates into three clusters each. Principal component analysis (PCA) was performed for the evaluation of variability and sensitivity of isolates collected from different states in India and also from the different regions of the same state against the fungicides under testing.

Results and discussion

Six fungicides, in which four individual fungicides (hexaconazole, difenoconazole, mancozeb, propiconazole) and two combination fungicides (ipriodine + carbendazim; carbendazim + mancozeb) were evaluated (at 50 ppm) in in vitro against *A. helianthi*, applying poisoned food technique and using sunflower leaf extract as a basal broth. Effect of these fungicides on mycelial growth (as fresh weight) of the test pathogen over untreated control was recorded and the results obtained are presented. All the fungicides tested recorded a range of inhibition of mycelia growth of the test pathogen, depending upon their efficacy was summarized in Table 1. Among the fungicides tested, propiconazole recorded significantly highest inhibition of mycelia growth which accounted 66.16% reduction over the control (Fig. 1). Mallikarjun (1996) also reported that propiconazole was best among the fungicides tested against *Alternaria alternata.* Waghe et al. (2015) reported that maximum inhibition was recorded with propiconazole which was in support of the above mentioned evaluation. Mesta et al. (2009) reported that hexaconazole and propiconazole were significantly effective over all other fungicides with respect to inhibition of spore germination of *A. helianthi*. Similarly reported by Arun kumar (2008) that propiconazole and hexaconozole were highly effective in inhibition of mycelia growth against *A. alternata*. The second and third best fungicides found were combination fungicide carbendazim + mancozeb (63.34%) and ipriodine + carbendazim (52.98%). These were followed by difenoconazole (52.10%) and hexaconazole (48.71%). The efficacy of iprodione alone was previously reported by several workers. Rao (2006) reported the combi product iprodione with carbendazim was effective fungicide against *A. helianthi.* Amaresh (1997) reported iprodione as the most effective fungicide for the management of Alternaria blight of sunflower, tested both in laboratory and field conditions. The lowest inhibition of mycelia growth/fresh weight was recorded by mancozeb (34.93%).

In combination fungicides, carbendazim + mancozeb fungicide was found most effective in inhibiting the mycelial growth of *A. helianthi*, while in individual fungicides, propiconazole and hexaconazole were found most effective. The results obtained in present studies in respect to in vitro effect of fungicides on mycelial growth inhibition of the *A. helianthi* for the combination of carbendazim + mancozeb, mancozeb, propiconazole, and hexaconazole fungicides is similar with earlier workers (Amaresh and Nargund 2004; Akbari and Parakhia 2007; Mathivanan and Prabavathy 2007).

The mean values of the fresh weight of mycelium growth were highly significant for tested fungicides ($P < .05$) (Table 1). The mean values of the tested fungicides ranged from .36–1.05 g with an average of .57 g irrespective of isolates. The result of the present study concluded that

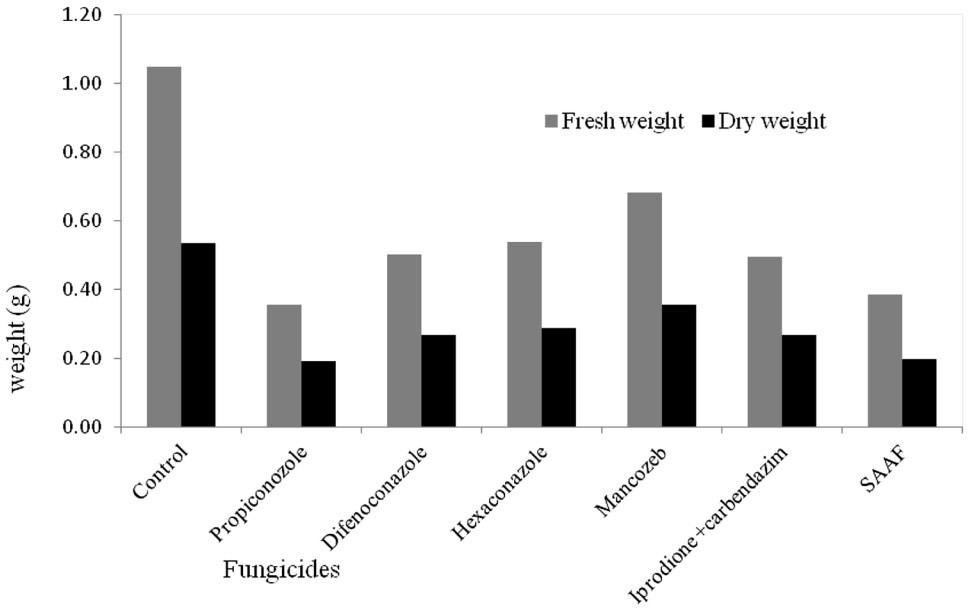

Fig. 1 In vitro evaluation of fungicides against isolates of *A. helianthi*. *Note* Fungicides were used at 50 ppm concentration

more than 50% (EC 50) of the mycelium growth was more effectively inhibited by four fungicides i.e., propiconazole, combi fungicide carbendazim + mancozeb, combi fungicide ipriodine + carbendazim and difenoconazole. Out of 220 *A. helianthi* collected from different areas, 92% isolates were effectively inhibited by fungicides under in vitro conditions.

Based on the results of fresh weights, K-means cluster analysis was done to know the sensitivity towards these fungicides on 220 isolates under study. Cluster analysis comparison of six different fungicides on mycelia growth of isolates revealed propiconazole and combi fungicide carbendazim + mancozeb are showing similar efficacies in controlling the growth was presented in Table 3. Based on the optimal cluster analysis (cubic clustering criterion) it was found that three clusters was optimum for grouping of the isolates. Similarly K-means clustering with three clusters was done for six fungicides separately. Cluster mean is lowest for cluster 1 i.e., .204 and the highest number of isolates under this cluster 127 indicates the best efficacy of propiconazole (Table 2) among the other, combi fungicide carbendazim + mancozeb with the same lowest cluster mean value of .204 grouped 121 isolates under that cluster (Table 2). It is also evident that combi fungicide carbendazim + mancozeb is quite effective for all the sunflower cultivated regions. Combi fungicide ipriodine + carbendazim has been observed as the third best with cluster mean of .264 including 130 isolates in the same cluster (Table 2). Least cluster mean value (.288) observed for difenoconazole with 144 isolates, while 137 isolates were clustered into one with lowest mean value (.301) by hexaconozole (Table 2). However least performed among the six fungicides, mancozeb with lowest cluster mean value (.400) added 156 isolates into a single cluster.

Among the *A. helianthi* isolates collected from different sunflower growing states of India, Karnataka i.e., *Ah*-130, *Ah*-131, *Ah*-48, *Ah*-98 collected from Bangalore, Kolar, Koppal, Hirapur regions, respectively, Maharashtra isolates *Ah*-105, *Ah*-107, *Ah*-108, *Ah*-109 from Kondi, two from Boramani and Kanapur *respectively*, Tamil Nadu isolate 74 collected from panjapatti, along with the isolate *Ah*-218 collected from West Bengal were clustered in a group with highest mean value clusters of all the six fungicides. Among all the isolates, the above mentioned has responded less to all the fungicides evaluated so it is suggested to use specifically either propiconazole or combi fungicide carbendazim + mancozeb in these specific regions as even though they were grouped under high mean cluster in these fungicides also but they were only around .986 and .939, respectively.

Propiconazole has clustered 77 isolates with mean .469 and 16 under cluster with mean .986. All the isolates from Bihar were clustered in the least cluster mean showing their sensitivity towards the chemical. Isolates from West Bengal *Ah*-213, *Ah*-218 and *Ah*-220 less sensitive to other fungicide were also less sensitive to propiconazole as they got clustered under high mean value group. Except two isolates (*Ah*-77, *Ah*-74) from Tamil Nadu and one isolate from Andhra Pradesh (*Ah*-19) all the other isolates got included within two low cluster mean (Table 2). Propiconazole is the best suggested in all regions apart from other chemicals as according to the clusters formed for this includes lowest number, i.e., 16 isolates which were under high mean cluster. Combination fungicide carbendazim + mancozeb has grouped 78 isolates in the second cluster with mean value .503 and only 21 isolates in the third cluster with mean value

Table 2 Cluster analysis for propiconazole, carbendazim + mancozeb, ipriodine + carbendazim, difenoconazole, hexaconazole

Fungicide	Cluster	Frequency	Mean	Standard deviation	Isolate number
Propiconazole	1	16	.986	.132	A—19; K—130, 131, 132, 48, 89, 98; M—105, 107, 108, 109; T—77, 74; WB—220, 213, 218
	2	127	.204	.073	A—10, 11, 13, 14, 144, 145, 146, 15, 16, 17, 20, 6, 61, 62, 63, 64, 8, 9 K—133, 134, 136, 137, 138, 139, 140, 142, 21, 26, 28, 31, 33, 34, 36, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 51, 52, 54, 55, 57, 58, 60, 82, 85, 87, 88, 90, 92, 93, 96, 99; M—101, 103, 104, 106, 111, 112, 114, 117 WB—197, 198, 199, 200, 201, 202, 204, 205, 206, 207, 208, 210, 214, 215, 217, 219; B—153, 154, 155, 156, 157, 158, 159, 160, 162, 164
	3	77	.469	.094	A—1, 12, 143, 147, 148, 149, 150, 18, 5, 7; B—151, 152, 161, 163; K—100, 129, 135, 141, 22,23, 24, 25, 27, 29, 30, 32, 35,37,38, 50, 53, 56, 59, 81, 83, 84,86, 91, 94, 95, 97; M—102, 110, 113, 115, 116, 119, 120, 121, 123, 126, 128, 166, 167, 2, 3, 4; T—169, 175, 176, 177, 178, 179, 182, 186, 187, 194, 196, 71, 75, 78, 79; WB—203, 209, 211, 212, 216
Carbendazim + mancozeb	1	121	.204	.080	A—10, 11, 14, 144, 145, 146, 15, 17, 6, 61, 62, 63, 64 B—153, 154, 155, 157, 158, 159, 160, 162, 164 K—100, 133, 134, 136, 137, 138, 139, 140, 142, 21, 22, 24, 27, 28, 31, 33, 34, 36, 39, 41, 42, 43, 44, 45, 46, 47, 49, 51, 52, 54, 55, 57, 58, 60, 82, 85, 87, 88, 89, 90, 92, 93, 96, 99; M—101, 104, 106, 111, 112, 114, 117, 118, 122, 124, 125, 127, 165, 65, 66 T—170, 171, 172, 173, 174, 180, 181, 183, 184, 185, 188, 189, 190, 191, 192, 193, 195, 67, 68, 69, 70, 72, 73; WB—197, 198, 199, 200, 201, 202, 204, 205, 206, 207, 208, 210, 211, 214, 215, 217, 219
	2	78	.503	.092	A—1, 12, 13, 143, 147, 148, 149, 150, 16, 18, 19, 20, 5, 7, 8, 9; B—151, 152, 156, 161, 163; K—129, 132, 135, 141, 23, 25, 26, 32, 35, 37, 38,40, 53, 56, 59, 81, 83, 84, 86, 91, 94, 95; M—103, 110, 113, 115, 116, 119, 120, 121, 123, 126,128, 166, 167, 168, 4; T—169, 175, 176, 177, 178, 179, 182, 186, 187,194, 196, 71, 76, 78, 79, 80; WB—203, 209, 212, 216
	3	21	.939	.109	K—130, 131, 29, 30, 48, 50, 97, 98 M-102, 105, 107, 108, 109, 2, 3; T—74, 75, 77; WB—213, 218, 220
Ipriodine + carbendazim	1	71	.694	.169	A—1, 143, 147, 149, 17, 19, 5, 6, 7, 8; B—151, 152, 156, 161, 163 K—100, 129, 135, 141, 23, 32, 35, 37, 38, 50, 53, 56, 59, 81, 83, 84, 86, 91, 94, 95 M—102, 103, 110, 111, 113, 116, 119, 120, 121, 122, 126, 166, 167, 168, 3, 4; T—169, 174, 175, 176, 177, 178, 179, 182, 186, 187, 194, 196, 71, 76, 77, 79, 80; WB—203, 209, 216
	2	130	.264	.099	A—10, 11, 12, 13, 14, 144, 145, 146, 15, 150, 16, 18, 20, 61, 62, 63, 64, 9; K—133, 134, 136, 137, 138, 139, 140, 142, 21, 22, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 36, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 51, 52, 54, 55, 57, 58, 60, 82, 85, 87, 88, 89, 90, 92, 93, 96, 99 M—101, 106, 112, 114, 117, 118, 124, 125, 127, 128, 165, 2, 65, 66 T—170, 171, 172, 173, 180, 181, 183,184, 185, 188, 189, 190, 191, 192, 193, 195, 67, 68, 69, 70, 72, 73, 78 WB—197, 198, 199, 200, 201, 202, 204, 205, 206, 207, 208, 210, 211, 212, 214, 215, 217, 219; B—153, 154, 155, 157, 158, 159, 160, 162, 164
	3	19	1.308	.134	A—148; K—130, 131, 132, 48, 97, 98 M—104, 105, 107, 108, 109, 115, 123; T—74, 75; WB—213, 218, 220

Table 2 (continued)

Fungicide	Cluster	Frequency	Mean	Standard deviation	Isolate number
Difenoconazole	1	17	1.376	.147	K—130, 131, 132, 48, 50, 84, 97, 98 M—102, 105, 107, 108, 109, 115; T—74; WB—218, 220
	2	59	.775	.168	A—1, 10, 12, 143, 147, 148, 18 B—151, 152, 156, 161, 163; K—129, 135, 22, 24, 25,27, 29, 30, 32, 37, 83, 91, 92, 94, 95; M—104, 111, 113, 116, 119, 120, 121, 123, 126, 128, 166, 167, 168; T—169, 174, 175, 176, 177, 178, 187, 194, 196, 71, 75, 77, 79, 80 WB—203, 209, 213, 216
	3	144	.288	.116	A—11, 13, 14, 144, 145, 146, 149, 15, 150, 16, 17, 19, 20, 5, 6, 61, 62, 63, 64, 7, 8, 9; B—153, 154, 155, 157, 158, 159, 160, 162, 164 K—100, 133, 134, 136, 137, 138, 139, 140, 141, 142,21, 23, 26, 28, 31, 33, 34, 35, 36, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 81, 82, 85, 86, 87, 88, 89, 90, 93, 96, 99 M—101, 106, 110, 112, 114, 117, 118, 122, 124, 125, 127, 165, 2, 3, 4, 65, 66 T—170, 171, 172, 173, 179, 180, 181, 182, 183, 184, 185, 186, 188, 189, 190, 191, 192, 193, 195, 67, 68, 69, 70, 72, 73, 76,78 WB—197, 198, 199, 200, 201, 202, 204, 205, 206, 207, 208, 210, 211, 212, 214, 215, 217, 219
Hexaconazole	1	65	.796	.184	A—1, 143, 147, 148, 149,20, 5, 6; B—151, 152, 156, 157, 161, 163 K—100, 129, 135, 21, 22, 24, 27, 32, 37, 40, 50, 53, 56, 83, 84,86, 91, 92, 94, 95; M—102, 103, 104, 111, 113, 116, 119, 120, 122, 166, 167, 168; T—169, 174, 175, 176, 177, 178, 179, 182, 186, 187, 194, 196, 77, 79, 80; WB—203, 213, 216, 220
	2	137	.301	.109	A—10, 11, 12, 13, 14, 144, 145, 146, 15, 150, 16, 17, 18, 19, 61, 62, 63, 64, 7, 8, 9 K—133, 134, 136, 137, 138, 139, 140, 141, 142, 23, 25, 26, 28, 29, 30, 31, 33, 34, 35, 36, 38, 39, 41, 42, 43, 44, 45, 46, 47, 49, 51, 52, 54, 55, 57, 58, 59, 60, 81, 82, 85, 87, 88, 89, 90, 93, 96, 99 M—101, 106, 110, 112, 114, 117, 118, 124, 125, 127, 128, 165, 2, 3, 4, 65, 66; T—170, 171, 172, 173, 180, 181, 183,184, 185, 188, 190, 191, 192, 193, 195, 67, 68, 69, 70, 71, 72, 73, 76, 78 WB—197, 198, 199, 200, 201, 202, 204, 205, 206, 207, 208, 210, 211, 212, 214, 215, 217, 219 B—153, 154, 155, 158, 159, 160, 162, 164
	3	18	1.410	.181	K—130, 131, 132, 48, 97, 98; M—105, 107, 108, 109, 115, 121, 123, 126; T—74, 75 WB—209, 218

A Andhra Pradesh, *B* Bihar, *K* Karnataka, *M* Maharashtra, *T* Tamil Nadu, *WB* West Bengal

.939 (Table 2). All the isolates from the states of United Andhra Pradesh and Bihar has shown sensitivity towards the fungicide by falling only in the first two clusters with mean values .204 and .503, respectively. Other than the three isolates each, all the isolates collected from Tamil Nadu and West Bengal, were under the first two clusters showing better sensitivity towards the same fungicide. Among the 37 isolates collected and tested from Maharashtra, 30 isolates were grouped under one and two clusters. Only 8 among the 74 isolates of Karnataka were grouped under the third cluster with mean value of .939. This shows the efficacy of combination fungicide carbendazim + mancozeb relatively better when compared with the other fungicides tested. Mancozeb is less suggested as the clusters formed were all with high mean values when compared with other fungicides evaluated, the three cluster means were 1.797, 1.133 and .400. However, six fungicides were found effective against the fungal mycelial growth when compared with the clusters with mean value .595, 1.628 and 2.590 in control (without chemical incorporated).

Principal component analysis revealed that PC1 and PC2 could explain 93% of variance, thus inferring only these two principal components would suffice to evaluate the diversity among the isolates. For carrying out the PCA results SAS 9.3 was used to plot the variables of isolates against the fungicides tested. PCA plot shows that there are two major groups (Fig. 2). For clarity the data of all states represented in separate graphs as in Fig. 3 and it is observed that the

groups include all the states. However, isolates from South 24 Paragana's region in West Bengal state *Ah*-213, *Ah*-218, *Ah*-220 showing dissimilarity from the other isolates of the same state with less sensitivity towards the fungicide which was in support with the clusters formed in the cluster analysis. Isolates from different regions of the Karnataka state (i.e., *Ah*-130, *Ah*-131, *Ah*-48, *Ah*-98 from Bangalore, Kolar, Koppal and Hirapur, respectively) has also shown diversity from the other isolates and they shows different response to fungicides. In Bihar *Ah*-151, *Ah*-152, *Ah*-156, *Ah*-163, *Ah*-161 collected from Dholi, Manipur, Kushiara and Garia were also proved to be responding less sensitive towards the fungicides. Except isolate 1 from Hyderabad, United Andhra Pradesh all the other isolates were responding in a similarly sensitive way against the fungicide. The isolates *Ah*-102, *Ah*-105, *Ah*-107 and *Ah*-108 from Solapur, Kondi and two isolates collected in Boramani from Maharashtra were different with the other isolates as with less sensitivity towards the fungicides. From Tamil Nadu *Ah*-75, *Ah*-74, *Ah*-77 collected from the regions Chinna Sengal and Panjapatti has also responded less sensitivity to the fungicide (Fig. 3). High sensitive isolates were clearly scattered from the components which were collected from the state of Karnataka viz., *Ah*-39, *Ah*-85, *Ah*-87, from Bihar *Ah*-153, *Ah*-154, *Ah*-159 and *Ah*-61, *Ah*-62, *Ah*-63, *Ah*-64, *Ah*-10, *Ah*-145, *Ah*-146 from Andhra Pradesh (Fig. 2).

According to the results revealed by Mathivanan and Prabavathy (2007) carbendazim and mancozeb can be used against the Alternaria leaf blight in sunflower. Singh (2000) also reported the use of fungicides in the management of sunflower Alternaria blight disease. Sudhakar (2000) found that, at higher concentration most of the fungicides inhibited maximum mycelial growth but their effect decreased with reduced concentration. The sterol biosynthesis pathway in fungi are known to inhibit by triazoles and carbendazim, being a benzimidazole fungicide (Nene and Thapliyal 1973). The efficacy of carbendazim against fungi is attributed to the inhibition of biosynthesis process and synthesis of DNA of fungi (Davidse 1973).

Under glass house conditions, the disease severity in two trails were subjected to ANOVA, the observed result showed that among the six fungicides evaluated propiconazole was recorded best with disease of 32.2% in first year as against 80.9% disease severity recorded in pathogen

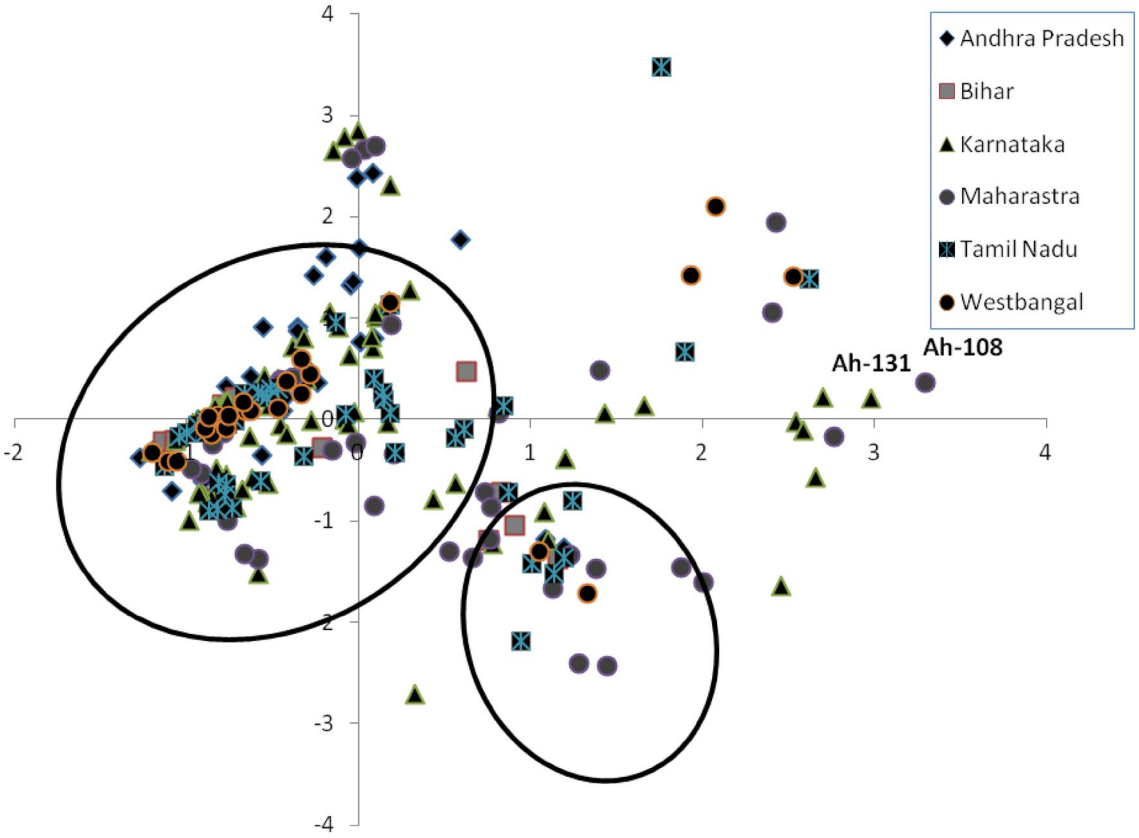

Fig. 2 Two dimensional non-linear map of principal component analysis (PCA) for the detection of similarity and dissimilarity matrix of isolates sensitivity against the fungicides under testing

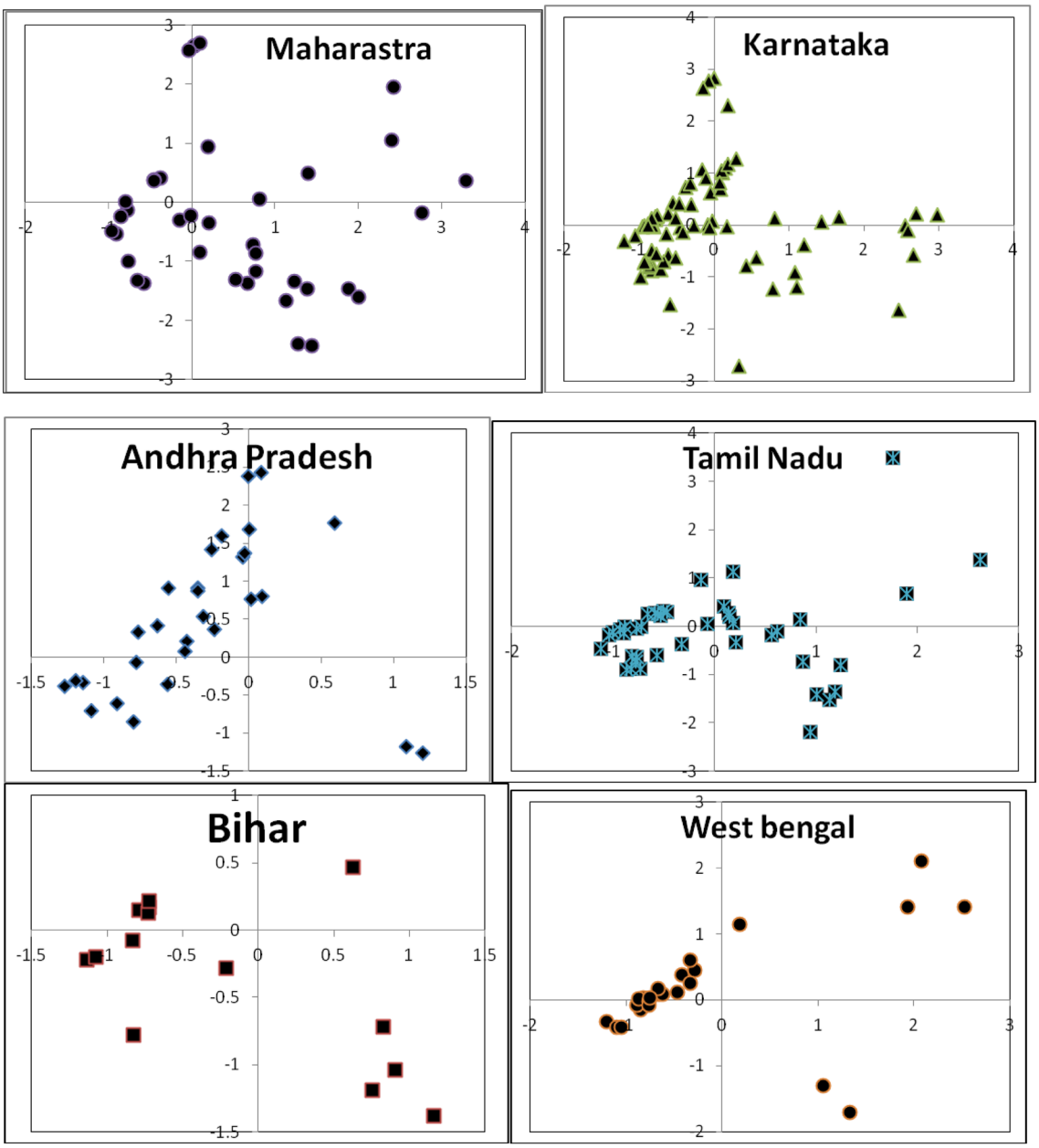

Fig. 3 Principal component analysis of isolates sensitivity against the fungicides under testing for *A. helianthi* collected from different states

check and 29.2% leaf blight in the second year against 75.9% disease severity observed in pathogen check. The results obtained in glass house studies were also identical with results recorded in in vitro screening stating that propiconazole as the best fungicide against highly aggressive *A. helianthi* pathogen (Table 3). Mesta et al. (2009) and Santha Lakshmi Prasad et al. (2015) reported that the foliar spray of fungicides, especially systemic fungicides like propiconazole gave maximum protection against leaf blight in sunflower. While second best fungicide in managing the disease incidence was carbendazim + mancozeb (SAAF) by recording 34.7% and 30.6% disease incidence in first year and second year, respectively. Combination fungicide carbendazim + mancozeb (SAAF) was found effective in managing leaf blight after propiconazole both under in vitro screening and glass house conditions. Same order of effectivity in managing the disease was observed in fungicides in both in vitro and glass house studies as difenoconazole (36.4, 34.6), followed by hexaconazole (39.6, 35.9) and mancozeb (42.1, 39.7). However the only exception was seen in ipriodine + carbendazim (quintal) with the mycelia growth of *A. helianthi* inhibited

Table 3 Evaluation of fungicides against leaf blight under glass house conditions

Treatment	Leaf blight (%)		
	I year	II year	Mean
Propiconazole (.1%)	32.2 (34.57)*	29.2 (32.71)	33.64
Mancozeb (.25%)	42.1 (40.46)	39.7 (39.06)	39.76
Carbendazim + mancozeb (.2%)	34.7 (36.09)	30.6 (33.58)	34.83
Difenaconazole (.1%)	36.4 (37.11)	34.6 (36.03)	36.57
Hexaconazole (.1%)	39.6 (39.00)	35.9 (36.81)	37.90
Ipriodine + carbendazim (.2%)	45.1 (40.51)	61.2 (51.47)	50.49
Pathogen check	80.9 (63.87)	75.9 (60.47)	57.67
CD (.05)		16.85	
CV %		16.58	

Average of three replications (10 plants/replication)

*Figures in parantheses are transformed values

considerably but under the glass house conditions it has recorded less inhibition of the disease which was only next to mancozeb. Glass house studies were in correlation with in vitro studies, results showed propiconazole as the effective fungicide in reduction of disease over control followed by combination fungicide carbendazim + mancozeb (SAAF) and least effective fungicide mancozeb with low reduction of leaf blight disease over control.

Development of new management techniques may emerge as a solution for many diseases, however, the use of fungicides has became an inevitable method in the management of sunflower in the absence of resistant cultivars to Alternariaster blight. The present study was undertaken to find out new fungicides in in vivo conditions to know their efficacy against the isolates collected from different regions of India for subsequent recommendation as per their region. Fungicide sensitivity study more extensively helps in developing a resistance map of isolates which would be helpful to incorporate the best fungicide according to the location of the disease. Thus the objective of the study was explained by suggesting the best fungicides in areas with less sensitive isolates and other fungicides in more sensitive isolate identified areas according to the cost and availability of the fungicides. As the indiscriminate use of the same fungicide may develop resistance in the isolates and also not cost effective therefore use of the other evaluated fungicides were suggested for low cost and the management of high sensitive minimal disease areas. Among the fungicides evaluated against all *A. helianthi* isolates propiconazole and combination fungicide carbendazim + mancozeb under laboratory condition effectively controlled the *Alternariaster* mycelial growth and regulate the blight of sunflower and could be exploited on large scale for the management of the disease in high disease prevalent areas and against the less sensitive isolates.

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