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Effect of Irrigation with Saline Water on Development of Pink Root of Garlic

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Laboratory and greenhouse experiments were conducted in El-Kassassin Res. Station, Ismailia, Egypt, in 2010/2011, to determine the effects of water salinity on linear growth of *Pyrenochaeta terrestris*, incidence of pink root and yield of garlic, as well as, the plant responses to the water salinity. *Pyrenochaeta terrestris* tolerates a high salinity level (35 mM NaCl) in culture. Irrigation with saline water increased pink root incidence in garlic inoculated with *P. terrestris* but yield was decreased. Irrigation of garlic until 20 mM NaCl did not inhibit plant growth development, but partial inhibition was observed at 25 mM NaCl level of garlic uninoculated with *P. terrestris*. Pink root increases by saline water are associated with the effect on plant response.

Keywords: Garlic, pink root, *Pyrenochaeta terrestris* and water salinity.

Pink root caused by *Pyrenochaeta terrestris* (Hans.) has been reported as disease to garlic in Egypt by Shalaby *et al*. (2002). Pink root pathogen fungus is soilborne and remains viable in the soil for many years (Rengwalska and Simon, 1986). Root infected by *P. terrestris* turns pink initially and then becomes brittle and dies. Although *P. terrestris* can be present in roots; of dose not invade the basal plate or stem of the bulb (Coleman and Ellerbrock, 1997).

Agricultural crops frequently are irrigated with saline water at various salinity levels in arid, semiarid areas and/or under deficiency of water (Essa *et al*., 1999; Shalaby, 2000 and Dotan *et al*., 2005). Irrigation with saline water may have adverse effect on the physical and chemical properties of the soil, on crop production, and on plant metabolism which may in turn affect disease severity and plant resistance (Kylin and Quatrono, 1975; Hasegawa *et al*., 2000 and Dotan *et al*., 2005). Salinity generally affects plant growth through either excess ions or water deficit, with different plant organ factors such as, the duration and degree of the stress and growth stage at stress exposure (Kamel *et al*., 1995).

Various pathogens are highly tolerant to salt in culture, including *Aspergillus*, *Penicillium* and *Fusarium* spp. (Tresner and Hayes, 1971); *Phytophthora* spp. (MacDonald, 1982 and Blaker and MacDonald, 1985); *Pythium* spp. (Rasmussen and Stanghellini, 1988) and *Urocystis* spp. (El-Ganieny *et al*., 1997). High salinity may increase the incidence of diseases, *i.e.* tomato wilt (Jones *et al*., 1993), early blight of potato (Nachmias *et al*., 1993), onion smut (El-Ganieny *et al*., 1997), sesame wilt (Shalaby, 2000) and cotton wilt (Turco *et al*., 2002). On the other hand, suppression of diseases under saline irrigation has been reported with Fusarium wilt of date palm (Brac de la Perrière *et al*., 1995) and Fusarium crown and root rot of asparagus (Elmer, 2003). The effect of salinity on plant diseases may result from its effect on one or more of the biotic components involved in the disease, the pathogen, the host, microbial activity in the soil, or abiotic components of the soil (Dotan *et al*., 2005).

This research was designed to study the effect of water salinity on the pathogen, disease incidence and the plant response to the pathogen of garlic plant.

Materials and Methods

Inocula:

An isolate of *P. terrestris* was isolated from an infected garlic fields in El-Ismailia governorate. The fungus was identified as mentioned by Barnett (1960). The isolated fungus was grown on Czapek's medium for 30 days. The mycelial and broth were blended for 30 second at low speed in a wiring Blender, then 200 ml inoculum and 1.400 ml of distilled water were mixed with 23.5kg of sterile soil (Rengwalska and Simon, 1986) and used as inoculum, the pathogenicity tests of the isolated fungus were done in the greenhouse. Garlic plants (cv. Seds-40), highly susceptible to the disease (Shalaby *et al*., 2002), were used in this study.

Laboratory experiments:

Petri dishes containing Czapek's agar (Rengwalska and Simon, 1986) amended with 0, 10, 15, 20, 25, 30, 35 and 40 m mole/l. NaCl, electrical conductivity (EC): 0.4dSm-1, 1.5dSm-1, 2dSm-1, 2.5dSm-1, 3dSm-1, 3.5dSm-1 and 4dSm-1, respectively, five plates for each salt concentration were inoculated in the centre by discs (0.5-cm-diam.) of *P. terrestris*. Both experimental and check plates were assigned according to the completely randomized design with three replicates/treatment. All plates were incubated in the dark at 28ºC for three days and the linear growth of the pathogen was assessed at 24 h intervals.

Greenhouse experiments:

This experiment was conducted in pots (30-cm-diam.×50-cm-height) in the greenhouse of El-Kassassin Res. Station, during the growing season 2010/2011. The inner surface of each pot was coated with bitumen, then 2kg of gravel were transferred into the bottom and infested (as previously mentioned) or uninfested 30kg of air-dry-sand-clay loam soil [sand 50%, silt 15%, clay 30%, CaCO3 4% and organic matter 1%] were added to each pot. Garlic cloves (cv. Seds-40) were sown in pots at the rate of two cloves/pot. Pots were irrigated with saline water at different rates of NaCl [0, 10, 15, 20 and 25m Mol]. Pots irrigated with tap water were served as check. The four salinity treatment levels were divided into two groups; first group was uninoculated with *P. terrestris*, while the second one was inoculated with *P. terrestris*. In each pot, a tube of 30cm length was vertically fixed through the soil with its lower part immersed in the gravels in order to obtain a good system for irrigation and drainage. Pots were irrigated to a moisture level of 65% of the total water holding capacity of the soil (W.H.C.). Both experimental and check pots were assigned according to completely randomized design with three replicates/treatment. Calcium superphosphate and potassium sulphate fertilizers were added to the soil before planting at the rate of 5gm and 2gm/pot, respectively, whereas ammonium nitrate at 1gm 33.5% N2/pot was applied as commonly known. Samples were taken

120 days after planting, each one represented by 3 plants/treatment. Seed germination (%), leaves dry weight (48h of drying at 80ºC) and plant height and total chlorophyll were assessed in the first group (Rangena, 1977), meanwhile diseased plants (%) and yield were determined in the second group. Obtained data were statistically analyzed according to Snedecor and Cochran (1972).

Results and Discussion

Effect of salinity on the pathogen growth in culture:

The *in vitro* linear growth of *P. terrestris* was not affected when the level NaCl was at or below 35mM until 72 h, as compared with the check (Table 1). The linear growth of the pathogen was reduced when NaCl levels exceeded 35mM. Cultural growth of *P. terrestris* was affected by saline water at 40mM NaCl, indicating that this pathogen tolerates a high salinity level.

Tolerances of fungi to high salinity levels have been demonstrated in other studies (Tresner and Hayes, 1971; MacDonald, 1982; Blaker and MacDonald, 1985; Rasmussen and Stanghellini, 1988 and El-Ganieny *et al*., 1997). Thus, there is no evidence that high salinity stimulates this pathogen. It is concluded that *P. terrestris* tolerates a high salinity level (35 mM NaCl) in culture.

Table 1. Effect of NaCl levels on the *in vitro* growth of *P. terrestris*

NaCl level (mM)	Linear growth (mm) after (hours)		
	24 h	48 h	72 h
Check (0)	60.06	74.01	87.02
10	60.05	74.03	87.11
15	62.11	75.04	87.11
20	62.10	75.03	88.02
25	62.11	76.31	88.01
30	61.09	75.43	88.01
35	60.06	74.34	87.04
40	52.01	52.01	52.01
L.S.D. 5%	5.61	5.91	6.82

Effect of salinity on garlic plants growth (cv. Seds-40) under greenhouse conditions:

Data presented in Table (2) show the effect of water saline irrigation [0,10, 15, 20 and 25 mM NaCl] on seed germination (%), plant height (cm), leaves dry weight (gm/plant) and total chlorophyll concentrations of garlic (cv. Seds-40) in non-infested soil under greenhouse conditions. Seed germination (%), plant height and dry weight of leaves a well as chlorophyll concentration of garlic plants were not affected when the level of NaCl was at or below 20 mM until 120 days from planting, except that of seed germination was measured, 10 days after sowing, relative to tap water. Therefore, irrigation of garlic plants with saline water 20 mM NaCl, is considered as a moderate salinity level. At 25 mM NaCl level seed germination (%), dry weight of leaves, plant height and total chlorophyll concentrations of garlic were reduced, as compared to the check treatment.

Table 2. Effect of NaCl levels on seed germination, plant height, leaves dry weight and total chlorophyll concentrations of garlic (cv. Seds-40) under greenhouse conditions

NaCl level (mM)	Seed germination (%)	Plant height (cm)	Dry weight (gm/plant)	Total chlorophyll (mg/gm. fresh/wt.)
Check	97	41.22	4.35	2.49
10	97	40.04	4.35	2.49
15	97	40.04	4.35	2.50
20	97	40.05	4.35	2.50
25	82	31.06	2.78	1.35
L.S.D. 5%	3.00	1.58	0.97	0.73

Irrigation of garlic plants grown in non-infested soil with saline water at or below 20 mM did not affect seed germination (%), leaves dry weight, plant height and total chlorophyll levels, however, at higher level (25 mM NaCl), garlic plants were adversely affected. In this concern, literature is not available, however, high salinity may affect plant physiology via morphological, metabolic and biochemical changes, such as water relations, number and size of stomata; stem, root and membrane structure; photosynthesis, protein synthesis, lipid metabolism, salt accumulation, metabolic enzymes and nucleic acids (Hasegawa *et al*., 2000; Bernstein and Kafkafi, 2002; Dotan *et al*., 2005 and Parida and Das, 2005).

Effect of salinity on pink root incidence and yield of garlic cv. (Seds-40) under greenhouse conditions:

The percentages of diseased garlic plants (cv. Seds-40) and yield were not significantly affected when NaCl levels were at or below 20 mM, relative to tap water. At 25 mM NaCl level, disease incidence was increased, but yield was decreased as compared with the check treatment (Table 3).

Table 3. Effect of NaCl levels on the percentage of pink root incidence and yield of garlic cv. Seds-40 under greenhouse conditions

NaCl levels (mM)	Disease incidence (%)	yield (gm/pot)
Check (0)	67.08	63.20
10	67.23	63.20
15	72.04	63.2
20	72.04	62.90
25	81.09	41.30
L.S.D. 5%	5.81	3.11

It is worth to mention that symptoms of pink root on garlic plants were appeared 90 days after planting under field conditions. In this concern, Thornton and Mohan (1996) found that symptoms of pink root were not usually noticeable during early stage of onion growth when temperature is below optimum for the pathogen growth. These results are confirmed by Shalaby *et al*. (2002). They concluded that *P. terrestris* pink root has been developed at the optimum temperature, 28ºC.

It may be noticed from the results of the *in vitro* growth of *P. terrestris* that this pathogen tolerates salinity of 35 mM NaCl level, even 25 mM NaCl affects crop growth. There is no evidence that high salinity stimulates this pathogen but there is a possible effect of saline water to increase plant susceptibility to the pathogen. Increased susceptibility of plants to the disease by exposure to high salinity has been reported with various pathogens (MacDonald 1982 and 1984). Thus, the increased number of diseased plants was attributed directly to the effect of salinity on the host without any concomitant salinity effect on the pathogen (Dotan *et al*., 2005). However, the effect of salinity on plant diseases may result from its effect on one or more of the biotic components involved in the disease, the pathogen, the host microbial activity in the soil or abiotic components of the soil (Shalaby, 2000 and Dotan *et al*., 2005).

The reduction in garlic yield due to irrigation with saline water may be attributed to the negative effects of sodium ions on plant growth and increased pink root disease of garlic plants. These results are confirmed by Shalaby (2000). The reduction in plant growth due to irrigation with salt water has been explained by a suppression of nutrient absorption due to uptake of sodium in competition with nutrient ions. Thus, even when the osmotic stress was eliminated, growth of *Phaseolus vulgaris*, *Pisum sativum* and *Citrus aurantium* was decreased by salt stress (Giorgi *et al*., 1967). In support of this explanation, many researchers (O'leary, 1970; Prisco and O'leary, 1972 and Storck *et al*., 1975) reported that the imbalance of hormone, *i.e.* GA3, sprayed on the leaves at 100 ppm counteract the negative effects of salinity on bean plants growth.

Conclusion

Pink root increases, due to using saline water, are associated with the effect on plant response. Water salinity could play an important role in the disease incidence on garlic plants. In this concern, further investigations are greatly needed to confirm this role.

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تأثير الري بالماء الملحي علي إصابة نباتات الثوم بمرض الجذر القرنفلي

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أجري هذا البحث بغرض معرفة تأثير الماء الملحي (كلوريد الصوديوم) علي نمو بيرينوكيتا ترستس (المسبب المرضي للجذر القرنفلي في نبات الثوم) تحت ظروف المعمل، ومعرفة تأثير الري بالماء الملحي على الإصابة بمرض الجذر القرنفلي والمحصول في نباتات الثوم المعدية بالمسبب المرضي، وكذلك معرفة تأثير الري بالماء الملحي علي النسبة المئوية للإنبات، وارتفاع النبات، والوزن الجاف للأوراق، ومحتوي النبات من الكلوروفيل في نبات الثوم غير المعدية بالمسبب المرضي تحت ظروف الصوبة خلال الموسم الزراعي ٢٠١١/٢٠١٠م. وأوضحت نتائج المعمل قدرة الفطر بيرينوكيتا ترستس على تحمل الملوحة حتى ٣٥ مليمول/لتر. وقد تبين من نتائج الصوبة أن الري بالماء الملحي أدى إلى زيادة الإصابة بمرض الجذر القرنفلي ونقص المحصول في نباتات الثوم المعدية بالمسبب المرضي. وأن الري بالماء الملحي أدى إلى التأثير بالسلب على الإنبات، وارتفاع النبات، والوزن الجاف للأوراق، ومحتوى النبات من الكلوروفيل في نباتات الثوم غير المعدية بالمسبب المرض عند تركيز ٢٥ مليمول/لتر. وأوضحت الدراسة أن زيادة حساسية نبات الثوم للإصابة بمرض الجذر القرنفلي تكون مرتبطة بتأثير الملوحة على النباتات وقدرة المسبب المرضي على تحمل الملوحة حتى تركيز ٣٥ مليمول/لتر.