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Screening of maize hybrids against turcicum leaf blight of maize caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs

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Abstract
Turcicum Leaf Blight (TLB) is a major foliar disease of maize caused by the fungus *Exserohilum turcicum*. The disease is most prevalent in all the major maize growing regions of India during rainy (*kharif*) as well as in winter (*rabi*) season since last two decades. However, management of the TLB through the continuous use of chemicals may alarm new problems in crop production. Hence, the host plant resistance is a cheap and environmentally reliable component to minimize the disease intensity below the threshold level. Keeping in view the above points, screening of 55 hybrids along with two standard checks i.e., CI 4 (Resistant check) and CM 202 (Susceptible check) were evaluated against *E. turcicum* under artificially inoculated field conditions. Using 1-9 rating scale disease reaction and AUDPC values of hybrids were also calculated. Among the 55 hybrids, five hybrids viz., DKC 7074, DKC 7173, DKC 8174, DKC 9145, DKC 9157 and CI 4 (RC) recorded resistant reaction.

Keywords: Area under disease progress curve (AUDPC), disease severity, *Exserohilum turcicum*, maize, turcicum leaf blight

1. Introduction
In India, maize is third most important cereal crop after rice and wheat. It is one of the most versatile emerging crops having wider adaptability under varied agro-climatic conditions. Globally, maize is known as queen of cereals because it has the highest genetic yield potential among the cereals. Nutrition in maize contains high level of starch, oils and also rich proteins. It is rich in calcium, potassium, zinc, iron, selenium, manganese and magnesium. Maize contains vitamins such as A, C and E. The increase in interest of the consumers in nutritionally enriched products, use of maize as feed and rising demand for maize seed are the core driving forces behind emerging importance of maize crop in India.

Among the maize growing countries, India ranks 4th in area and 7th in production, representing around 4% of world maize area and 2% of total production. During 2018-19 in India, the maize area has reached to 9.2 million ha. During 1950-51 India used to produce 1.73 million MT maize, which has increased to 27.8 million MT by 2018-19, recording close to 16 times increase in production. The average productivity during the period has increased by 5.42 times from 547 kg/ha to 2965 kg/ha, while area increased nearly by three times.

Globally, turcicum leaf blight (TLB) or northern corn leaf blight (NCLB) disease has emerged as a constraint to maize production in many temperate and tropical environments. It is caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs. the anamorph of the Deuteromycete. The telomorph of the ascomycete, *Setosphaeria turcica* (Luttrell) Leonard and Suggs. First time, it was reported by Passerine (1876) [10] in Perma, Italy, this was followed by a serious outbreak of TLB in Connecticut, New England in 1889 (Drechsler, 1923) [4]. The disease appears particularly during growing season in the areas of high humidity and moderate temperature. Incidence of turcicum leaf blight in the pre-harvest stage is prominent. The disease is responsible for premature death of blighted leaves and results in significant yield reductions.

Early symptoms of disease are oval, water-soaked spots on leaves. Mature symptoms of turcicum leaf blight are characteristic cigar shaped lesions that are 3 to 15cm long. Lesions are elliptical and tan in colour, developing distinct dark areas as they mature that are associated with fungal sporulation. Lesions typically first appear on lower leaves, spreading to upper leaves and the ear sheaths as the crop matures. Under severe infection, lesions may coalesce, blighting the entire leaf may occur.

Although breeding for TLB resistance started much earlier, more efforts are still needed as new challenges arise. There is a possibility of emergence of new races of pathogens and some available resistance sources may become susceptible. Therefore, following the difficulty in controlling TLB due to high input prices and arising of new races is unreliable. Therefore breeding for maize resistance to TLB was more demanded as it is cheap and reliable approach for combating losses due to the disease.

2. Material and Methods

2.1 Collection of diseased samples

The leaves of maize plants severely infected by *E. turcicum* showing typical leaf blight necrotic lesion type symptoms were collected from experimental fields of All India Coordinated Maize Improvement Project, Main Agricultural Research Station, University of Agricultural Sciences, Dharwad and further used for isolation of the pathogen. The pathogen *E. turcicum* was isolated by standard hyphal tip isolation procedures and then nucleus culture was maintained on potato dextrose agar slants, kept in refrigerator at 5 °C which was further used in all the laboratory and field studies.

2.2 Isolation of the pathogen

The fungus was isolated following standard tissue isolation technique. The necrotized leaf bits along with healthy portions were surface sterilized in 1:1000 sodium hypochlorite solution for 30 sec and washed thoroughly thrice in sterile distilled water to remove the traces of sodium hypochlorite. Then sterilized bits were aseptically transferred to sterile Petri plates containing PDA media. The inoculated Petri dishes were incubated at room temperature (25 ± 1 °C) and observed periodically for fungal growth. The growth of the fungus was conspicuous after 24 hr of incubation. The pure colonies which developed from the bits were transferred to PDA slants and incubated at room temperature for 15 days. After the incubation period, abundant sporulation was observed and the pathogen was purified following hyphal tip isolation technique as described below.

2.3 Hyphal tip isolation

The pure culture of the pathogen was obtained by hyphal tip isolation method. The spore suspension was diluted in sterilized distilled water to get eight to ten spores per ml from 15 days old culture. One ml of such suspension was spread uniformly on two per cent solidified water agar plates and incubated at 27 ± 1 °C for 12 hr. Single spore was marked with a marker pen on back side of the Petri plate with the aid of microscope and it was allowed to germinate. Such plates were periodically observed for spore germination under microscope. The hyphae coming from each cell of the single spore was traced and marked. The tip of the hyphae was cut carefully with cork borer and transferred to PDA plates and incubated at 27 ± 1 °C for 10 days. Later, mycelial bits of the fungus from incubated plates were transferred to the Petri plates containing PDA and incubated at 27 ± 1 °C for 10 days. The pure culture thus obtained was free from saltation or sectoring. In order to confirm the identity of *E. turcicum*, spore morphology and colony characteristic studies were done on PDA. Further, the conidia of *E. turcicum* was observed under microscope (Fig. 1).

2.4 Maintenance of the culture

The hyphal tip cultures of *E. turcicum* were sub-cultured on PDA slants and kept in laboratory at 28 ± 1 °C for 15 days. Such mother culture slants were preserved at 5 °C in refrigerator. Further, these cultures were sub-cultured once in a month to maintain viability and used for future studies.

2.5 Mass multiplication of inoculum

The mass multiplication of *E. turcicum* was prepared on sterilized sorghum grains (Joshi *et al.*, 1969) [6]. About an inch layer of sorghum grains (nearly 40 to 45 g) was dispensed in a 500 ml conical flask, soaked in water for about 3-4 hours and excess water was drained off. The flasks containing sorghum grains were autoclaved twice at 15 pounds per inch square pressure for one hour, seeded with fungus under aseptic condition and kept for incubation at 25- 27^{0}C. The flasks were shaken once in 2-3 days to facilitate uniform growth of *E. turcicum* on grains. After incubation of about a fortnight, the material was ready for inoculation. The above impregnated sorghum grains were allowed for drying by spreading them on a clean paper sheet in shade at room temperature. After drying, fine powder of these grains was prepared with the help of mixer - grinder and put a pinch of this powder in the leaf whorl. The inoculum was directed into the whorl of the plant @ 2g/plant followed by water spray in the whorls so as to maintain adequate moisture for longer period to permit spore germination. Artificial inoculation was done twice *i.e*, at 30 and 40 days after sowing (Fig. 2).

a) Five days old culture **b) Sporulating culture (10 days old)**

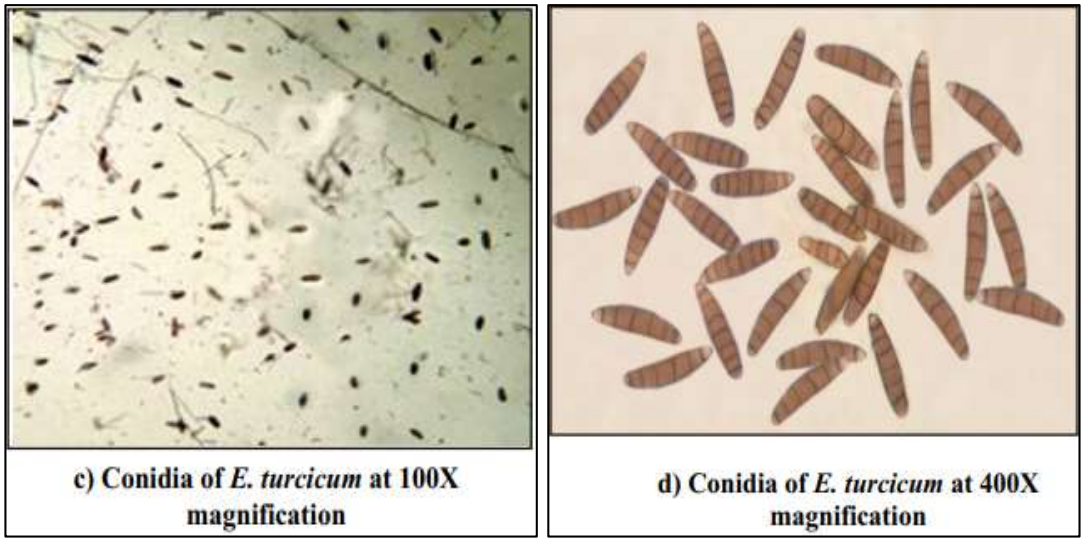


Fig 1: Cultural and morphological characters of *E. turcicum*

2.6 Screening of hybrids

A field experiment was conducted to screen maize hybrids for turcicum leaf blight at MARS, Dharwad during *kharif* 2018. Fifty five hybrids were screened against turcicum leaf blight under artificial inoculated field conditions. The resistant check CI-4 and susceptible check CM-202 were planted along with test entries in plot size of 4.8 sq. m and replicated twice. The crop was raised by following the recommended agronomic practices except disease management. The test hybrids were inoculated by *E. turcicum* inoculum multiplied on sorghum grains in the leaf whorls at 30 and 40 days after sowing at the rate of 2 g per plant during evening hours. A light water spray was given immediately after the inoculation to create optimum humidity for infection. The observations on the disease severity of turcicum leaf blight were recorded on the basis of 1-9 modified disease rating scale (Anonymous, 2016) [2].

Modified disease rating scale for TLB (1-9)

Rating scale	Degree of infection
1	Very slight infection (≤ 10%)
2	Slight infection, a few lesions scattered on two lower leaves (10.1 -20%)
3	Light infection, moderate number of lesions scattered on four lower leaves (20.1 - 30%)
4	Light infection, moderate number of lesions scattered on lower leaves, a few lesion scattered on middle leaves below the cob (30.1 - 40%)
5	Moderate infection, abundant number of lesions scattered on lower leaves, moderate number of lesions scattered on middle leaves below the cob (40.1 - 50%)
6	Heavy infection, abundant number of lesions scattered on lower leaves, moderate infection on middle leaves and a few lesions on two leaves above the cob (50.1 - 60%)
7	Heavy infection, abundant number of lesions scattered on lower and middle leaves and moderate number of lesions on two to four leaves above the cob (60.1 - 70%)
8	Very heavy infection, lesions abundant scattered on lower and middle leaves and spreading up to the flag leaf (70.1-80%)
9	Very heavy infection, lesions abundant scattered on almost all the leaves, plant prematurely dried and killed (> 80%)

Further the hybrids were categorized into resistant, moderately resistant, moderately susceptible and susceptible. Further, disease scores were used to calculate the area under disease progress curve (AUDPC) using the following formula given by Wilcoxson *et al.* (1975) [12].

$$AUDPC = \sum_{i=1}^{k} \left[\frac{1}{2} (S_i + S_{i-1})\, d \right]$$

Where,

S_i = Disease severity at the end of time

S_{i-1} = Number of successive evaluations of blight

d = Interval between two evaluations

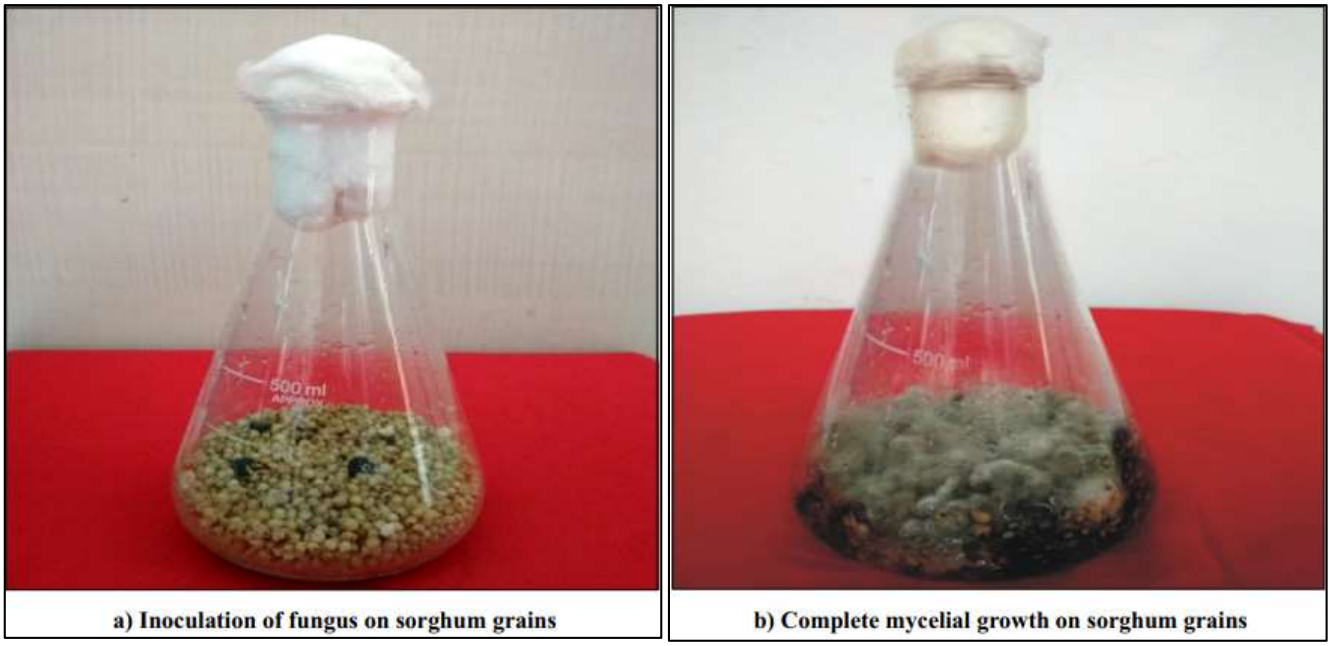


Fig 2: Mass multiplication of *E. turcicum* on sorghum grains

3. Results and Discussion

Significant differences in disease reaction were observed among different hybrids for TLB. Of the fifty five hybrids evaluated, five hybrids *viz.*, DKC 7074, DKC 7173, DKC 8174, DKC 9145, DKC 9157 and CI 4 (RC) showed resistant reaction to TLB. Whereas, twenty one hybrids *viz.*, GEMH 16222, GEMH 16211, GEMH 16220, GEMH 16204, GEMH 16214, GEMH 16219, GEMH 16210, GEMH 16202, PAC 751, DKC 8144, DKC 8164, P 3550, DKC 9141, DKC 9133, DKC 9164, DKC 9178, D 4244, DKC 8161, NK 6240, PAC 740 and NK 30 were found to be moderately resistant. Nine hybrids *viz.*, GEMH 16203, GEMH 16209, GEMH 16208, CP818, DMH 8255, Hi-Shell, S 6668, DKC 9155 and DKC 8101 were found to be moderately susceptible and remaining twenty hybrids *viz.*, GEMH 16215, GEMH 16216, DKC 9125, DKC 9081, P 3396, DKC 9150, DKC 8171, DKC 9126, PAC 753, P 3501, NK 6607, CP 999, P 3401, P 3377, S 7750, S 6217, GH 0727, GH 1101, D 4142, DKC 9144 and CM 202 (SC) exhibited susceptible reaction to turcicum leaf blight (Table 1a, 1b and Fig. 3).

AUDPC values showed significant differences among different maize hybrids ranging from 137.25 to 549.00. The least AUDPC value was recorded in DKC 9145 (137.25) showing resistant reaction and highest AUDPC value was recorded in GEMH 16216 (527.86) which was depicted as susceptible reaction. Hence, hybrids with lower AUDPC values *viz.*, DKC 9145, DKC 7074, DKC 7173, DKC 8174 and DKC 9157 could be used for rating them as slow blighters (Table 1a).

The present findings are in corroborative with the studies of Wani *et al.* (2017) [11], who evaluated sixty maize genotypes against TLB disease using 1-5 disease rating scale. Among them, two inbred lines *viz.*, NAI-112 and NAI-147 and one hybrid i.e., HQPM-1 were found resistant with pooled disease intensity of 4.12 per cent, 4.04 per cent and 4.38 per cent respectively. DKC 7074, DKC 9108, DKC 9106 with disease score 2.0 were found to show moderately resistant reaction. Four inbred lines, *viz.*, KDM 381 A, KDM 918 A, NAI-152 and NAI-167 were found susceptible with pooled disease intensity of 52.82 per cent, 51.02 per cent, 58.58 per cent and 61.33 per cent respectively. The remaining genotypes were moderately resistant to moderately susceptible. Harlapur *et al.* (2008) [5] conducted field experiment to study the TLB response of thirty maize genotypes based on latent period, lesion density, lesion size, apparent rate of infection and area under disease progress curve (AUDPC). The genotypes *viz.*, Allrounder, IB- 8501, Cargill 900M, Hi shell, NAC- 6004, C-111, KH- 517, Kaveri 235 and NK -6240 were identified as slow blighters.

Thus from the present investigation, new sources of resistance were identified through artificial epiphytotics. This can cater to the resistance breeding programme by combating with the new races of pathogens that would be emerging continuously and susceptibility of some resistance sources. This result would also be useful in improvement of maize hybrids through population improvement programmes for sustainable productivity.

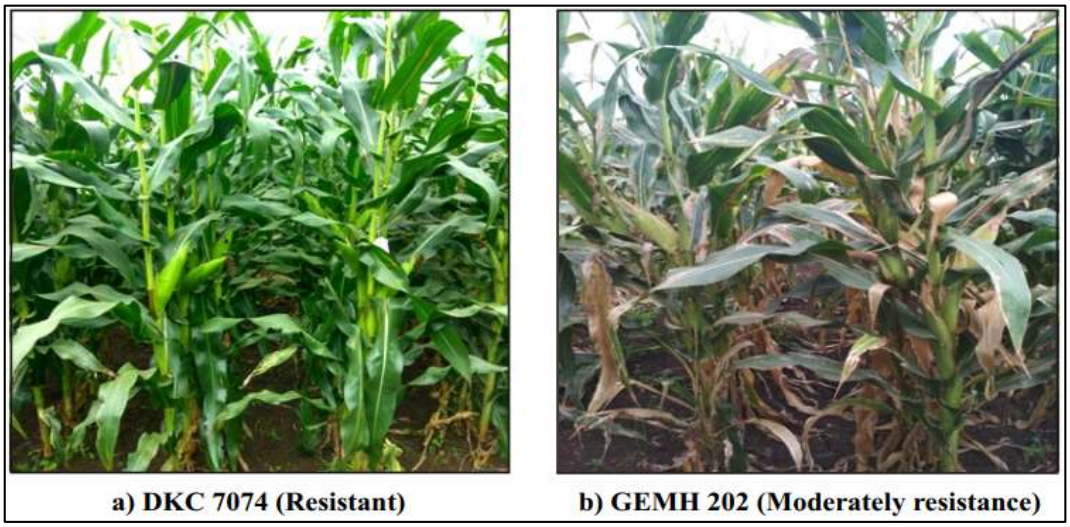
Table 1a: Screening of maize hybrids against turcicum leaf blight of maize

Sl. No.	Hybrid	Disease score (1-9 scale)	AUDPC value	Sl. No.	Hybrid	Disease score (1-9 scale)	AUDPC value
1	GEMH 16222	4	284.27	30	PAC 740	5	325.75
2	GEMH 16211	5	303.33	31	NK 6240	4	293.25
3	GEMH 16220	4	294.70	32	DKC 9141	4	282.75
4	GEMH 16204	5	328.96	33	DKC 9144	7	411.75
5	GEMH 16215	7	437.09	34	P 3501	8	457.50
6	GEMH 16216	8	527.82	35	DKC 9133	4	282.75
7	GEMH 16203	6	429.37	36	DKC 9155	6	370.58
8	GEMH 16208	6	424.94	37	DKC 8101	6	386.00
9	GEMH 16209	6	391.22	38	DKC 9164	5	328.23
10	GEMH 16219	4	283.00	39	DKC 9178	5	365.50
11	GEMH 16210	4	295.40	40	DKC 7074	2	141.83
12	GEMH 16214	5	308.40	41	DKC 7173	2	146.40
13	GEMH 16202	4	291.30	42	DKC 8161	4	297.05
14	DKC 9126	7	416.33	43	DKC 8174	3	192.15
15	CP 818	6	379.73	44	DKC 8171	4	284.48
16	DMH 8255	6	383.65	45	DKC 9081	9	503.25
17	P 3401	7	416.33	46	DKC 9145	2	137.25
18	P 3396	8	494.10	47	DKC 9157	2	146.40
19	Hi Shell	6	396.00	48	NK 30	4	297.90
20	DKC 9150	7	435.15	49	NK 6607	7	421.75
21	S 6668	6	373.13	50	CP 999	7	434.63
22	PAC 753	8	471.23	51	S 7750	8	475.80
23	PAC 751	5	329.08	52	D 4244	5	367.50
24	S 6217	8	494.10	53	D 4142	8	457.50
25	DKC 9125	7	430.05	54	GH 0727	8	503.38
26	DKC 8144	5	288.23	55	GH 1101	7	433.25
27	DKC 8164	5	292.80	56	Resistance check CI 4	3	137.25
28	P 3550	5	301.95	57	Susceptible check CM 202	9	549.00
29	P 3377	9	474.50				

AUDPC - Area under disease progress curve

Table 1b: Reaction of maize hybrids against turcicum leaf blight of maize

Disease rating	Reaction	No. of hybrids	Hybrids
≤ 3.0	Resistant	5	DKC 7074, DKC 7173, DKC 8174, DKC 9157, DKC 9145, CI 4 (RC)
3.1- 5.0	Moderately resistant	21	GEMH 16222, GEMH 16211, GEMH 16220, GEMH 16204, GEMH 16214, GEMH 16219, GEMH 16210, GEMH 16202, PAC 751, DKC 8144, DKC 8164, P 3550, DKC 9141, DKC 9133, DKC 9164, DKC 9178, D 4244 , DKC 8161, NK 30, PAC 740, NK 6240
5.1-7.0	Moderately susceptible	9	GEMH 16203, GEMH 16209, GEMH 16208, CP 818, DMH 8255, Hi-Shell, S 6668, DKC 9155, DKC 8101
≥7.0	Susceptible	20	GEMH 16215, GEMH 16216, DKC 9125, DKC 9081, P 3396, DKC 9150, DKC 8171, DKC 9126, PAC 753, P 3501, NK 6607, CP 999, P 3401, P 3377, S 7750, S 6217, D 4142, DKC 9144, GH 0727, GH 1101, CM 202 (SC)



a) DKC 7074 (Resistant) b) GEMH 202 (Moderately resistance)

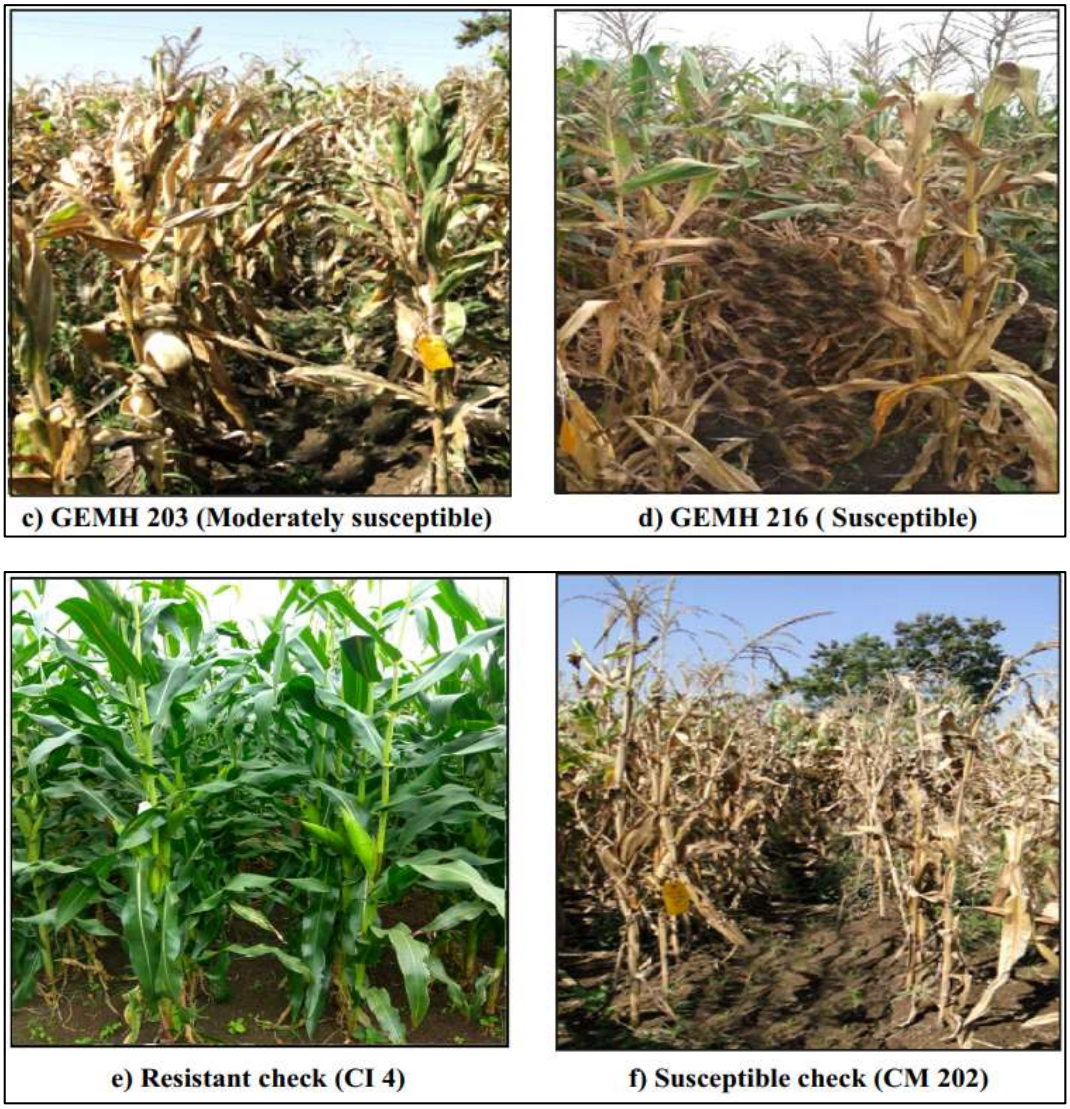


Fig 3: Screening of hybrids against turcicum leaf blight disease

4. Conclusion

Among fifty five hybrids evaluated, five hybrids *viz.*, DKC 7074, DKC 7173, DKC 8174, DKC 9145, DKC 9157 and CI 4 (RC) were recorded resistant reaction. The AUDPC values differed considerably for different maize hybrids ranging from 137.25- 549.00. The least AUDPC value was recorded in DKC 9145 (137.25) under resistant reaction and highest AUDPC value was recorded in GEMH 16216 (527.86) which is depicted as susceptible reaction. Hence, hybrids with lower AUDPC values *viz.*, DKC 9145, DKC 7074, DKC 7173, DKC 8174 and DKC 9157 could be used for rating them as slow blighters.

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6. References

1. Anonymous. Annual progress report. All India Coordinated Maize Improvement Project, Indian Council of Agricultural Research, Indian Institute of Maize Research, Ludhiana; c2004. p. 80-83.
2. Anonymous. Annual progress report. All India Coordinated Maize Improvement Project, Indian Council of Agricultural Research, Indian Institute of Maize Research, Ludhiana; c2016-2017. p. 214-216.
3. Butler EJ. Fungi and diseases in Plants. Calcutta and Simla, India Thacker, Spink and Co., Kolkatta (India); c1918.
4. Drechsler C. Some graminicolous species of *Helminthosporium.* Journal of Agricultural Research. 1923;24:641-739.
5. Harlapur SI, Kulkarni MS, Wali MC, Kulkarni S, Yashoda H, Patil BC. Status of turcicum leaf blight of maize in Karnataka. Karnataka Journal of Agricultural Sciences. 2008;21(1):55-60.
6. Joshi LM, Goel LB, Renfro BL. Multiplication of inoculums of *Helminthosporium turcicum* on sorghum seeds. Indian Phytopathology. 1969;22:48.
7. Kachapur RM, Kulkarni MS, Wali MC, Kalappanavar IK, Timmanna O. Genetic evaluation of maize germplasm lines against turcicum leaf blight (*Exserohilum turcicum* (Pass.)). In: National Symposium on Plant diseases: New perspectives and innovative management strategies. University of Agricultural

Sciences Dharwad; c2014. p. 25.
8. Laxminarayana C, Shankerlingam S. Turcicum leaf blight of maize, techniques of scoring for resistance to important diseases of maize. Proceedings of AICRP. ICAR, New Delhi; c1983. p. 16-24.
9. Mitra M. *Helminthosporium* species on cereals and sugarcane in India. Part I Diseases of *Zea mays* and *Sorghum vulgare* caused by species of *Helminthosporium.* Memoirs of the Department of Agriculture. India, Botanical Series. 1923;11:219-242.
10. Passerini. Lanebbia Delgranotur Co. Bol. Comiz, Agriculture. Parmense. 1876;10:3.
11. Wani TA, Bhat GN, Ahmad M, Anwar A. Integrated disease management of turcicum leaf blight of maize caused by *Exserohilum turcicum.* Indian Journal of Currrent Microbiology and Applied Sciences, 2017;6(9):149-155.
12. Wilcoxson RD, Skovmand B, Atif AH. Evaluation of wheat cultivars for ability to retard development of stem rust. Annals of Applied Biology. 1975;80:275-281.