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Assessment of Impact of Late Wilt Caused by *Harpophora maydis* (Samra, Sabet and Hing) on Grain Yield and its Attributing Traits in Maize (*Zea mays* L.)

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ABSTRACT

Harpophora maydis (Samra, Sabet and Hing) is one of the devastating pathogens causing post-flowering stalk rot (PFSR) complex disease in maize. The disease caused by *Harpophora maydis (H. maydis)* is referred to as Late Wilt Disease (LWD). It is relatively a new and emerging disease of maize in India. To assess the effect of LWD on maize productivity, an investigation was carried out at Kallinayakanahalli (KNH) and Muppadighatta (MPG) during 2018 post-rainy season. Twenty-six commercial hybrids from both private and public sectors were evaluated for grain yield and its component traits under LWD inoculated and control sets. Hybrids differed significantly for grain yield and its component traits under both LWD infection and control conditions in both the locations, suggesting LWD significantly influenced by the environment and genotype x environment interaction. An increase in LWD score by one unit caused a decrease in grain yield per plant by 11.36 g and 3.26 g at KNH and MPG locations, respectively. An estimated reduction in grain yield losses by 7.5 quintals and 5 quintals acre^{-1} could be possible by developing and deploying tolerant and moderately tolerant hybrids for commercial production, respectively.

Keywords : Late wilt disease, Yield loss, Inoculation, *Zea mays* L.

As many as 112 diseases affect maize production on a global basis and about 35 diseases are recorded in India (Kumar *et al.,* 2013). Among these, late wilt disease (LWD) caused by *Harpophora maydis* is one among the most destructive pathogens of post-flowering stalk rot (PFSR) disease complex. Infection by *H. maydis* is characterized by relatively rapid wilting of maize plants typically after tasselling and shortly before maturity (Degani and Cernica, 2014). The first LWD symptoms appear approximately 60 days after sowing (Sabet *et al*., 1970). Leaves become dull green, eventually lose colour and dry with inward rolling from the edges. Later, drying-out ascends upwards in the plant with yellow-brown discoloration of the vascular bundles followed by the appearance of red-brown stripes advancing up to fifth internode or further (Sabet *et al*., 1966). With disease progression, the lower stem dries out (particularly at the internodes) and has a shrunken and hollow appearance with dark yellow to brownish macerated pith and brownish-black vascular bundles. Because of the delay in the appearance of initial symptoms until about flowering, this disease has been designated as "late wilt" (Samra *et al*., 1963). Since the pathogen aggravates during the grain filling stage, it is reported to affect grain yield due to shrunken and unfilled cobs (Drori *et al*., 2013).

The LWD has been reported to occur in the tropics of Tanzania, Pakistan, Hungary, Kenya (Freeman and Ward, 2004), Egypt and India (Samra *et al.,* 1963; Payak *et al*., 1970; Pecsi and Nemeth, 1998; Ward and Bateman, 1999), Portugal and Spain (Molinero-Ruiz *et al.,* 2010), Romania (Bergstrom *et al*., 2008), and Israel (Drori *et al*., 2013) and is now considered as endemic throughout the maize growing areas. In India, the disease is sporadically prevalent in most of the maize growing areas including Andhra Pradesh, Karnataka, Uttar Pradesh, Bihar, Rajasthan, Madhya Pradesh, Tamil Nadu, West Bengal, Jammu and Kashmir, Punjab, Haryana, and Delhi with disease incidence ranging from 10 to 42 per cent (Desai *et al.,* 1991), 25 to 32 per cent (Kumar *et al.,* 1998) and 10.18 to 31.08 per cent (Harlapur *et al.,* 2002). It is emerging as a serious biotic constraint of maize production in India.

Hitherto, the estimates of yield losses attributable to LWD are mostly based on surveys. For instance, economic losses up to 100 per cent have been reported in Egypt (Galal *et al.,* 1979), up to 51 per cent in India (Johal *et al*., 2004) and 40 per cent losses in susceptible cultivars (Labib *et al*., 1975). A solitary report based on empirical study using four genotypes indicated 3.5 - 38.4 per cent loss in grain yield attributable to soil inoculation by *H. maydis* (El-Naggar *et al.,* 2015). This report on estimates of grain yield losses is based on fewer genotypes and the symptoms that appear on stalks and leaves only. However, assessment of yield losses based on these symptoms without splitting the stalks may often be confused for those attributable to other pathogens or physiological stresses. Hence, the objective of the investigation is to assess grain yield losses due to LWD using appropriate LWD screening protocols (Rakesh *et al.,* 2016) with a large number of genotypes.

Material and Methods

Experimental Material

The experimental material included 26 commercial maize hybrids from both the public and private sectors. The identity of hybrids has not been mentioned due to proprietary issues and hence they have been indexed as Hybrid-1 to Hybrid-26. Among these, two hybrids (Hybrid-25 and Hybrid-26) which have been identified as susceptible and tolerant, respectively by our previous experiments were used as checks.

Evaluation of Hybrids

The hybrids were evaluated in two separate sets following Randomised Complete Block Design (RCBD) (Fisher, 1949) with four replications at two locations *viz*., at Kallinayakanahalli (KNH), Chikkaballapur District, Karnataka, India and Muppadighatta (MPG), Bengaluru Rural District, Karnataka, India during *Rabi*, 2018. Seeds of each hybrid in each set were dibbled in single rows of 4.00 m length with a spacing of 0.60 m between the rows and 0.25 m between plants within a row. One set of hybrids were artificially inoculated with the spores of *H. maydis* and another set of hybrids without inoculation were used as control. In the inoculated set, the hybrids were inoculated twice at 45 Days After Sowing (DAS) and 55 DAS by injecting 2 ml of *H. maydis* (4×10^6 spores ml^{-1}) inoculum to the second internode from the base using a syringe (Plate 1) after making a hole using screwdriver to dispense the inoculum in to stem. All the recommended production

Plate 1 : Injection of *Harpophora maydis* inoculum (4×10^6 spores/ml) to the second internode of the plant at 45 and 60 days after sowing

practices were followed to raise a healthy crop in control set and except measures to control LWD in the inoculated set.

Sampling and Data Recording

The data on disease severity was recorded on all the plants of each hybrid and replication of inoculated set

Plate 2 : Representative disease specimen showing *Harpophora maydis* infection scoring from 2-9 LWD scores

by splitting the stalks at 110 DAS based on the symptoms typical to LWD using modified 1-9 scale (Plate 2) (Rakesh *et al.,* 2016). Data were recorded on grain yield per plant (g), cob length (cm), kernel rows per cob and kernels per row on five random plants of each hybrid in each replication.

Statistical Analysis

Average data on LWD scores of hybrids of inoculated set and grain yield and its component traits of both the sets were used for statistical analysis. Pooled analysis of variance was performed to detect hybrid x location interaction on the expression of LWD in the inoculated set and for grain yield and its component traits of both the sets. Effect of disease on grain yield and its component traits was assessed by comparing the average grain yield performance of hybrids of inoculated and control sets using two-sample 't' test. Further, the extent of loss in grain yield and its component traits attributable to LWD in the inoculated set was assessed following linear regression model (Snedecor and Cochran, 1956) using 'GENSTAT' software version 18.

To assess the impact of resistance on reduction in yield losses, the hybrids were grouped into three response groups (tolerant, susceptible and highly susceptible) based on the mean LWD scores across two locations. The significance of differences in average LWD of the three response groups of the hybrids was examined using the 'F' test to justify the classification. Further, the significance of differences in grain yield reduction between the three hybrid response groups was examined using Dunnet's T3 test for unequal variances using SPSS software version 16.

RESULTS AND DISCUSSION

Response of Hybrids to LWD Disease

Substantial differences among the hybrids for LWD infection in both KNH and MPG locations as evident from wide range of disease score (4.00 to 8.50 at KNH and 3.67 to 8.67 at MPG) and significant mean squares attributable to mean LWD score suggested sufficient disease infection in both the locations (Table 1). Significant mean squares attributable to hybrids x location interaction suggested differential response of hybrids to LWD at two locations viz., KNH and MPG (Table 2). These results justified the choice of hybrids to address the objective of assessing the impact of LWD on grain yield reduction.

Relative Performance of Hybrids under LWD Infection and Control Sets

The hybrids differed significantly for grain yield and its component traits under both LWD inoculated and control sets in both the locations. The hybrids responded differentially for LWD expression to environments that prevailed in the two locations as evident from significant mean squares due to hybrids × location interaction. However, the hybrids interacted significantly with locations only for cob length under both inoculated and control sets. On the other hand, the hybrids interacted significantly with locations for grain yield only under controlled sets. These results indicated that hybrids are comparable for cob length and grain yield under inoculated conditions in both the locations (Table 2). As expected and reported by Drori *et al.* (2013) and El-Naggar *et al.* (2015), grain yields of hybrids evaluated under control sets were better than those evaluated under LWD inoculated sets in both the locations (Table 1). These results provided adequate evidence to show that LWD is a complex disease whose occurrence is significantly influenced by the environment and genotype x environment interaction.

Effect of LWD on Grain Yield

The difference between the LWD inoculated and control hybrid sets for grain yield per plant was significant in both the locations as suggested by two-sample t-test (Fig. 1). For other traits such as cob length and kernels per row, hybrids' performance in control was better than those of LWD inoculated hybrids only at MPG. These results suggested the differential response of hybrids to LWD in two locations attributable to hybrid x location interaction. Further, regression analysis indicated that increase in LWD by unit score resulted in the decrease of grain yield plant^{-1} by 11.36 g and 3.26 g at KNH (Fig. 2) and MPG (Fig. 3), respectively. The grain yield

TABLE 1

Response of maize hybrids for LWD and reduction in grain yield plant^{-1} due to LWD infection

Hybrid	Kallinayakanahalli (KNH)					Muppadighatta (MPG)				
	Mean LWD score	Grain yield plant^{-1} (g)				Mean LWD score	Grain yield plant^{-1} (g)			
		Control (C)	Inoculated (I)	Reduction (C-I)	Per cent reduction		Control (C)	Inoculated (I)	Reduction (C-I)	Per cent reduction
Hybrid-1	5.25	164.48	128.98	35.49	21.58	5.00	169.56	129.53	40.03	23.61
Hybrid-2	5.50	153.01	110.27	42.73	27.93	5.75	152.12	114.19	37.94	24.94
Hybrid-3	5.00	175.89	130.34	45.55	25.90	4.00	172.19	134.75	37.44	21.74
Hybrid-4	5.33	159.12	137.98	21.14	13.28	5.75	176.96	148.25	28.71	16.22
Hybrid-5	6.00	134.89	108.31	26.58	19.70	6.75	160.16	101.70	58.45	36.50
Hybrid-6	5.00	154.03	124.55	29.49	19.14	5.67	163.39	121.21	42.18	25.81
Hybrid-7	6.00	155.13	124.39	30.74	19.82	7.00	138.06	120.82	17.24	12.49
Hybrid-8	4.67	149.65	123.07	26.58	17.76	4.25	142.85	134.49	8.35	5.85
Hybrid-9	4.75	186.99	144.50	42.49	22.72	6.25	153.76	132.96	20.80	13.53
Hybrid-10	5.00	173.23	119.34	38.33	24.31	4.00	175.44	128.19	29.60	18.36
Hybrid-11	4.33	135.38	107.76	27.62	20.40	3.67	145.42	100.81	44.61	30.68
Hybrid-12	5.33	166.79	110.31	56.47	33.86	5.67	173.23	121.51	51.72	29.86
Hybrid-13	6.50	148.11	94.98	53.13	35.87	7.67	177.30	108.41	68.89	38.85
Hybrid-14	6.00	130.65	102.36	28.28	21.65	7.50	148.21	126.71	21.50	14.51
Hybrid-15	5.33	132.76	103.58	29.19	21.99	6.67	129.80	106.30	23.50	18.11
Hybrid-16	6.67	134.03	92.72	41.32	30.83	7.50	169.51	117.89	51.62	30.45
Hybrid-17	7.33	133.44	104.65	28.79	21.57	8.67	138.99	104.33	34.65	24.93
Hybrid-18	5.00	136.87	111.44	25.43	18.58	4.50	132.18	114.90	17.28	13.07
Hybrid-19	5.33	156.36	88.68	67.68	43.29	6.00	148.67	91.57	57.10	38.41
Hybrid-20	5.67	178.65	110.64	68.00	38.07	6.25	145.02	104.58	40.43	27.88
Hybrid-21	4.75	155.80	107.86	47.95	30.77	5.50	149.42	126.73	22.70	15.19
Hybrid-22	6.25	100.34	77.90	22.44	22.36	5.63	143.06	91.96	51.10	35.72
Hybrid-23	7.33	123.38	85.33	38.05	30.84	7.75	145.07	101.71	43.36	29.89
Hybrid-24	6.00	130.75	120.11	10.64	8.14	6.67	128.07	108.39	19.68	15.37
Hybrid-25 (Susceptible)	8.50	127.18	88.24	38.93	30.61	8.25	206.72	115.43	91.28	44.16
Hybrid-26 (Resistant)	4.00	163.90	145.68	18.22	11.12	4.00	129.42	111.24	18.17	14.04
Mean	5.65	148.49	111.69	36.20	24.31	6.01	154.41	116.10	37.63	23.85
Lowest	4.00	100.34	77.90	10.64	8.14	3.67	128.07	91.57	8.35	5.85
Highest	8.50	186.99	145.68	68.00	43.29	8.67	206.72	148.25	91.28	44.16
SEm	1.54	27.10	22.61	-	-	0.43	33.61	13.37	-	-
CD at P=0.05	3.63	63.83	53.24	-	-	1.01	79.17	31.49	-	-

TABLE 2

Pooled ANOVA of hybrids for LWD, grain yield and its component traits in two locations

Source of variation	Degrees of freedom	Cob length (cm)		Kernel rows per cob		Kernels per row		Grain yield per plant (g)		Mean LWD score
		Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control	
Hybrids (G)	25	12.49 **	19.57 **	12.77 **	13.75 **	70.84 **	105.96 **	1689.5 **	1597 **	10.79 **
Locations (L)	1	1.56	15.37 *	22.40 *	9.55 **	7.11	465.35 *	660	2104	7.83 **
Hybrids × Locations (G×L)	25	2.29 **	2.53 **	2.76	0.73	23.15 *	60.79	330.8	1163 **	1.17 **
IPCA 1	25	2.29 **	2.53 **	2.76	0.73	23.15 *	60.79	330.8	1163 **	1.17 **
Error	150	0.96	1.14	3.28	0.53	13.44	46.78	287.6	587	0.526

*Significant at P = 0.05 **Significant at P = 0.01

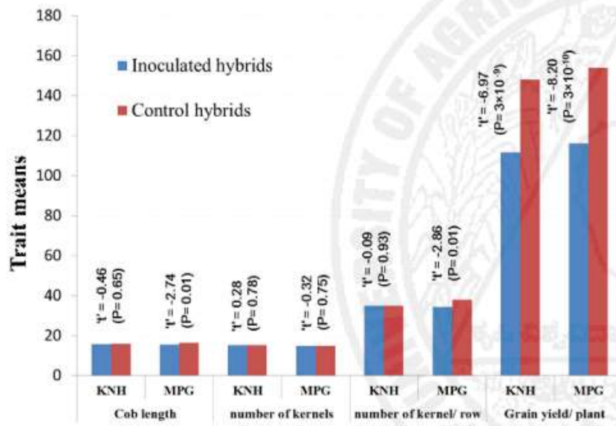

Fig. 1 : Comparison of trait means between LWD inoculated and control hybrid sets at Kallinayakanahalli (KNH) and Muppadighatta (MPG)

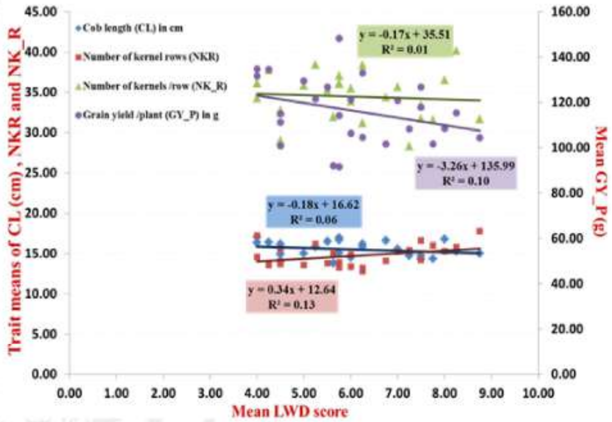

Fig. 3 : Effect of LWD on cob length, number of kernel rows per cob and number of kernels per row, and grain yield per plant at Muppadighatta (MPG)

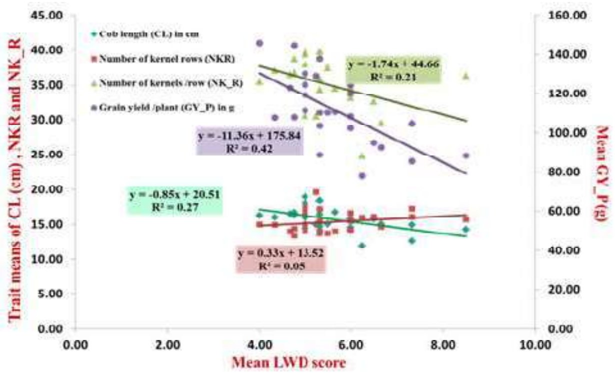

Fig. 2 : Effect of LWD on cob length, number of kernel rows per cob and number of kernels per row, and grain yield per plant at Kallinayakanahalli (KNH)

reduction ranged from 10 to 68 g per plant at KNH and 8 to 91 g per plant at MPG. The disease caused considerable grain yield reduction up to 44 per cent in some hybrids (Table 1). These results highlighted the variation in yield reduction attributable to LWD not only varied with the genetic background but also with locations. These results are in agreement with those of Callaway *et al.* (1992) for Anthracnose stalk rot and Nagy *et al.* (2011) for *Fusarium* ear infection; Drori *et al.* (2013) and El-Naggar *et al.* (2015) for LWD in maize who also reported that extent of yield reduction differed with the genetic background. Our results signify that LWD is an economically important biotic constraint in maize production.

TABLE 3

Impact of hybrids with different LWD response levels on average grain yield loss in maize

Hybrids' LWD response groups	Number of hybrids	Average LWD score	Average Grain yield loss (in grams/ plant)	% reduction in grain yield
Tolerant	06	4.37 [a]	28.10 [a]	18.52 [a]
Moderately tolerant	15	5.82 [b]	35.64 [b]	23.63 [b]
Highly susceptible	05	7.62 [c]	50.50 [b]	33.25 [c]
F test	-	$P = 4.7\times10^{-10}$	P = 0.014	P = 0.006

[a,b,c] significance of multiple comparison tests at $P<0.05$

Impact of LWD Resistance on Grain Yield Losses

The hybrids were grouped into tolerant, moderately tolerant and highly susceptible classes based on their mean LWD response scores across locations. These hybrids' groups differed significantly for their LWD scores, thus justifying their classification (Table 3). Susceptible group of hybrids suffered highest average grain yield losses compared to those of moderately tolerant and tolerant hybrid groups in that order. These results warrant and justify research interventions to develop markers to mitigate losses caused by LWD. Genetic options are considered as most eco-friendly and economical for reducing grain yield losses. As a short-term breeding strategy, even if moderately tolerant hybrids are identified and deployed for commercial production, it is possible to reduce the grain yield losses by 14.86 grams per plant which amounts to about 5 quintals per acre on large scale. If tolerant hybrids are deployed, it is possible to reduce grain yield losses by 22.41 grams per plant which amounts to 7.5 quintals per acre. This work demonstrates that LWD can lead to high yield losses. The study is first of its kind to quantify yield losses in maize due to LWD under field conditions.Financial support in the form of 'Monsanto student fellowship' and extension of field facilities by Monsanto India Limited under collaborated project with University of Agricultural Sciences, Bangalore, Karnataka, India are gratefully acknowledged.

REFERENCES

BERGSTROM, G., LESLIE, J., HUBER, D., LIPPS, P., WARREN, H., ESKER, P., GRAU, C., BOTRATYNSKI, T., BULLUCK, R., FLOYD, J., BENNETT, R., BONDE, M., DUNKLE, L., SMITH, K., ZELLER, K., CARDWELL, K., DABERKOW, S., BELL, D. AND CHANDGOYAL, T., 2008, Recovery plan for late wilt of corn caused by *Harpophora maydis* syn. *Cephalosporium maydis*. Washington, DC: National Plant Disease Recovery System.

CALLAWAY, M. B., SMITH, M. E. AND COFFRNAN, W. R., 1992, Effect of anthracnose stalk rot on grain yield and related traits of maize adapted to the northeastern United States. *Canadian J. plant sci.,* **72** : 1031 - 1036.

DEGANI, O. AND CERNICA, G., 2014, Diagnosis and Control of *Harpophora maydis*, the cause of late wilt in Maize. *Advances Microbiol.*, **4** : 94 - 105.

DESAI, S., HEGDE, R. K. AND DESAI, S., 1991, A preliminary survey of incidence of stalk rot complex of maize in two districts of Karnataka. *Indian Phytopathol.,* **43 :** 575 - 576.

DRORI, R., SHARON, A., GOLDBERG, D., RABINOVITZ, O., LEVY, M. AND DEGANI, O., 2013, Molecular diagnosis for *Harpophora maydis*, the cause of maize late wilt in Israel. *Phytopathologia Mediterranea,* **52** (1) : 16 - 29.

EL-NAGGAR, A. A. A., SABRYR, A. M. AND YASSIN, M. A., 2015, Impact of late wilt disease caused by *Harpophora maydis* on maize yield. *J. Biol. Chem. Environ. Sci.,* **10** (3) : 577 - 595.

FISHER, R. A., 1949, The design of experiments, *Ed.* 5: pp. 242.

FREEMAN, J. AND WARD, E., 2004, *Gaeumannomyces graminis*, the take-all fungus and its relatives. *Mol. Plant Pathol.,* **5** (4) : 235 - 252.

GALAL, A. A., EL-ROUBY, M. M. AND GAD, A. M., 1979, Genetic analysis of resistance to late wilt (*Cephalosporium maydis*) in variety crosses of maize. *Zeitschriftfür Planzenzüchtung*, **83** : 176 - 183.

HARLAPUR, S. I., WALI, M. C., PRASHANT, M. AND SHAKUNTALA, N. M., 2002, Assessment of yield losses in maize due to charcoal rot in Ghataprabha Left Bank Cannal (GLBC) command area of Karnataka. *Karnataka J. Agric. Sci.,* **15 :** 590 - 591.

JOHAL, L., HUBER, D. M. AND MARTYN, R., 2004, Late wilt of corn (maize) pathway analysis: intentional introduction of *Cephalosporium maydis*. *In* : Pathways Analysis for the Introduction to the U.S. of Plant Pathogens of Economic Importance. U.S. Department of Agriculture, animal and plant health inspection service technical report no. 503025.

KUMAR, M., LAL, H. C AND JHA, M. M., 1998, Assessment of yield loss due to post-flowering stalk rots in maize. *J. Appl. Biol.,* **8** : 90 - 92.

KUMAR, R., HOODA, K. S., OLAKH, D. S., KAUR, H., MALIK, V. AND KUMAR, S., 2013, Reaction of QPM inbred lines against Maydish leaf blight (MLB) and charcoal rot. *Electronic J. Plant Breed.,* **4** (4) : 1280 - 1283.

LABIB, H. A., ABDEL-RAHIM, M. F., SALEM, A. AND ABDEL-FATTAH, A., 1975, DC 19, a new maize hybrid seed resistant to late wilt disease caused by *Cephalosporium maydis. Agric. Res. Rev.,* **53** (8) : 1 - 4.

MOLINERO-RUIZ, M. L., MELERO-VARA, J. M. AND MATEOS, A., 2010, *Cephalosporium maydis*, the cause of late wilt in maize, a pathogen new to Portugal and Spain. *Plant Diseases,* **94** : 379.

NAGY, E., HAS, V., HAS, I., SUCIU, A. AND FLORIAN, V., 2011, The influence of *Fusarium* ear infection on the maize yield and my cotoxin content (transylvania-romania). *Plant Breed. Seed Sci.,* **64** : 35 - 44.

PAYAK, M. M., LAL, S., LILARAMANI, J. AND RENFRO, B. L., 1970, *Cephalosporium maydis* - a new threat to maize in India. *Indian Phytopathol.,* **23** : 562 - 569.

PECSI, S. AND NEMETH, L., 1998, Appearance of *Cephalosporium maydis* samra, sabet and hingorani in Hungary. Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen, *University Gent*, **63**: 873 - 877.

RAKESH, B., GANGAPPA, E., GANDHI, S., VEERESHGOWDA, R. P., SWAMY, S. D., RAMESH, S., HEMAREDDY, H. B. AND NAGARAJU, N., 2016, Modified method of screening maize inbred lines to late wilt disease caused by *Harpophora maydis*. *Mysore J. Agric. Sci.,* **50** (4) : 684 - 690.

SABET, K. A., SAMRA, A. S. AND DAWOOD, N. A., 1966, Combined infection with stalk-rot fungi, *In*: Sambra A S and Sabet K A, Eds., Investigations on stalk-rot disease of maize in U.A.R., Ministry of Agriculture, Government Printing Offices, Cairo, pp. 195 - 204.

SABET, K. A., ZAHER, A. M., SAMRA, A. S. AND MANSOUR, I. M., 1970, Pathogenic behavior of *Cephalosporium maydis* and *C. acremonium*. *Annals Appl. Biol.,* **66** : 257 - 263.

SAMRA, A. S., SABET, K. A. AND HINGORANI, M. K., 1963, Late wilt disease of maize caused by *Cephalosporium maydis*. *Phytopathology,* **53** : 402 - 406.

SNEDECOR, G. W. AND COCHRAN, W. G., 1956, Statistical methods applied to experiments in agriculture and biology. 5th ed. Ames, Iowa: Iowa State University Press.

WARD, E. AND BATEMAN, G. L., 1999, Comparison of Gaeumannomyces and Phialophora - like fungal pathogens from maize and other plants using DNA methods. *New Phytologist,* **141** : 323 - 331.

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