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INTERACTION OF NITROGEN FERTILIZERS WITH WHEAT GROWTH STAGE AND FOLIAR TREATMENT WITH UREA EFFECTS ON WHEAT CROWN ROT INDUCED BY *FUSARIUM CULMORUM*

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Abstract

Wheat crown rot disease (*Triticum durum* Desf.), induced by *Fusarium culmorum* is a serious constraint in most arid and semi-arid cereal regions of the world. Disease severity is related to water stress and excessive use of nitrogen especially in the absence of recommendations where inoculum is present in the soil. Therefore, a reasonable nitrogen management throughout the right type, the right moment and the right method of its application were the major goal of this work to control wheat crown rot. Two trials were conducted under greenhouse conditions. The first one dealt with site effect of nitrogen applied as three types of nitrogen fertilizer at sowing as baseline fertilizer or broadcasted with two different doses, at two growth stages of durum wheat inoculated plants. In the second trial, a urea solution was applied as a foliar treatment in comparison to a soil application. All treatments were applied either at heading or flowering growth stages. Fertilizers' effect on disease development and biological yield was evaluated once at flowering stage in the first experiment and at maturity at the second one. Results showed that ammonitrate applied before sowing or broadcasted at stem elongation or jointing stage, were the most effective in controlling this disease. On the other hand, broadcasting urea or applied as a foliar fertilizer when the inoculum of root rot is present in the soil should be avoided, because it has significantly enhanced disease development and consequently reduced yield and its components. Application of these results along with a tentative nitrogen recommendation for regions at risk of wheat crown rot is pointed out in the discussion section.

Key words : Durum wheat, *Fusarium culmorum*, nitrogen, site application, foliar application.

Introduction

Dry rot of durum wheat (*Triticum durum* Desf.), induced by *Fusarium culmorum*, is a worldwide constraint in most cereal regions, especially in the arid and semi-arid regions (Cook 1980; El Yousfi 1984; Rossi *et al.,* 1995; Backhouse *et al.,* 2004; Lamprecht *et al.,* 2006; Hollaway and Exell 2010; Moya *et al.,* 2011). The damage attributed to this disease is significant and highly variable from one year to another and from one region to another (Wearing and Burgess 1977; Tinline and Ledingham 1979; Diehl *et al.,* 1982; Piening *et al.,* 1983; Wiese *et al.,* 1984; Kidambi *et al.,* 1985; Stack 1991). Economically, this root rot can induce yield reductions of up to 50% (Cook 1968) in the U.S.A, and in Morocco, they are in the order of 12 to 17% (El Yousfi 1984).

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In this regard, several control measures have been developed against the causal agents of this disease, which are mainly *Fusarium culmorum* and *Fusarium pseudograminearum* (Strausbaugh *et al.,* 2004), these methods are either genetic by adoption of resistant or tolerant varieties (Sallans and Tinline 1965; Dodman and Wildermuth 1987; Purss 1965; Mergoum 1991; Mergoum *et al.,* 1994), biological with biological agents (Anwar 1949; Mergoum 1991; Uoti 1976), chemical (Watking and Kerr 1980; Stack 1982; Mergoum 1991) or through cultural techniques (Vincent and Panneton 2001), including the application of crop rotation and rational use of fertilization (Lamprecht *et al.,* 1995; Duveiller *et al.,* 2007; Burgess *et al.,* 2010). Many reports showed that nutrients may have variable effects to diseases and may be important factors in the development of disease susceptibility or resistance (Singh 2015). However, the effects of nutrients on plant diseases are directly related to their efficient use, taking the case of nitrogen that is affected not only by climate and certain soil characteristics, but also by crop management practices, such as type of tillage systems and timing and method of application (Usherwood and Segars 2001; Huber and Haneklaus 2007; Borghi *et al.,* 1995; Duncan *et al.,* 2018; Fageria and Baligar 2005; Blackshaw *et al.,* 2002). The study conducted under the agro-climatic conditions of Sind showed that the efficient use of fertilizers, especially urea, is a foliar one which gave positive results on wheat yield and its components (Parvez *et al.,* 2009). In addition, Mosluh *et al.,* (1978), and under irrigated conditions in Iraq, found that urea nitrogen applied both to soil and as a foliar fertilizer is more effective than the one applied to soil. On the other hand, overuse of this nutrient may aggravate wheat crown rot in plants that have suffered water stress from excessive nitrogen application (El Yousfi 1984; Cook 1992; Davis *et al.,* 2009).

In Morocco, the recommendations concerning nitrogen fertilization are given by region, by crop and according to the achievable yields (Karrou 2003; Guennouni 2017). For semi-arid wheat, the requirement is 60 to 80 kg N / ha and the inputs are split: 2/3 at the time of sowing and the remaining at the tillering stage. The dose is relative to sufficient moisture in the soil and the choice of urea (46% N) is advisable for a price reason (Karrou 2003). In contrast and for arid zones, nitrogen is only an option if rainfall is favorable (Karrou 2003), it is clear that these recommendations are insufficient because we are talking about wheat, but does not define whether it is for durum wheat or common wheat and even the choice of fertilizer type is based only on an economic reason and not on a biological and scientific basis. Consequently, recommendations should be based on a rational management of nitrogen fertilization through the choice of the right type of nitrogen, the right moment and the good method of its application especially when most frequent biotic constraints are present like those that are related to nitrogen fertilization such as root rot. Thereafter, nitrogen use may have a positive impact on agricultural production.

In this context, the present work aimed to study the effect of site application of different types of nitrogen fertilizer (Ammonium sulphate 21% N, Ammonitrate 33.5% N and Urea 46% N) at the root system, and also a foliar treatment effect of urea on development of wheat crown rot induced by *Fusarium culmorum*.

Materials and Methods

A durum wheat variety "Ourgh" (ONSSA 2019) used in this study is known for its susceptibility to wheat crown rot disease (Oslane *et al.,* 2014). This variety was fertilized using commercial fertilizers: DAP (Di-ammonium phosphate 18N, 46P, 0K), which was used as a base fertilizer and ammonitrate (33.5% N), ammonium sulfate (21% N) and urea (46% N) as surface fertilizers.

Isolation of the fungus

Roots or sub-crown plants' parts showing typical symptoms of the disease were sampled from field surveys of different cereal zones in Morocco. Those plant parts underwent 5 successive washes for 15 minutes in a soap solution containing 10% bleach, and 3 rinses with sterile distilled water. Then, the roots were dried on sterile filter paper, cut into small pieces and immediately deposited on PDA (Potato-Dextrose-Agar). After 4 to 5 days of incubation at a temperature of 20°C and at a photoperiod of 12h, five isolates of *Fusarium culmorum* of different origins were identified according to the procedures of Leslie *et al.,* (2006) and purified on PDA, then incubated on PDA for 10 days under the same conditions mentioned previously.

Inoculum preparation

After mycelial multiplication, clumps of the isolates were added to 100 g of barley seeds previously imbibed and sterilized in erlenmeyer flasks, and the whole is incubated for 2 months. Following incubation, the erlenmeyer flasks were transferred to a greenhouse and emptied for a moderate drying of their contents for 15 days. Once well dried, the inoculated seeds were milled to obtain a fine powder called inoculated organic matter (IOM) or inoculum which is stored at a temperature of 5°C for subsequent uses.

Inoculation

Plastic unperforated pots were half filled with a vertisol soil brought from Sidi El Aidi-INRA, Settat experimental station, and 10 seeds of durum wheat were sown, then a layer of soil of 2 cm was added to cover the seeds. Afterwards and to initiate seed germination, a volume of 100 ml is poured on pots. Two days later, a thin layer of soil is broadcasted on the surface of the pots to avoid wet soil contact with the inoculum which was evenly scattered at a rate of 0.4 g per pot. In this way, the added inoculum would initiate the infection the disease plants' crown level. Then, the added inoculum was covered with a layer of the same soil of a height of about 1 to 2 cm.

Experiment 1: Site of nitrogen application

This experiment was carried out under a greenhouse condition using black pots of top 10.5 cm and lower 9 cm in diameters. DAP was applied to all pots beneath the sowing bed and all plants underwent the same inoculation process. This experiment was carried out as a complete blocks design with 4 repetitions with 20 treatments composed of four different nitrogen fertilization treatments: unfertilized (control), ammonium sulphate (21% N), ammonitrate (33.5% N) and urea (46% N), applied at five levels: 25 cc added at tillering stage, stem elongation and at both growth stages, and either 25 cc 50 cc were added before sowing. All solutions were of a concentration of 120 g/5 liters of water for each type of fertilizer. This experiment was repeated 3 times and plants were sufficiently irrigated as needed.

At early heading stage, plants in each pot were pulled out, their root system thoroughly washed, and lower internodes were well cleared to ease evaluation of the disease. The assessment was based on lesions length within the first three nodes from the base of the stems. The severity of the disease was graded from 0 to 3, and the scoring ranged from 0, 0.1, 0.2 to 3 according to a continuous progression of the disease from the first to the third node. Once the severity assessment was completed, the plants were well air dried in the greenhouse to measure plant dry weight biomass of each treatment.

Experiment 2: Urea foliar application

This experiment was carried out in three sets under the same conditions as for the first one, except that each set was laid out in a different greenhouse. Pots with diameters of top 15.5 cm and a lower 11.5 cm were all prepared for inoculation as previously described. Each set was of a randomized complete block design of four repetitions with four treatments composed of a combination of urea applications foliar or irrigation with two different growth stages heading and flowering which are compared to an untreated control. All urea applications were of 25 cc at a concentration of 120 g by 5 liters of water. Foliar application was applied with a hand sprayer at leaf, and for the control treatment water was used instead. Ammonium sulphate was applied one as soil fertilization at tillering and stems elongation growth stages in the first experiment.

At maturity, the number of whiteheads was counted for each treatment as an effect of the disease on yield, and the severity of the disease was evaluated between the nodes according to the same method previously mentioned, and at the same time the remaining filled ears were harvested to determine grain yield and its components.

Statistical analysis

The data collected from each experiment were analyzed according to the adopted experimental design. In the first experiment, the first analysis focused on the effect of the 16 treatments composed of three types of fertilizer 21% N, 33.5% N, 46% N plus the control, which were combined with the four levels of the interaction of the two doses (25 cc and 50 cc) and the two stages of application (tillering and stem elongation). This analysis also included a contrast test of the analysis variance between fertilizer types. A second analysis looked at the effect of the interaction between the 4 fertilizer levels (21% N, 33% N, 46% N and the unfertilized control) and the 5 levels of the dose-site factor as follows: the 25cc applied at tillering, stem elongation, tillering + stem elongation, and 25 and 50 cc applied before sowing. For the second experiment, the main effect of the individual treatment and their interaction; 3 application levels of 46% N (untreated control, foliar treatment (46% N) and treatment at ground level) and the two growth stages were evaluated.

In both experiments, all the factors and their interaction were taken as fixed while the repetitions and blocks were considered as random. The differences in means were tested according to (LSD) the smallest significant difference at a probability of 5%.

Results

Site of nitrogen application

In this experiment we examined the effect of the 16 treatments on the severity of the disease, and the analysis showed a highly significant effect of fertilizers and their site of application both at ($Pd \leq 0.0001$) and also noticed their significant interaction ($pd \leq 0.0001$). Application of urea (46% N) or ammonium sulphate (21% N) at the tillering and stem elongation stages was found to be 25 cc per stage and the use of urea alone as before sowing fertilizer at a dose of 50 cc respectively significantly increased the severity of the disease by more than 3-fold, almost 2-fold and once compared to the unfertilized control, however the use of Ammonitrate (33.5% N) did not affect the severity of the disease regardless of the dose used or its mode of application (Fig. 1).

Similarly, for the dry weight of the biomass measured for each treatment, nitrogen fertilization treatments and their location showed a highly significant effect on this variable ($p \leq 0.0001$), but this time urea (46 % N) is the fertilizer that affected the dry weight of biomass the most and was significantly negative when applied at a 25cc at the tillering stage, 25cc at the tillering and stem elongation stage, and 50 cc as before sowing fertilizer compared to the control (Fig. 2).

The contrast test that compared the effect of the

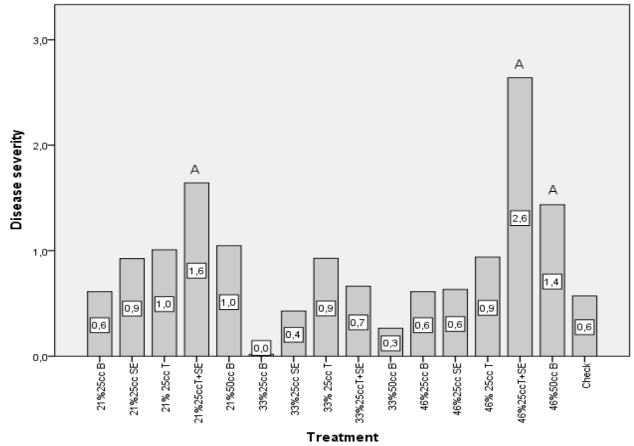

Fig. 1: Effect of nitrogen fertilization treatments and application site on the severity of wheat crown. Note: T: Tillering; SE: Stem Elongation growth stage and B: before sowing.

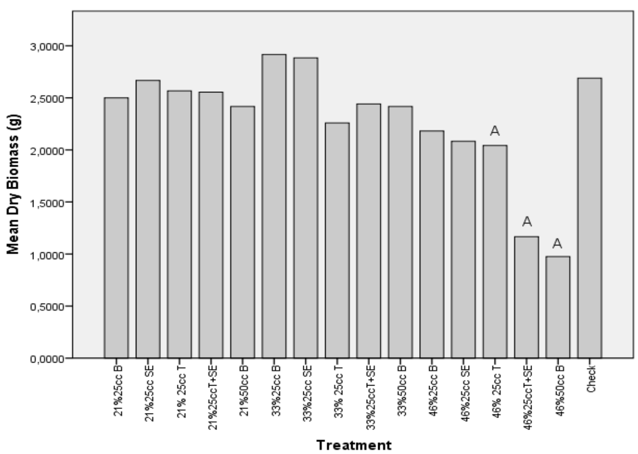

Fig. 2: Effect of nitrogen and application site effects on dry biomass of wheat Ourgh. Note: Only column with (A) are significantly different from the check; T: tillering stage; M: Stem elongation; B: Before sowing.

three types of nitrogen on the severity of the disease and biomass dry weight was significant at $p \leq 0.0001$, reflecting a different effect of nitrogen types on disease development and biomass production. Disease severity was not significantly affected by ammonitrate application. However, the use of urea or ammonium sulphate significantly induced disease severity by 41% and 34%, respectively. With respect to dry weight of biomass, only urea application induced a loss of almost 59% (Table 1).

Since the contrast test of types on nitrogen revealed that ammonitrate fertilization (33.5% N) did reduce disease severity, a correct location and dose of application was also looked for. Therefore, a comparison between the five dose-locations treatments of 33.5% N in terms of the effect on disease severity and dry weight biomass was performed. Result showed that the application of 33.5% N at tillering and tillering + stem elongation with 25 cc increased significantly the severity of the disease

Table 1: Comparison between the different types of nitrogen fertilizers in terms of their effect on the severity of the disease and the dry biomass weight of durum wheat.

Fertilizer	**Severity (%)**	**Dry biomass weight (g)**
Check (0%N)	19.033	2.688
Ammonium sulphate (21%N)	34.900*	2.498
Ammonitrate (33,5%N)	15.333	2.583
Urea (46%N)	41.433*	1.692*
Standard error	0.357	0.201

Note: the values of the same column and followed by a star are significantly different from the control (0% N) at 5% probability.

Table 2: Comparison between the five ammonitrate location-dose (33.5%) in terms of their effect on the severity of the disease and the dry biomass weight of durum wheat.

Location-Dose	**Severity (%)**	**Dry biomass weight (g)**
Before sowing-25 cc (Check)	0.60	2.92
Before sowing -50 cc	8.83	2.42
Tillering	30.93*	2.26
Stem elongation (SE)	14.27	2.88
Tillering + Stem elongation	22.10*	2.44
Standard error	0.274	0.281

Note: Values in the same column and followed by a star are significantly different from the treatment Before sowing-25 cc (taken as a check) at 5% probability.

to almost 31% and 22% respectively, unlike its application with 50 cc at stem elongation. Note that disease severity was almost nil when this type of nitrogen was applied before sowing at a dose of 25 cc. However, none of the treatment showed a significant effect on dry weight biomass (Table 2).

Foliar treatment with urea (46% N)

Data analysis revealed a significant effect of urea growth stage application on grain weight and thousand kernel weight (TKW), with a decrease of 23% at the flowering stage (Table 3). However, site (soil or foliar) and growth stage application interacted significantly only for TKW, with a decrease of 18% and 25% when compared to the untreated check table 4 for foliar and soil application, respectively (Fig. 3).

Nitrogen fertilization is essential for good management of a wheat crop (Karrou 2003; Ryan *et al.,* 1997; Guennouni 2017; Fageria and Baligar 2005; Ladha *et al.,* 2005), and the improvement of its production requires good management of nitrogen fertilizers. In this study we focused on the effect of the location of three types of nitrogen fertilizers by stage of growth and method of application of urea (46% N) on the severity of root rot

Table 3: Effect of urea (46% N) application growth stage on severity of wheat crown rot severity, number of whiteheads, grains number, grain weight and TKW of durum wheat Ourgh.

Application stage	Severity (%)	Whiteheads number	Grain number	Grain weight (g)	TKW (g)
Heading	39.57	2	57	2.75	46.26
Flowering	47.67	2	52	2.13	40.22
Probability	0.090	0.408	0.399	0.018	0.001
Standard error	0.160	0.504	12.808	0.722	3.235

Table 4: Application site effect of urea (46% N) on severity of wheat crown rot, number of whiteheads, the grain number, grain weight and TKW of durum wheat.

Application level	Severity (%)	Whiteheads number	Grain number	Grain weight (g)	TKW (g)
Foliar	38.30	2	60	2.67	43.14
Soil	46.63	2	53	2.14	38.76*
Untreated control	42.53	3	51	2.51	47.82
Probability	0.643	0.149	0.348	0.188	0.001
Standard error	0.176	0.560	13.010	0.732	3.344

Note: Values in the same column followed by a star are significantly different from the untreated check at 5% probability.

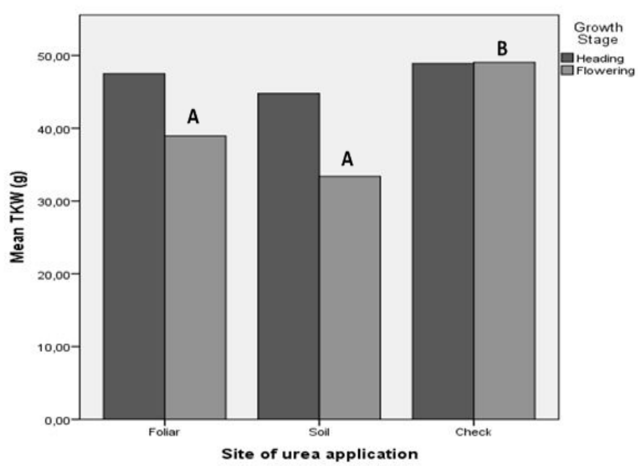

Fig. 3: Site effect of urea application and growth stages on TKW of durum wheat. Note: columns with (A) are significantly different from those with (B).

in durum wheat induced by *Fusarium culmorum* in order to find the right way for the beneficial applications of nitrogen without aggravating the severity of root rot (El Yousfi 1984; Davis *et al.,* 2009; Cook 1992).

The results showed that nitrogen in the form of urea (46% N) favored the development of the disease, whether it was provided as cover during the tillering and stem elongation stage at a dose of 25 cc or under the root system at a dose of 50 cc, and this increase in severity has resulted in a highly significant reduction in the weight of dry biomass produced, which shows that root rot has a negative influence on plant vigor by inhibiting assimilation of nutrients through the root system. And although urea (46% N) did not affect the severity of the disease when applied at 25cc at the tillering stage, it caused a significant reduction in the dry biomass weight, and this, unlike ammoniumsulphate (21% N) which increased the severity more than once compared to the control without significantly reducing the biomass production (Fig. 1 and 2).

In addition, ammonitrate (33.5% N) did not affect the development of the disease, whatever the dose and mode of application compared with the unfertilized control, and its use at a dose of 25 cc. or 50 cc under the seed or at the stem elongation stage as cover manure seems the best choice both for the severity of the disease that was minimal and the vegetative production improved (Table 1, 2). This allowed us to say that it is a question of dose and type of nitrogen.

The contrast made (Table 1, 2) clearly shows the utility of adopting (33.5% N) as nitrogen fertilizers with these salient effects on the reduction of the disease and on the improvement of the biological yield which are all two related to the impact of this fertilizer on the physiology of plants in terms of improving the architecture of the root system, and especially on the lengthening of lateral roots to increase the ability of plants to explore the soil profiles and put available nutrient resources (Lynch 1995; Hackett 1972).

On the other hand, and with a direct impact on the pathogen responsible for root rot, urea stimulates growth, sporulation, persistence and subsequent spread of *Fusarium culmorum* spores, which easily renders the disease uncontrollable and consequently the reduction of the yield (Cook 1992; Butler 2008; Davis *et al.,* 2009; Palmero *et al.,* 2009).

From our results and in an environment harboring the causal agents of the disease, a rational nitrogen fertilization must not include nitrogen in the form of urea and if it will be applied it must be as far as possible from the contact with the pathogen and that it be at a minimum rate of 25 cc applied at sowing.

The insignificant effect of treatment with urea on the severity of the disease, either foliar or as top dressing at the heading or flowering stages means that at those growth stages, the disease was already installed and any treatment cannot control it or give any differential results (Table 3, 4). Furthermore, grain weight loss is explained by the differential effect on TKW of urea application either as foliar or cover crop related to growth stages. In contrast, studies of Mosluh *et al.,* (1978) and Parvez *et al.,* (2009) have shown that foliar application of urea increases yield of wheat and its components, and their results were obtained under irrigation conditions with healthy plants experiencing no biotic stress.

Our results could serve as a fertilization guide, especially in the presence of root rot induced by *Fusarium culmorum* and based on the recommendations of the fertilization of Morocco's cereal zones (Karou 2003; Guennouni 2017). Thus, for semi-arid areas, it would be proposed to use 87 fertilizer units (FU) of nitrogen. Before sowing, fertilization with DAP (21% N), at the tillering stage 1 quintal of ammonitrate (33.5% N) and at the stage of return another quintal of ammonitrate according to the importance and the distribution of precipitations. Along the agricultural campaign (Karrou 2003). In arid zones where nitrogen fertilization is little used, it would be recommended to add 54 FU before sowing, which consists of one quintal of DAP (21% N) and one quintal of ammonitrate (33.5% N) and if the water conditions of the soil are favorable from the point of view of precipitation and temperature, up to 15 FU can be added in the form of ammonitrate. Under the prevalence of root rot, urea (46% N) should be avoided as a cover fertilizer as it increases the severity of root rot. But for arid zones it can be tolerated as a base fertilizer at a dose of 23 FU.

For our results to be reliable, these trials must be scaled up under natural conditions within different cereal regions of Morocco, so that any recommendation developed here is transferable to farmer's field.

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