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Downy Mildew of Sunflowers in Tunisia and evaluation of four fungicides

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***Plasmopara halstedii*, the downy mildew pathogen of sunflower (*Helianthus annuus* L.) causes significant economic losses world-wide, mostly through soilborne systemic infection of seedlings. Natural infection of sunflower with *P. halstedii* was monitored in sunflower fields cultivated in many regions of Beja, during a survey in the spring of 2010. *P. halstedii* were obtained from diseased sunflowers seeds in 2010 and characterized by virulence and by reaction to four fungicides: Ridomil MZ 58, Ridomil Gold 48%, Aliette 80% and Triziman 80%. These fungicides have significantly reduced the colony growth of the fungi compared to the control. The significant reduction of mycelia growth were obtained with Ridomil M, Triziman 80% and Aliette 80% with a completely inhibition.**

Keywords: Downy mildew, *Helianthus annuus* L., pathogen, *Plasmopara halstedii*, fungicides.

Sunflower downy mildew (SDM) is caused by *Plasmopara halstedii* ((Farl.) Berl. et Toni), an Oomycete specific to sunflower (*Helianthus annuus* L.), infected by soil- and seed- borne dissemination. Different physiological races (pathotypes) can attack a variable range of sunflower genotypes (Tourvieille de Labrouhe et al., 2000). To date, there are at least 35 races in different parts of the world (Gulya, 2007). SDM is one of the principal diseases causing economic losses in sunflowers fields. The significant hosts are the wild and cultivated species of *Helianthus*, including sunflower (Leppik, 1966), but 100 host species from a wide range of genera in the family Asteraceae have been reported. This disease had spread to all sunflower growing countries mainly through seeds (Viranyi, 1990).

Primary infection is caused by zoospores which are released from a primary sporangium produced by an oospore. Within the *Peronosporales* this is one of three possible ways for oospores to germinate. Alternatively, zoospore production in sessile vesicles was found to be typical for *Albuginaceae*, and direct infection with the formation of a germ tube was reported from some *Pythium* and *Phytophthora* species ((Leppik, 1966).

The majority of systemically infected plants either die prematurely or hardly produce viable seed, They make no contribution to yield. Furthermore, reduction in seed yield may also be due to pre-or post-emergence damping-off of severely mildewed seeding, a symptom often overlooked and underestimated. Disease severity may vary considerably

according to region, year and growing conditions. The incidence of downy mildewed sunflowers in a field may range from traces to near 50% or even up to 95% (Sackston, 1981). Sakr (2010) noted that percentage infection and dwarfing could be used to differentiate aggressiveness in P. halstedii, but these criteria played a limited role in defining P. halstedii isolates according to their aggressiveness. The disease affects young plants when water content of the soil is high and maximum air temperature is between 15 and 18°C. *P. halstedii* shows asexual propagation by liberation of zoosporangia produced on the lower surfaces of sunflower leaves and sexual reproduction by oospores which are found in crop residues. Virulence has been defined as specific disease-causing abilities and aggressiveness as non-specific disease-causing abilities according to Van Der Plank (1968). Sakr et al. (2009) used two aggressiveness criteria, latent period and sporulation density, to differentiate between two field isolates of races 100 and 710.

In Europe, after its first appearance in 1941, the disease increased rapidly and by 1977 it was rated a "major disease" in all sunflower producing countries of Europe (Sackston, 1981). *P. halstedii* is seed-borne and soil-borne fungus, with its spores capable of surviving for as long as 8-10 years in the soil. Thus, the disease is extremely difficult or impossible to eradicate once it is established in an area. Fungicides with definite systemic and long-lasting properties seemed to be efficient in controlling the disease (Viranyi and Oros, 1990). Field isolates and single sporangium lines of the biotrophic Oomycete *Plasmopara halstedii*, differing in host preference and fungicide sensitivity, were used simultaneously for infection of sunflower. In many crops like sorghum, maize, pearl millet etc., downy mildew has been managed by seed treatment with metalaxyl. This fungicide is especially toxic to oomycetous fungi (Venugopal and Safeeulla, 1978). Patil *et al*. (1992) have also reported the efficacy of Apron 35 W.S. (a powder form of metalaxyl) applied at the dose of 6 g/kg of sunflower seed. Based on that, the objectives of this study were (i) to assess the variation of virulence and aggressiveness of collected *Plasmopara halstedii* isolates (ii) to find the appropriate dose of the four fungicides tested *in vitro* and *in situ* against Sunflower Downey Mildew.

Materials and Methods

Measurement of aggressiveness in *P. hasltedii* isolates

Collection of Seed Samples

Seeds of sunflower cultivars sown in April 2010 were obtained from farmers located in Beja in Northern of Tunisia.

Fungal Isolates

Fungi isolation from sunflower seeds was made in PDA Petri plate. In each one, ten seeds were used and replicated four times. The Petri plates were incubated at 25-27°C in darkness for 7 days.

Fungi were identified on the basis of their typical structure and basic characters as suggested by Spring and Zipper (2000).

Inoculum Preparation

Field samples of the pathogen were collected from 10 fields of sunflower originated from 4 areas in Beja. The zoospores of the pathogen were recovered directly from plants that showed pathogen sporulation on leaves. These one were washed with a brush and distilled water. The inoculum was a zoosporangial suspension. Concentration was adjusted to $2x10^5$ zoospores/ml with Malassez cell.

Artificial infection

Plants were raised from seed in pots containing peat and perlite (1:1). To evaluate the isolates pathogenicity, 90 plants with two or three pairs of sunflowers leaves were used. Whole plants were inoculated by spraying (15 ml/plant) with zoospores suspended in distilled water. Inoculated plants were covered with plastic film in a saturated atmosphere and placed

in a chamber (15° to 20°C) for 24h and then transferred to a green house (24 to 27°C) for 14 days to allow for disease development. Plants were fertilized once a week with N:P:K (20:20:20) fertilizer.

Two weeks after inoculation, the number of plants expressing symptoms (showing sporulation on leaves) was noted. This experiment was repeated three times. The number of replications is 3 with 30 plants per replicate.

Chemical control test *in vitro*

Fungicides

The fungicides used for *in vitro* and *in situ* experiments were Ridomil MZ 58 (Metalaxyl + Mancozebe), Ridomil Gold 48% (Mefenoxam), Aliette 80% (Fosytel-Al) and Triziman 80% (Mancozebe). Fungicides concentration used for each experiment is indicated in tables 1 and 2.

Mycelial growth assay

Culture of *P. halstedii*, previously isolated from sunflowers seeds was used in this experiment. Appropriate volumes of each fungicide and bio-fungicides were added to PDA at approximately 50°C in amounts to achieve final concentrations. Control PDA plates were prepared similarly but adding sterile distilled water (SDW) instead the fungicide. Mycelia plug (5 mm in diameter) of *P. halstedii*, aged 5 days, was inoculated in the center of each Petri plate. Three concentrations were used with with four replicatesThe plates were incubated at 25± 2°C and radial growth of the fungus was measured when the control plates were completely developed by the fungus. Growth inhibition (%) was calculated following the formula below:

$$\% \text{ Inhibition} = \frac{\text{Diameter of colony in control} - \text{diameter of colony of fungi in fungicide}}{\text{Diameter of colony of control}} \times 100$$

Chemical control test *in situ*

Disease management trials were conducted in greenhouse on one month-old sunflowers. Plants were grown in plastic pots containing peat and perlite (1:1). Two applications methods were used, foliar applications and plants were sprayed to runoff (~ 10ml/plant) with sprayer and the second application was soil drenches and were made by pouring 50 ml of aqueous solution of fungicide into each pot. Plants were inoculated with a zoosporangial suspension, by spraying (15 ml/plant), after 5 days from applications fungicides. The experiment was arranged in a completely randomized design. Fifty plants were used for each fungicide/ treatment

Results and discussion

Symptoms and morphological identification

P. halsteddi is an obligatory biotrophic plant pathogen. Leaves of infected plants develop chlorotic mottling which spreads from the veins near the petiole across the lamina, and increases in area and intensity as leaves old (Fig. 1).

Fig. 1: Green and chlorotic mottling along the main veins due to *P. halstedii* on sunflower

Plants become stunted, having thin stems, very much smaller capitula without seeds, and smaller and darker roots. The disease is primarily systemic and mycelium can be found throughout the plant from roots to

capitulum and achenes. Under humid conditions, a white felt of sporangiophores develops on the undersurface of chlorotic areas (Fig. 2).

Fig. 2: Young sunflower plants affected by downy mildew (*P. halstedii*) in the field, with fungal sporulation on the lower leaf surface.

Localized secondary infection of the leaves and heads occasionally develops, resulting in spots, delimited by veins. Such secondary infection may also become systemic. Some infected plants show no disease symptoms, but produce lower yields of poorer quality seeds, which lose vitality and have lower germination rates (latent infection).

Sporangiophores were monopodially branched at right angles with acutely tapering termini (Fig. 3). Sporangia were ovoid to elliptical (Fig. 3) and measured 20 to 25 × 13 to 19 µm. Based on these features, the organism was identified as *P. halstedii* (Farl.) Berl. & de Toni.

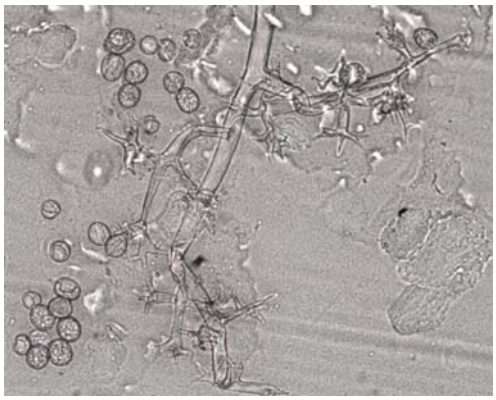

Fig. 3: Sporangiophore and sporangia of *Plasmopara halstedii*.

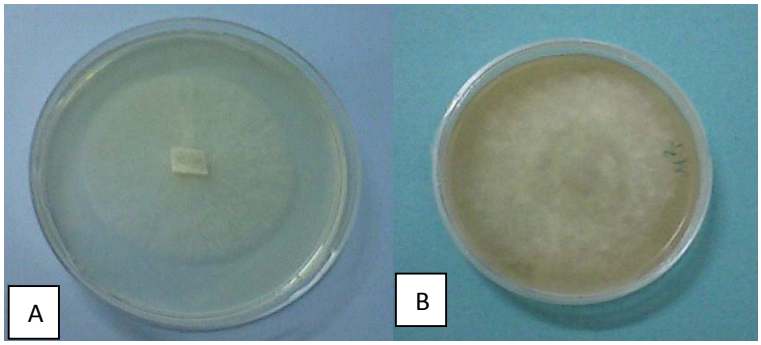

Fig. 4: Colony pattern on Potato dextrose agar (A) and PARBH (B) of *P. halstedii*

Measurement of aggressiveness in *P. hasltedii* isolates

The artificial infection by zoosporangia showed chlorotic symptoms, after two week after inoculation. A microscopic investigation of mycelium distribution in leaves confirmed the installation of pathogen.

Chemical control test *in vitro*

The effect of four fungicides noted Ridomil MZ 58, Ridomil Gold 48%, Aliette 80% and Triziman 80% was assessed against *P. halstedii* isolated from sunflower plants. These fungicides have significantly reduced the colony growth of the fungi compared to the control. The significant reduction of mycelia growth were obtained with Ridomil M, Triziman 80% and Aliette 80% with a completely inhibition (Fig. 5).

P. halstedii isolates collected in Beja have showing an atypical reaction to metalaxyl , and their level of sensitivity to this

fungicide was tested in the laboratory for. The infections by *P. halstedii* were not controlled by the Mefenoxam treatment. There was no reduction of the isolates agressiveness. Albourie et al., (1998) have showed that Ridomil M, systemic fungicides (Metalaxyl and Mancozeb) and the mixed formulations : Dimethomorph + Mancozeb; Cymoxanil + Mancozeb and Ofurace + Folpet were effective against primary infections but not against secondary infections . Metalaxyl mixed with Fluazinam, Folpet or Mancozeb were more effective against primary infections with the resistant isolate than Metalaxyl alone. The EC50 of five other isolates ranged from 5 800 to 32 900 mg a.i. kg-1, indicating a variability in metalaxyl sensitivity of resistant sunflower downy mildew isolates.

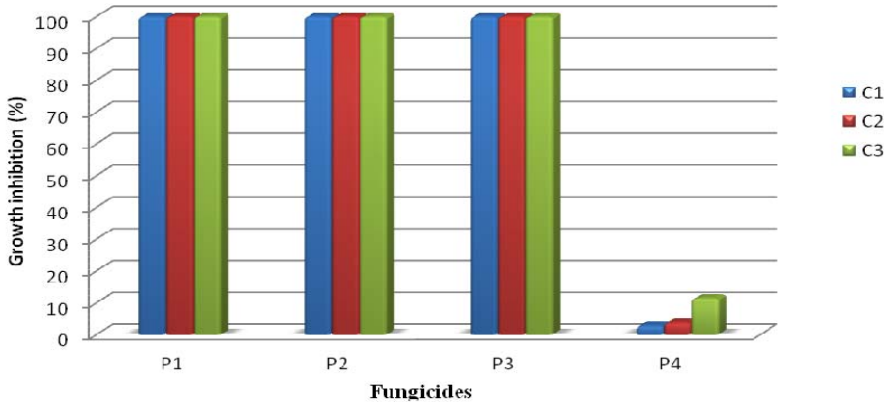

Fig. 5: Effect of 4 fungicides Ridomil MZ 58, Ridomil Gold 48%, Aliette 80% and Triziman 80% against *P. halstedii* isolated from sunflower.

Table 1: Doses used for the four fungicides in the *in vitro* experiments

Concentration / Product	D1	D 2	D 3
Ridomil MZ 58	0,2 g/l	0,4 g/l	0,8 g/l
Aliette	0,3 g/l	0,6 g/l	1,2 g/l
Triziman	0,25 g/l	0,5 g/l	1 g/l
Ridomil Gold 480	10µl	20µl	40µl

Table 2: Method and dose used for the four fungicides in the *in situ* experiments

Treatment	Dose
Aliette - foliar spray	3 g/l
Triziman - foliar spray	2, 5 g/l
Ridomil MZ 58 – soil drench	2 g/l
Ridomil Gold 480– soil drench	0,1 ml/l

Metalaxyl-M is a phenylamide that is applied extensively as a seed dressing for controlling sunflower (*Helianthus annuus*) downy mildew caused by *P. halstedii* (Molinero-Ruiz et al., 2005). The identification of the pathogen isolates expressing resistance to metalaxyl in recent years has raised. This study examined the virulence of *P. halstedii* populations and their sensitivity to metalaxyl and metalxyl-

M. The levels of resistance to metalaxyl and to metalaxyl-M were compared with four populations in three *in vivo* experiments. Race 310 was the most frequent in the 3 years in southern Spain (68% of the populations), and races 100 and 330 were identified also. The only two populations collected outside this area had different virulences (races 703 and 710). Thirty-one percent of the populations were not controlled by metalaxyl-M when applied at the recommended dose (2.0 g a.i./kg seed), and subpopulations collected on plants from treated seed were the same race as those from the initial populations. The incidence of downy mildew depended significantly on the dose. Resistance to metalaxyl-M in almost one-third of the populations may be attributed to the extended and frequent use of the fungicide in Spain. The authors recommended that the identification of resistance to metalaxyl-M in highly virulent populations of *P. halstedii* should be considered in the management of both genetic and chemical strategies for the control of sunflower downy mildew (Amzalek and Cohen, 2007). In Italy, it is obligatory that the sunflower seeds are treated with metalaxyl when imported fro; others countries. In greenhouse trials, it was not observed a reduction in sensitivity to this systemic fungicide used as seed treatment against *P. helianthi* race 2. Consequently, the susceptibility of some commercial hybrids, indicated as resistant to downy mildew by importers, could be attributed to irregularity in the production of seeds. Also, an insufficient seed dressing with metalaxyl does not completely control the pathogen (Baldini et al., 2008).

Research conducted by Amzalek and Cohen (2007) showed that *P. halstedii* causes extensive damage to sunflowers, and the absence of efficient antifungal treatments has led to a search for new varieties of sunflower resistant to the fungus. Several races of *P. halstedii* have been identified, and some resistant sunflower hybrids have been produced, but the resistance is not durable. Moreover, metalaxyl is the only commercial antifungal agent available, and resistant strains of *P. halstedii* have been described.

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