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Garlic Pink Rot Disease and Crop Yield as Affected by Salinity and Irrigation Water Deficit

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Greenhouse experiments were conducted in Ismailia governorate (El-Kassassine county), Egypt, during 2017/2018 growing seasons to determine the effect of both water salinity or water deficit at different treatments consisted of four levels of salinity (10, 15, 20 and 25 mM NaCl/L) and 3 rates of water stress (80, 60 and 40% W.H.C.) on pink root incidence and yield of garlic (*Allium sativum* L.) cv. (Sids-40), as well as the garlic plant responses. Irrigation with saline water increased pink root incidence in garlic inoculated with *Pyrenochaeta terrestris,* and yield was decreased. Irrigation of garlic until 20 mM NaCl/L did not affect cloves germination, plant height, leaves dry weight, cell sap concentration and proline concentration, but partial effect was observed at 25 mM NaCl/L of garlic grown in soil uninfested with the tested pathogen. Garlic plants exposed to 80% rate of W.H.C. weren't significantly affected, disease incidence, cloves germination, plant height, leaves dry weight, cell sap concentration and proline concentration and yield produced from infested or uninfested soils showed insignificant variation in comparison with 100 % W.H.C. At 60 and 40 % rates of W.H.C., noticeable increases in pink root incidence were found on plants inoculated with *P. terrestris,* and their yield was decreased. Cloves germination, plant height and leaves dry weight values were significantly reduced, whereas cell sap concentration and proline concentration values were significantly increased under water deficit of plants grown in uninfested soil comparing to the check. In this respect, 40% of W.H.C. highly affected the disease incidence and crop yield compared to the other tested rates.

Key words: Garlic, *Allium sativum,* Pink root, *Pyrenochaeta terrestris,* Water deficit, Proline, Salinity stress, Yield.

Pink root disease of garlic caused by *Pyrenochaeta terrestris* (Hansen) Walker, has been reported in Egypt (Shalaby *et al*., 2002). Pink root pathogen is a soil borne fungus that 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 latent infected bulbs (Coleman and Ellerbrock, 1997).

Environmental stress can predispose plants to disease from relatively weak parasites due to their negative effects on host physiology (Goudarzi *et al*., 2011).

Drought and salinity as abiotic stress are multidimensional in nature, plant and organisms at various levels.

Agricultural crops are frequently 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 Triky-Dotan *et al*., 2005). Irrigation with saline water may have adverse effect on plant metabolism which may, in-turn affect disease on crop production and plant resistance (Kylin and Quaerono, 1975; Hasegawa *et al.,* 2000 and Triky-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 and Triky-Dotan *et al.,* 2005). In saline soil, although water is present and is unavailable to plant because it is retained by the ions in the soil such as Na and Cl (Perez -lepza *et al.,* 2009).

Drought and salinity are considered as the major predisposing factors in plant diseases (Triky-Dotin *et al.*, 2005). Drought is defined as period without significant rainfall or soil moisture. Drought stress usually occurs when soil water content is less than 50 % of field capacity (Levitt, 1980). Several investigators indicated that water stress influences growth, physiological and biochemical processes of many plants which had been reflected on plant yield (Levitt, 1980), but the relation between water stress and plant disease is not known. However, water deficit had a predisposing effect on the severity of Phytophthora root rot in safflower (Duniway, 1977). Meanwhile, various pathogens are highly tolerant to water deficit, *i.e*., *Macrophomina sp*., *Sclerotinia sp*. and *Pythium sp*. (Park *et al*., 2001).

Various pathogens are highly tolerant to salt in culture, including *Aspergillus* spp. *Penicillium* spp. and *Fusarium* spp. (Tresner and Hayes, 1971); *Phytophthora* sp. (MacDonald, 1982 and Blaker and MacDonald, 1984); *Pythium* sp. (Rasmussen and Stanghellini, 1988) and *Urocystis* sp. (El-Ganieny *et al*., 1997). High salinity increases 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 disease under saline irrigation has been reported with Fusarium wilt of date palm (Brac de la Perriere *et al*., 1995) and Fusarium crown and root rot of asparagas (Elmer, 2003). Accumulation of organic solute mainly proline, free amino acids, and carbohydrates are among the non-specific mechanisms which increased under a range of stressful conditions (Khan, 2007). This mechanism has a positive effect on the daily carbon balance of a stressed plant, since it allows the plant to photosynthesize down to lower leaf water potentials than would otherwise have been possible (Mac Cree, 1986). Meanwhile, 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 (Triky-Dotan *et al*., 2005).

The objective of the present study was to study the effect of water salinity and water deficit stresses on the pink root incidence and the plant responses to the pathogen on garlic plant.

Materials and Methods

1-Laboratory experiment:

1-1-Source of the pathogen:

An isolate of *P. terrestris* was isolated from field grown garlic roots showing pink root symptoms in Ismailia governorate (El-Kassassine county)

1-2-Isolation, purification and identification of the isolated fungus:

Pieces of infected garlic roots were immersed in 1% sodium hypochlorite solution for two minutes, washed in sterilized water then, removed from the solution, drained, and placed directly on the surface of modified Czapek's medium solidified with 2 % agar in Petri dishes. The plates were incubated at 2 7°C. for 5 days. The isolated fungus was purified and identified as according to Watson (1961). Isolates were kept in soil tubes under cold conditions to avoid mutation incidence McKeen and Wensley (1961). The purified isolates of *P.terrestris* were replanted into PDA plates or tubes before using in future studies.

1-3-Preparation of Inocula:

The isolated fungus was grown in Czapek's liquid medium for 30 days. The mycelium and broth were blended for 30 sec. at low speed in a wiring with blender then 200 ml inoculum and 1.400 ml of distilled water were mixed with 23.5 kg of sterile soil (Rengwalska and Simon, 1986), and used as inoculum. The pathogenicity tests of the isolated fungus were done under greenhouse conditions. Garlic plant cv. (Sids-40), highly susceptible to the disease (Shalaby *et al*., 2002), were used in this study.

2- Greenhouse experiment:

Two experiments were conducted in plastic pots (30 cm diameter) under greenhouse conditions during, 2017/2018. In each pot, 1.5 kg of gravel were put in the bottom of each pot and then 2.5 kg of air-dried sand-clay-loamy soil (sand 50 %, silt 15%, clay 30%, $CaCO_3$ 4% and organic matter 1%) were added. Potted soils were infested by the tested fungus as mentioned before. Garlic cloves were sown in the pots on September 26th at two cloves /pot. In each pot, a tube of 40 cm length was vertically fixed through the soil with its lower part immersed in the gravel in order to obtain a good system for irrigation and drainage.

In the first experiment, pots were irrigated with saline water at different rates of NaCl (10, 15, 20 and 25 mM/L), NaCl electrical conductivity (E.C.): 1 dsm^{-1}, 1.5 dsm^{-1}, 2 dsm^{-1} and 2.5 dsm^{-1}, respectively. Pots irrigated with tap water served as check. The four salinity treatment levels were divided into two groups; the first group was un-infested with *P. terrestris*, while the second group was infested with *P. terrestris.* Pots were irrigated to a moisture level of 65% of total water holding capacity of the soil (W.H.C.). Both experimental and check pots were assigned according to a completely randomized design with ten replicates/treatment (based on thirty pots contained sixty plants). The disease incidence (D.I) was calculated with the following formula:

$$\text{Disease incidence (D.I) \%} = \frac{\text{Number of diseased plants}}{\text{Total number of observed plants}} \times 100$$

In the second experiment, pots representing the four treatments (100,80, 60 and 40%) at water holding capacity (W.H.C.) were used. The stressed treatments were foure different levels, i.e., 100,80, 60 and 40% of W.H.C. (Duniway, 1977). The foure stress treatment rates were divided into two groups, the first group was un-infested, while the second group was infested with *P. terrestris,* pots were assigned according to completely randomized design with three replicates for each treatment as mentioned before.

In both experiments, calcium superphosphate and potassium sulphate fertilizers were added to the soil before sowing at the rate of 5 gm and 2 gm / pot, respectively, whereas ammonium nitrate at 1 gm / pot was applied after planting. Samples were collected 120 days after planting and each sample was represented by three plants/treatment, except that of cloves germination was measured 10 days after sowing. Cloves germination %, leaves dry weight (48 h of drying at 80˚C), plant height, cell sap concentration in the leaves (Gusev, 1960) and proline concentration in the leaves (Bates *et al*., 1973) were assessed in the first grouping of each experiment, whereas percentages of diseased plants and yield were determined in the second one.

3- Statistical analysis:

Obtained data were statistically analyzed using the L.S.D. methodaccording to Snedecor and Cochran (1972).

Results and Discussion

- Effect of salinity on pink root incidence and yield of garlic c.v. Sids-40 under greenhouse conditions:

The percentage of diseased garlic plants c.v. Sids-40 and yield were not significantly affected when NaCl levels were below 20 mM/L relative to tap water while, at 25 mM NaCl/L level, disease incidence was increased but yield was decreased as compared with the check treatment (Table 1).

Table (1): Effect of NaCl levels on the percentage of pink root incidence and yield of garlic c.v. (Sids-40) under greenhouse conditions.

NaCl level (mM/L)	Disease incidence (%)	Yield (gm/pot)
Check (0) Tap water	68.33	63.20
10	68.33	61.40
15	71.66	60.50
20	73.33	59.80
25	83.33	41.30
L.S.D. at 5 %	4.77	1.28

It's worth to mention that symptoms of pink root on garlic plants appeared 90 days after planting under greenhouse conditions. In this concern, Thornton and Mohan (1996) found that symptoms of pink root were not usually noticeable during the early stage of onion growth when the temperature is below the optimum for the pathogen growth. These results were also confirmed by Shalaby *et al.* (2002) as they concluded that *P. terrestris* the causal of pink root has been developed at the optimum temperature, 28 ˚C. It may be noticed from the result of the in vitro growth of *P. terrestris* that this pathogen tolerates salinity of 35 mM/L level NaCl that affects crop growth (Shalaby, 2013). 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 (Mac.Donald, 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 (Triky-Dotan *et al*., 2005). However, the effect of salinity on plant disease may result from its effect on one or more of the biotic components involved in the disease, the pathogen, the highest microbial activity in the soil or abiotic components of the soil (Shalaby 2000 and Triky-Dotan *et al*. 2005).

The reduction in garlic yield due to irrigation with saline water may be attributed to the negative effect 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. O'leary (1970), Prisco and O'leary (1972) and Strock *et al*. (1975) reported that the imbalance of hormone, *i.e*., GA_3 sprayed on the leaves at 100 ppm counteract the negative effect of salinity on bean plants growth.

- Effect of salinity on cloves germination, plant growth, leaves dry weight, cell sap concentration and proline concentration:

Data presented in Table (2) show the effect of saline water irrigation (10, 15, 20 and 25 mM/L NaCl) on cloves germination %, plant height (cm), Leaves dry weight (mg/plant), cell sap concentration % and proline concentration (mg/gm fresh weight) of garlic c.v. (Sids -40) in non-infested soil under greenhouse conditions.

Table (2): Effect of NaCl levels on cloves germination, plant growth, leaves dry weight; cell sap concentration and proline concentration of garlic c.v. (Sids-40) under greenhouse conditions.

NaCl level (mM/L)	*Cloves germination (%)	** Plant height (cm)	** Leaves dry weight (mg/plant)	** Cell sap concentration %	** Proline concentration (mg/gm fresh weight)
Check (0) Tap water	95.00	43.30	4.50	8.50	27.9
10	95.00	41.20	4.50	8.50	27.9
15	94.00	41.10	4.15	11.10	29.9
20	94.00	41.10	4.95	11.22	30.2
25	82.00	36.70	2.80	14.3	37.3
L.S.D. at 5 %	3.20	2.32	0.42	1.25	1.90

* Cloves germination at 10 days after sowing.; ** assayed at 120 days after sowing.

Cloves germination, plant height, leaves dry weight, cell sap concentration and proline concentration of garlic plants were not affected when the level of NaCl was at or below 20mM/L until 120 days from planting, except that clove's 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. On the other hand, at 25 mM/L NaCl level, cloves germination (%), leaves dry weight (mg/plant) and plant height(cm) of garlic were reduced, as compared to the check treatment. 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, photosynthetic, protein synthesis, lipid metabolism, salt accumulation, metabolic enzymes and nucleic acids (Hasegawa *et al*., 2000; Bernstein and Kafkafi, 2002; Triky-Dotan *et al*., 2005 and Parida and Das, 2005). The aforementioned changes were noticed on the plants irrigated with high salinity might be associated with increased susceptibility to the pathogen. Salinity may lead to decrease in other nutrient ions in the tissues, including K^+ which frequently is connected with resistance to pathogens (Parida and Das, 2005).

Many researchers studied the consequences of salt stress on enzyme activities involved in proline metabolism could administer valuable information on the physiological importance of its accumulation. In the present study, increasing NaCl levels showed higher proline content in the garlic leaves. These results were confirmed by those recorded by Prakash *et al.* (2010) who found that salinity induced a significant increase in NaCl and proline concentrations, while reduced the accumulation of K^+ and Ca_2^+ in leaves of all the tested cultivars of cowpea.

In fact, proline accumulation in leaf tissues has been suggested to result from a reduction in proline degradation, arise in proline biosynthesis, a decrease in protein synthesis or proline employment and hydrolysis of protein (Charest and Phan,1990). The upper accumulation of proline might be due to enhanced activities of ornithine aminotransferase (OAT) and pyrroline-5-carboxylate reductase (P5CR), the enzymes involved in proline biosynthesis (Kohl *et al.,* 1991), also may be due to inhibition of proline oxidase and proline dehydrogenase (PDH), proline catabolizing enzymes (Kandpal *et al.*, 1981). In addition, Huang and Cavalier (1979) reported that proline dehydrogenase also functions as pyrroline-5-carboxylate reductase (proline synthesizing enzyme) and catalyses the reaction with equivalent reactants and co-enzymes but operating in another way.

Effect of water deficit rates on pink root incidence and yield:

The percentages of diseased garlic plants and yield were not significantly affected when plants were exposed to the rates of 100% and 80% W.H.C. At rates of 60 and 40% W.H.C., disease incidence was significantly increased, but yield was decreased as compared with the100% treatment (Table 3). On the other hand, plants grown under rate of 40% W.H.C. were strongly affected comparing to the other ones.

It is worth to mention that symptoms of pink root in garlic were appeared 90 days after planting under greenhouse conditions. As demonstrated in this work, the developed pink root disease of garlic was correlated with the soil water content during the experiment, in other words; the predisposing of plants to the disease was greater at low than at high soil water content (Abd -El Razik *et al*. ,1988 and Abd El-baky2005). The effects of water stress on plant disease may result from the effect on one or more of biotic components involved in the disease, namely the pathogen, the host, microbial activity in soil or biotic components of soil (Triky-Dotan *et al.*, 2005).

Table (3): Effect of water deficit rates on the percentage of pink root incidence and yield of garlic c.v. (Sids-40) under greenhouse conditions.

Treatments	Disease incidence %	Yield (gm/pot)
100 W.H.C.	67.67	63.20
80% W.H.C.	66.67	63.00
60% W.H.C.	76.33	50.00
40% W.H.C.	80.00	38.70
L.S.D. at 5 %	4.88	3.71

It may be noticed from the obtained results that there was no evidence that water stress stimulates this pathogen, but Khaled *et al*. (1996) indicated that pink root on onion was widely prevalent in sandy soil whereas it was limited rather than increased by wet soil as indicated by the fact that the pink root in more common and more severe in drier area of the world (Agrios, 1988). Similarly, Shalaby *et al.* (2008) found that a negative relationship was found between the pink root incidence and increase of soil moisture of garlic. In addition, drought stress increased the disease caused by *P. terrestris* and this may result from the effect on plant growth or spore's germination of the pathogen. There is no evidence to support these aspects. However, saturated conditions may affect the sclerotial survival of other plant pathogenic fungi, *i.e*., *Rhizoctonia solani* (Ploetz and Mitechell, 1988) and *M. phaseolina* (Gouderzi *et al*., 2011).

The reduction in garlic yield due to water stress might be attributed to the negative effect of drought on plant growth (especially roots) which becomes dry and brittle and easy to be penetrated by pink root pathogen. Literatures that support this explanation are not available.

***- Effect of water holding capacity rates on some vegetative characters, cell sap concentration and proline concentration of garlic c.v. (Sids-40) green house, artificially infested soil*:**

Data presented in Table (4) show the effect of water deficit at different rates (100,80, 60 and 40% of W.H.C.) on cloves germination, leaves dry weight, plant height, cell sap concentration and proline concentration of garlic c.v. (Sids-40) under greenhouse conditions.

Table (4): Effect of water deficit rates on cloves germination, leaves dry weight, plant height, cell sap concentration and proline concentration of garlic c.v. (Sids-40) under greenhouse conditions.

Treatments	*Cloves germination (%)	** Plant height (cm)	** Leaves dry weight (mg/plant)	** Cell sap concentration %	** Proline concentration (mg/gm fresh weight)
100% W.H.C.	97	41.22	4.5	8.5	27.9
80% W.H.C.	97	41.22	4.5	8.5	27.9
60% W.H.C.	85	38.02	3.9	10.1	29.3
40% W.H.C.	62	31.11	2.7	15.8	31.3
L.S.D. at 5 %	4.14	0.59	0.98	1.35	1.88

* Cloves germination at 10 days after sowing.; ** assayed at 120 days after sowing.

Cloves germination, leaves dry weight, plant height, cell sap concentration and proline concentration of garlic plants were not affected when plants were exposed to 100, and 80% of W.H.C. 120 days after planting, except that of cloves germination which was measured, 10 days after sowing. At 60% and 40% rates of W.H.C. cloves germination, plant height and leaves dry weight were significantly reduced. Whereas the opposite trend was found in cell sap and proline concentrations in garlic leaves, as compared to the 80 and 100% treatment. Significant decreases in the values of the afore-mentioned parameters were obtained when plants were grown at 40% rates of W.H.C. comparing to the other tested W.H.C. rates. In fact, there is a considerable amount of evidence indicating that garlic plants appeared to be more sensitive to water deficit when plants were exposed to 40% rate of W.H.C. Since, drought symptoms, *i.e*., wilting, rolling and/or browning of leaves tips were usually occurred in garlic, 55 days after planting.

The reduction of dry matter production under water stress conditions was mainly due to the reduction of net photosynthesis and increasing photorespiration ratio (Perry *et al.*, 1983). Moreover, El-Shafey *et al.* (1981) stated that when plants were subjected to water stress, certain physiological responses including wilting and stomata closure usually enhanced rates of leaf senescence and therefore decreased net assimilation rate and plant growth. All these proceses might be attributed to hormonal change addition to other factors such as mineral deficiencies and dehydration (Kamel *et al.*, 1995). The highest values of cell sap concentration can be easily noticed for garlic plants exposed to water deficit comparing to the check one. These results are in harmony with those obtained by Abd El-Rahman *et al*. (1986) and Kamel *et al.* (1995). Similarly, Stankova (1976) reported that subjecting plants to water stress increased cell sap concentration possibly due to the increase of transpiration rate under these conditions. It could be clearly noticed from the present results that garlic plants grown under drought conditions showed a progressive increase in proline concentration in the levels especially at the rate of 40 % W.H.C. It is quite reassembled to believe that the accumulation of proline in garlic under such condition may be due to the hydrolysis of protein or probably to the enhancing of proline synthesis (Stewart, 1972).

It is worth to mention that garlic plants exposed to 40% rate of W.H.C. were most sensitive, where cloves germination, leaves dry weight and plant height were progressively reduced but the opposite trend was found in cell sap and proline concentration, as well as drought symptoms were appeared.

The obtained results indicate that water deficit increased cell sap and proline concentration. However, cloves germination, leaves dry weight and plant height were reduced in garlic plants grown in uninfested soil.

Conclusion

In conclusion, pink root increases due to using saline water and water deficit as associated with the effect of plant response to salinity levels and water deficit rates. Water salinity and water deficit are factors contributing to outbreaks of pink root incidence on garlic plants. In this concern, further investigations are greatly needed to explain this role.

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تأثير الرى بالماء الملحى ونقص المياه على اصابة نباتات الثوم بمرض الجذر القرنفلى والمحصول

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اجرى هذا البحث بغرض معرفة تأثير كل من الرى بماء ملحى (كلوريد الصوديوم) وكذلك نقص المياه بمعدلات مختلفة على الاصابة بمرض الجذر القرنفلى والمحصول فى نباتات ثوم مزروعة في تربة معدية بالمسبب المرضى *Pyrenochaeta terrestris* وكذلك معرفة تأثير الماء الملحى ونقص المياه على النسبة المئوية لإنبات الفصوص وارتفاع النبات والوزن الجاف للأوراق ومحتوى النبات من العصير الخلوى والحمض الأمينى برولين فى نباتات ثوم منزرعة في تربة غير معدية بالمسبب المرضى تحت ظروف الصوبة خلال الموسم الزراعى ٢٠١٨/٢٠١٧. وقد تبين من نتائج الصوبة أن الرى بماء ملحى ادى الى زيادة الاصابة بمرض الجذر القرنفلى ونقص المحصول فى نباتات الثوم المنزرعة في تربة معدية بالمسبب المرضى وذلك عند معدل 25 ملليمول/لتر من كلوريد الصوديوم وأن الرى بالماء الملحى ادى الى التاثير بالسلب على انبات الفصوص وارتفاع النبات والوزن الجاف للأوراق ومحتوى النبات من العصير الخلوى وحمض البرولين فى نباتات الثوم غير معدية بالمسبب المرضى. واوضحت النتائج ان تعرض نباتات الثوم لنقص الماء بمعدل ٨٠% من السعة الحقلية لم يؤثر على النسبة المئوية للإصابه المرضية وانبات الفصوص وارتفاع النبات والوزن الجاف للأوراق ومحتوى النبات من العصير الخلوى والحمض الأمينى برولين سواء كانت النباتات منزرعة في تربة معدية او غير معدية مقارنة بالكنترول وان تعرض نباتات الثوم لنقص الماء بمعدل ٦٠% و ٤٠% من السعة الحقلية ادى الى زيادة الاصابة بمرض الجذر القرنفلى ونقص المحصول فى نباتات الثوم المزروعة في التربة المعدية بالمسبب المرضى وكذلك التاثير السلبى على انبات الفصوص وارتفاع النبات والوزن الجاف للاوراق بينما زاد تركيز كل من العصير الخلوى والحمض الامينى برولين بنباتات الثوم المنزرعة في التربة غير المعدية بالمسبب المرضى. وقد كان معدل النقص عند ٤٠% من السعه الحقلية اكثر تاثيرا بالمقارنة بالمعدل ٦٠% من السعة الحقلية.