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ORIGINAL PAPER

Effect of *Magnaporthiopsis maydis* inoculum density on late wilt disease development and maize yield

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ABSTRACT

Powder of *Magnaporthiopsis maydis* diseased plants (*Mm*-PDP) was employed as an inoculum for soil infestation instead of *M. maydis*-colonized sorghum seeds (*Mm*-CSS). Inoculum density levels of 5~100g /pot (0.071 m²) and 10~200g/box (0.15 m²) were used in the greenhouse and 150~3000 g *Mm*-PDP /row/2.1 m² in the field. *Mm*-CSS were used in the positive control (PC) and un-infested treatments served as negative control (NC). Pioneer; SC3062 late wilt-sensitive maize hybrid was used and the disease incidence (DI%) was assessed at the age of 90 and 104 days, and yield component at 115 days. In the greenhouse, plants that emerged from PDP-infested soil (20g-100g or 40-200g, respectively) were insignificantly different in their DI % at the two assessing dates (DI ranged from 86.7-100 to 76.7-100%, respectively). On the other hand, use of 3000g *Mm*-PDP /row/2.1 m² exhibited the highest DI (34 and 59 % at the age of 90 and 104 days, respectively) in the field. Ear weight (EW), net kernels weight (NKW), and 100 kernels weight (100 KW) loss following this density were 47.5, 49.1 and 17.7%, respectively. Moreover, DI% resulted from 600g *Mm*-PDP was higher than that obtained from both 1200g *Mm*-PDP and 600g *Mm*-CSS (PC). In this study, the optimal *Mm*-PDP densities for obtaining more than 50% of DI were 3000g followed by 600g and 1200g, respectively. Fortunately, 50% of DI or more are acceptable to differentiate among maize genotypes when screened for resistance against the disease. So, the use of 600g *Mm*-PDP is less cost than the other densities (600g *Mm*-CSS as well as 1200g and 3000g *Mm*-PDP) and adequate for this purpose.

Keywords: Corn, Soil-borne fungi, Powder of diseased plants.

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INTRODUCTION

Maize (*Zea mays* L.) is one of the main cereal crops in Egypt contributes by 7.1 million tons of total cereal production (FAO, 2024). It is considered one of the most important food cereals due to its content of an appropriate calories, proteins and fats (Galani *et al.*, 2020). So, it is used for human food, livestock and poultry feed as well as it provides a raw materials for many industries such as starch and oils (Ranum *et al.*, 2014; Ayiti and Babalola, 2022; Purewal *et al.*, 2022). However, maize production in Egypt dose not sufficient to meet the local excessive demands (FAO, 2024). Furthermore, one of the most important fungi attacks maize and limit its productivity in Egypt is *Magnaporthiopsis maydis* (Klaubauf, *et. al.*, 2014); the cause of late wilt disease (Samra *et al.*, 1962; Samra *et al.*, 1968; El-Naggar *et al.*, 2015; El-Naggar, 2019). This disease is significantly affects the sensitive maize hybrids (El-Shehawy, *et al.*, 2014; El-Hosary and El-Fiki, 2015) and subsequently increases the loss in the final grain yield (El-Naggar *et al.*, 2015). Due to the risk of this disease (Payak *et al.*, 1970; Pecsi and Nemeth, 1998; Molinero-Ruiz *et al.*, 2010, and Drori *et al.*, 2013; El-Naggar *et al.*, 2015), several attempts have been interested to reduce its negative impact (Abd-El-Rahim, *et al*, 1982; Shalaby, *et al.*, 2009; Ghazy and El-Nahrawy, 2020; El-Naggar and Yassin, 2024). Although of these efforts, no registered product for its control is known until now. So, the use of resistant or tolerant verities against late wilt disease remain the essential way for avoiding or minimizing the final yield loss (El-Lakany, *et al.*, 2009; Degani *et al.*, 2018; 2022).

Screening for resistance against late wilt is successfully achieved by planting of maize genotypes under causal agent stress. So, soil infestation by a suitable form and density of the fungus is required in this respect. Since the disease was discovered (Samra *et al.*, 1962), different *M. maydis* colonized substrates such as wheat bran and sorghum seeds were employed for fungal reproduction for soil infestation under greenhouse conditions (Samra *et al.*, 1971; Abd-El-Rahim *et al.*, 1982; El-Naggar and Yassin, 2024). At the same time, little information is available until now in respect of soil infestation under field conditions (El-Shafey, *et al.*, 1988; Fayzalla, *et al.*, 1994; Agag, 2022). Of these, El-Shafey, *et al.* (1988) used maize diseased roots and stalks as a starting point or field activation followed by the addition of *M. maydis*-colonized wheat bran at the rate of a handful of inoculum per hill before planting. Also, Fayzalla *et al.* (1994) used 1800 g of *M. maydis*-colonized crushed sorghum seeds/plot (150 x 280 cm) for soil infestation. Additionally, in evaluation study of 25 maize hybrids against late wilt disease by Agag (2022), 10 kg of *M. maydis*-colonized grain sorghum seeds were mixed with 12 kg clay soil, afterwards 250 g of the obtained mixture were added per row (3m long). All of these attempts did not interest or refer to the inoculum density demand for achieving high level of disease incidence. However, all substrates used for fungal reproduction are expensive and labor consuming for their preparation. Interestingly, the relationship between inoculum density per unit of cultivated area and the late wilt incidence was not considered. Therefore, find out a low cost substrate insure *M. maydis* reproduction and survival for a long period (Singh and Siradhana, 1987) is firstly needed for disease nursery preparation. So, this work aimed to evaluate the effect of various densities of powder of *M. maydis*-diseased maize plants (per unit of the cultivated area) on late wilt incidence and subsequent yield.

MATERIALS AND METHODS

Source of *M. maydis*

Single spore *M. maydis* isolate (*Mm*-36), historically has virulence behavior (El-Naggar and Yasin, 2024), was chosen from the culture collection of Maize and Sugar Crops Diseases Research Section, Plant Pathology Research Institute, A.R.C, for use in the present study.

Soil infestation:

Source of *M. maydis*-diseased plants (*Mm*-DP)

A preliminary experiment was conducted in greenhouse using soil infestation technique (SIT) by *M. maydis*-colonized sorghum seeds (*Mm*-CSS) to obtain numerous diseased plants sufficient to conduct further greenhouse and field experiments. Pieces (2 cm^2) of 5-days old *M. maydis* culture were used to inoculate autoclaved 100 g of wet sorghum seeds (*Sorghum bicolor* L.) in glass bottles (500 ml in capacity). Then, inoculated bottles were incubated at room temperature (27-30^{o}C) for about 3-4 weeks (El-Shafey, *et al*., 1979, El-Naggar and Yasin, 2024). *Mm*-CSS were transferred from the bottles and mixed with loam soil at 100 g/pot (10 kg soil capacity and 30 cm in diameter). Ten kernels of the sensitive maize hybrid Pioneer SC3062 (El-Naggar *et al.*, 2015) were sown and irrigated. After 21 days, the emerged plants were reduced to 5 plants / pot. All recommended agricultural practices were applied. Later, at the age of 90-100 days, plants had typical symptoms of late wilt disease were harvested from the above ground level of soil, dried and maintained at room temperature (RT) in paper pages until use. Afterwards, diseased plants were grounded to powder using grinding machine (Dietz-motoren Grob H & Co. KG, D-7319 Dettingen u. Teck), then the obtained *Mm*-PDP was used as a fungal inoculum.

Greenhouse experiments:

Effect of Inoculum density on late wilt incidence:

The *Mm*-PDP was added to the loam soil at the appropriate amounts to achieve 11 inoculum densities in pot experiment, and 4 inoculum densities in both box and field experiments. The common inoculum form (*Mm*-CSS) was used at 20 g/pot to compare its efficacy with that of *Mm*-PDP as a new and a cheap inoculum source. As a same time, un-infested soil of all experimental units served as negative control (NC). Afterwards, kernels of the sensitive maize hybrid Pioneer SC3062 (El-Naggar *et al.*, 2015) were sown and irrigated. All recommended agricultural practices were carefully followed and disease incidence percent (DI %) was recorded at the age of 90 and 104 days.

Pots Experiment (PE):

According to the formula of area of circle (pot) = πr^2 [where, $\pi = 3.14$ and r = the half diameter of pot (30 cm)], the surface area of the used pot was 0.071m². The 11 *Mm*-PDP, inoculum densities incorporated into the potted loam soil were 5, 10, 20, 30, and 40 ~ 100 g (Table 1). Un-infested soil served as negative control (NC) and infested soil by *Mm*-CSS (20 g/pot/0.071m²) was used as positive control (PC). Ten kernels of the sensitive maize hybrid were sown with three replicates, and after 21 days plants were reduced to 5 /pot.

Wooden boxes experiment (WBE):

Boxes made from wood (50 x 30cm and 30cm height**)** were used in this study. Since the area of pot (0.071 m²) was approximately half that of the box (0.15m²), boxes were filled with infested soil contain fold densities of that used per pot. So, the following *Mm*-PDP densities; 10, 40, 80 and 200 g/box/ 0.15m² were used (Table 2). As designed in pots experiment, un-infested soil and infested soil by *Mm*-CSS (40 g/box/0.15m²) served as NC and PC, respectively. Stand plants were ten at the age of 21 days and three boxes were used per each level of each inoculum type.

Field experiment (FE).

The experiment was conducted in a separate area in Giza Agricultural Research Station, Plant Pathology Research Institute, A.R.C., to originate an experimental field to serve as a reliable disease nursery to screen maize genotypes for resistance against late wilt disease. In addition, the experimental plots were free from *M. maydis* (previously tested for soil infestation with the fungus by sowing the susceptible hybrid Pioneer SC3062) and all treatments were separated from each other (2.1m) to avoid crossing over among inoculum levels. The same four *Mm*-PDP densities that used in boxes experiment were used in the field relative to the cultivated area (CA) along with the PC and NC treatments. Randomized complete block design was conducted with three replicates. The plot consisted of one row (3 m long) spaced 70 cm from adjacent one and the row area was 2.1 m² (3 m long x 0.7 m width). Since the row area was about 30 fold of that of the pot (30cm in diameter), so the amount of four selected *Mm*-PDP densities were multiplied by 30 (*i.e.* 150, 600, 1200 and 3000 g/row/ 2.1 m²) before adding to the row soil surface and immediately mixed through 10 cm depth (Table 3). Then, two kernels of SC 3062 were sown in a hill, 2-3 cm depth, spaced 20 cm apart. Twenty one days after planting, plants thinned manually to one and all recommended agricultural practices were performed. DI% was recorded after 90 and 104 days of planting. *Mm*-CSS (PC) was added by the same way at 600 g/row/2.1 m² and un-infested rows served as NC.

Yield parameters assay:

At the age of 110 days, maize plant ears were harvested. Then, ear weight (EW), 100 kernels weight (100KW) and net kernels weight of ear (NKW) in gram were recorded. Kernels moisture content (MC %) were recorded after harvest (El-Naggar *et al.,* 2015) and NKW was adjusted to 15.5% of moisture content (Abdallah, 2014; Callaway *et al.,* 1992). The following modified formula (Hudon *et al.,* 1992) of

Carangal *et al.*, (1971) was used to express the yield in g plant^{-1} :

$$\text{Grain yield (g plant}^{-1}) = \frac{\text{Fresh ear weight (g plant}^{-1}) \times (100\text{-mc}) \times 0.8}{(100\text{-}15.5)}$$

Where, mc: moisture content % in kernels at harvest, 0.8: shelling coefficient and 100 - 15.5: standard value of grain moisture at 15.5%.

Statistical Analysis

The whole work was conducted in Giza Agricultural Research Station, ARC, Egypt. Obtained data were statistically analyzed using Web Based Agricultural Statistics Software Package (WASP) and Duncan`s multiple range test (P = 5%) was used to compare means (Jangam and Thali, 2004).

RESULTS

Pots experiment (PE):

Obtained data (Table 1) show that all inoculum densities were effective and significantly varied in their capability of producing late wilt disease compared to the NC. Using of *Mm*-PDP densities (5 ~ 100 g / 0.071m² CA) resulted in DI ranged from 60 to 100%. DI% obtained at 5 g of *Mm*-PDP / 0.071m² was significantly lower than those obtained from other *Mm*-PDP densities. Besides, DI% at 5g of *Mm*-PDP / 0.071m² (60 and 80% at the age of 90 and 104 days, respectively) was insignificant compared with that obtained from 20 g of *Mm*-CSS (73.33 and 86.67% at the two assessing dates, respectively). At the same time, DI (100.0 %) resulted from infested soil by 20 g *Mm*-PDP / pot was significantly superior that recorded (86.67%) at the same density of *Mm*-CSS after 104 days of planting. Noticeably, late wilt disease incidence values were increased with the delaying of disease assessing date.

Table 1: Effect of *M. maydis* inoculum density on the incidence of late wilt disease under greenhouse experimental pots (30 cm diameter = 0.071m² area).

Inoculum		**Late wilt incidence (%) at the age of**	
density (g)	**type**	**90 days**	**104 days**
5	*Mm*-PDP[1]	60.00^{c}	80.00^{c}
10	*Mm*-PDP	80.00^{abc}	93.33^{ab}
20	*Mm*-PDP	86.67^{ab}	100.00^{a}
30	*Mm*-PDP	93.33^{ab}	100.00^{a}
40	*Mm*-PDP	100.00^{a}	100.00^{a}
50	*Mm*-PDP	93.33^{ab}	100.00^{a}
60	*Mm*-PDP	100.00^{a}	100.00^{a}
70	*Mm*-PDP	93.33^{ab}	100.00^{a}
80	*Mm*-PDP	93.33^{ab}	100.00^{a}
90	*Mm*-PDP	93.33^{ab}	100.00^{a}
100	*Mm*-PDP	100.00^{a}	100.00^{a}
20	*Mm*-CSS[2]	73.33^{bc}	86.67^{bc}
0	NC[3]	0.00^{d}	0.00^{d}

[1]*Mm*-PDP= Powder of *M. maydis*-diseased plants; [2] *Mm*-CSS= *M. maydis*-colonized sorghum seeds, served as positive control; [3]NC= Negative control (un-infested soil); Values with the same letter in the same column are insignificant at 5%.

Boxes experiment (BE):

Data of WBE (Table 2) exhibit that all tested *Mm*-PDP densities were effective for producing DI (36.67 - 86.67% and 83.33 - 100% at 90 and 104 days after sowing, respectively) significantly different from the NC (00.00%). DI% obtained from the lowest *Mm*-PDP density (10g/box/0.15m²) was significantly less than that obtained from the remaining densities (20, 40 and 100g/box/0.15m², respectively) at the age of 90 days. On the other hand, DI (36.67 and

83.33 %) obtained from 10g of *Mm*-PDP was insignificantly different from DI (26.67 and 86.67 %) of 40g *Mm*-CSS at the two reading dates, respectively. As noticed in pots experiment, DI% was increased with the delaying of disease assessing date. It was also increased as inoculum density was increased.

Table 2: Effect of *M. maydis*-inoculum density on the incidence of late wilt disease under greenhouse experimental boxes (50 cm length x 30 cm width x 30 cm height = 0.15m² area).

Inoculum		**Late wilt incidence (%) at the age of**	
density (g)	**type**	**90 days**	**104 days**
10	*Mm*-PDP[1]	36.67[b]	83.33[c]
40	*Mm*-PDP	76.67[a]	96.67[ab]
80	*Mm*-PDP	86.67[a]	100.00[a]
200	*Mm*-PDP	70.00 [a]	93.33[abc]
40	*Mm*-CSS[2]	26.67[b]	86.67[bc]
0	NC[3]	0.00[c]	0.00[d]

[1] *Mm*-PDP= Powder of *M. maydis*-diseased plants; [2] *Mm*-CSS= *M. maydis*-colonized sorghum seeds, served as positive control; [3] NC= Negative control (un-infested soil); Values with the same letter in the same column are insignificant at 5%.

Field experiment (FE):

Table (3) shows that the four *Mm*-PDP densities along with *Mm*-CSS density, caused late wilt in maize plants. Significant differences were noticed among the obtained DI% that ranged from 14.69 to 59.28% during the two assessing dates. On the other hand, High DI (34 and 59 % at the age of 90 and 104 days, respectively) were obtained from the use of 3000 g/row/2.1m² of *Mm*-PDP followed by 600g (29 and 55% after 90 and 104 days from sowing, respectively). Besides, DI% obtained from the use of 600g *Mm*-PDP was significantly higher than that obtained from the same *Mm*-CSS density. Noticeably, DI% was increased with delaying of the assessing date from 90 to 104 days after sowing.

Table 3: Effect of *M. maydis*-inoculum density on the incidence of late wilt disease under field experimental rows (3m long x 0.7m width = 2.1 m²).

Inoculum		**Late wilt incidence (%) at the age of**	
density (g/ row)	**type**	**90 days**	**104 days**
150	*Mm*-PDP[1]	14.6[e]	39[e]
600	*Mm*-PDP	29[b]	55[b]
1200	*Mm*-PDP	19[d]	47[d]
3000	*Mm*-PDP	34[a]	59[a]
600	*Mm*-CSS[2]	24[c]	51[c]
0	NC[3]	0[f]	0[f]

[1] *Mm*-PDP= Powder of *M. maydis*-diseased plants; [2] *Mm*-CSS= *M. maydis*-colonized sorghum seeds, served as positive control; [3] NC= Negative control (un-infested soil); Values with the same letter in the same column are insignificantly different at 5%.

Effect of inoculum densities on maize yield components:

Table (4) shows that yield parameters (EW, NKW and 100 KW) of maize plants emerged from infested soil by all of *Mm*-PDP densities and *Mm*-CSS density (PC) were significantly lower (118 – 157, 84-109 and 27.3-29 g, respectively) than those obtained from the NC plants (225, 165 and 32.3g, respectively). EW, NKW and 100 KW (118.2, 84 and 26.3 g, respectively) of the plants sown in infested soil at density of 3000 g/row/ 2.1 m² of *Mm*-PDP showed the lowest values at all and were lower than the control. On the other hand, EW and NKW of plants cultivated in infested soil at 600g of *Mm*-PDP were significantly higher than those obtained from the same density of *Mm*-CSS. At the same time, no significant differences were found in EW and NKW obtained from plants sown in infested rows at densities of 600 and 1200g of *Mm*-PDP. Also, no significant differences appeared between the 100KW of maize plants emerged from infested soil at 150, 600g of *Mm*-PDP and 600g of *Mm*-CSS.

Concerning to the significance of yield losses, EW, NKW and 100 KW losses (30.7- 47.5, 33.9 - 49.1 and 10 - 17.7 %, respectively) were high in general. However, the highest percentages of loss in EW, NKW and 100 KW (47.5, 49.1 and 17.7%, respectively) were obtained from plants sown in *Mm*-PDP infested soils at density of 3000g. There were no significant differences appeared between EW and NKW losses of plants that emerged from soil infested by each of 600 and 1200g of *Mm*-PDP.

Table 4: Effect of inoculum density on maize yield components and loss % in the field experiment: ear weight (EW), net kernels weight of ear (NKW) and 100 kernels weight (100KW).

Inoculum		**Yield components (g)**			**Loss% in Yield components (g)**		
density (g/row)	**type**	**EW**	**NKW**	**100 KW**	**EW**	**NKW**	**100 KW**
150	*Mm*-PDP[1]	153.7[c]	109[b]	28.7[b]	31.7[c]	33.9[c]	10.4[c]
600	*Mm*-PDP	156[b]	109[b]	28.7[b]	30.7[d]	33.9[c]	10.4[c]
1200	*Mm*-PDP	157[b]	108[b]	27.3[c]	30.2[d]	34.5[c]	14.6[b]
3000	*Mm*-PDP	118.2[e]	84[d]	26.3[d]	47.5[a]	49.1[a]	17.7[a]
600	*Mm*-CSS[2]	146[d]	104.3[c]	29[b]	35.1[b]	36.7[b]	9.3[d]
0	NC[3]	225[a]	165[a]	32.3[a]			

[1]*Mm*-PDP= Powder of *M. maydis*-diseased plants; [2]*Mm*-CSS= *M. maydis*-colonized sorghum seeds, served as positive control; [3] NC= Negative control (un-infested soil); Values with the same letter in the same column are insignificantly different at 5%.

DISCUSSION

M. maydis causing maize late wilt disease continues to be a difficult agent to control by fungicides and there is no registered product for this purpose until now (El-Naggar and Yassin, 2024). So, breeding for resistance to late wilt is still the major way for disease management (El-Lakany *et al.*, 2009; Degani *et al.*, 2018; 2022). To achieve this goal, screening of maize genotypes under stress of fungal propagules should be follow. This requires two essential factors: firstly, an enough inoculum density for acceptable disease incidence (Maitlo *et al.*, 2016); secondly, low cost fungal inoculum. In this study, powder of late wilt diseased plants was used as a cheap source of inoculum to infest the soil at numerous densities relative to the cultivated area in greenhouse and field trials. The obtained results showed that the tested *Mm*-PDP densities were effective for developing maize late wilt disease in all experiments. In greenhouse experiments, 20g of *Mm*-PDP /pot/ 0.071 m² and 40g of *Mm*-PDP /box/0.15m² were enough to produce high DI (86.67-100% and 76.67-96.67% at the two assessing dates, respectively) that insignificantly different from those obtained with the higher densities used. At the same time, the later DI% was either equal or higher than that caused by the common *Mm*-CSS at the same density (20 and 40g, respectively). This result confirms that the use of 20g of *Mm*-PDP can facilitate reliable and accurate screening of *M. maydis* susceptible maize genotypes under greenhouse condition (Matthews *et al.*, 2023). Also, our results are consistent with the results of Yaqub and Shahzad (2005) on *Sclerotium rolfsii* root rot of mungbean and sunflower. They noted that there were no significant differences in root colonization index in infested soil contained 1, 5 and 10 sclerotia /g soil.

Besides, using 3000g of *Mm*-PDP /row/2.1 m² was also enough to cause the disease with the highest incidences (34-59%) which were reflected on increasing the reduction of EW, NKW and 100KW. Similarly, Maitlo, *et al.* (2016) reported that one (10^4 spores g^{-1} soil) out of five inoculum densities of *F. oxysporum* f.sp. *ciceris* used to infest chickpea soil was enough to produce maximum degrees of disease parameters. Also, these results were some-consistent with those obtained by others (Yaqub and Shahzad, 2005; Chang *et al*., 2014; Altaf *et al*., 2016; Brantner and Chanda, 2021; and Matthews *et al*., 2023). All of them stated that increasing of the pathogen inoculum level was negatively correlated with the growth parameters and yield components of mungbean and sunflower (Yaqub and Shahzad, 2005), faba been fresh weight (Chang *et al*., 2014), sugar beet roots and sucrose % (Brantner and Chanda, 2021), maize grains yield (Altaf *et al*., 2016), and ginger root size and weight (Matthews *et al*., 2023). On the other hand, diseased plants were previously used as a source of inoculum in screening of maize genotypes for resistance against northern corn leaf blight disease caused by *Exserohilum turcicum* (Hooker, 1975; Schechert *et al*., 1999). Furthermore, *M. maydis*-colonized sorghum seeds (*Mm*-CSS) are prepared annually for late wilt disease nurseries over five experimental stations for maize resistance screening as a part of the continuous programs of our department. But, *Mm*-CSS required the excessive use of the expensive sorghum seeds for this purpose in addition to the workers efforts and time depletion. As reported by Paul *et al*., (2013), 50% of DI or more are acceptable to differentiate among maize genotypes when screened for resistance against most diseases. In this study the optimal *Mm*-PDP density for obtaining more than 50% of DI were 3000g followed by 600g and 1200g, respectively. On the other hand, using 600g of *Mm*-PDP /row was more efficient (DI; 29 - 55%) than 1200g (DI; 19-47%) for producing late wilt incidences at the two assessing dates. So, the use of 600g of *Mm*-PDP/row/2.1 m² is less cost and adequate than the use of 1200 and 3000g of *Mm*-PDP as inoculum density required for screening maize genotypes against late wilt in the field.

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