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Suppression of Bacterial Soft Rot Disease of Potato

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Bacterial soft rot disease of potato caused by *Erwinia carotovora* subsp. *carotovora* can cause great losses to potato yield, especially during storage. This study aimed to management of the disease using salicylic acid and BION as resistance inducing factors, potassium sulfate, ammonium phosphate and calcium chloride as salt compounds and isolates of *Pseudomonas fluorescens* (Pf1 and Pf5), *P. aeruginosa* (Pa1), *Serratia marcescens* (Sm1 and Sm2) and *Bacillus subtilis* (Bs3) as bioagents, under artificial inoculation condition. All tested agents decreased the disease compared with the control. Disease severity was completely reduced when salicylic acid was applied at 0.9 mM, before or at the same time or after inoculation with the pathogen. When potassium sulfate and calcium chloride were applied at 0.5 g/l, at the same time of inoculation by the pathogen and when *P. fluorescens* (Pf5), *B. subtilis* (Bs3) and *S. marcescens* (Sm2) were applied after or before or at the same time of inoculation with the pathogen, respectively. Efficiency of the inducer agent and salt compounds were increased against the disease by increasing their rates. Salicylic acid, calcium chloride and potassium sulfate were the most effective against the disease compared with BION and ammonium phosphate. Isolates of *P. fluorescens* (Pf1), *S. marcescens* (Sm1) and *P. aeruginosa* (Pa1) were moderately effective against the disease when they were applied before, after and at the same time of inoculation with the pathogen, respectively.

Keywords: Bioagents, *Erwinia carotovora*, resistance-inducing factors, potato, salt compounds, soft rot and *Solanum tuberosum*.

Bacterial soft rot, caused by *Erwinia carotovora* subsp. *carotovora* (Van Hall) Dye, is one of the most important and widespread bacterial diseases of a wide variety plants either in the field or during storage. Losses due to bacterial soft rot have been seriously complained by potato growers in Egypt. Currently, the control of this pathogen relies on (1) Seed technology, *i.e.* tuber pasteurization, sanitation and use of certified seeds, (2) Application of chemical pesticides in the field and (3) The use of potato cultivars resistant to *E. carotovora* (Cronin *et al.*, 1997).

A number of compounds that do not have direct antimicrobial activity increase resistance or at least decrease symptoms in some host-pathogen interaction (Hammerschmidt and Smith, 1997). From these compounds benzothiodiazole (BTH), which induces systemic resistance (Gorlach *et al.*, 1996 and Bokshi *et al.*, 2003) in many plants and against a broad spectrum of pathogens.

Increased resistance in potato tubers against *E. carotovora* subsp. *carotovora* was observed when tubers were dipped in acetylsalicylic acid (ASA) (Abd El-Sayed *et al.* 1996 and Bokshi *et al.*, 2003). Salt treatments can inhibit plant pathogens or suppress toxin production (Olivier *et al.*, 1998). Salts including calcium propionate and calcium chloride reduced tissue maceration of potato tubers caused by *E. carotovora* (McGuire and Kelman, 1986; Biggs *et al.*, 1997 and Droby *et al.*, 1997). The efficacy of salt treatments depends on the concentration, the target organism and the method of application (Punja and Gaye, 1993 and Olivier *et al.*, 1998).

Application of fluorescent pseudomonads reduced soft rot incidence, improved plant growth and increased tuber yield in field trials (Xu and Gross, 1986 and Kloepper *et al.*, 2004). Liao (1989) mentioned that an isolate of *P. putida* inhibited the growth of *E. carotovora* on potato slices. Also, *P. putida* isolate survived on the tubers and roots of potato plant for more than 5 weeks. Application of fluorescent pseudomonads to seed pieces to reduce infection of daughter tubers with soft rot bacteria has suppressed the population of *E. carotovora* on roots and daughter tubers (Kloepper, 1983). In the present work, it was planned to suppress bacterial soft rot disease on potato using different treatments, i.e. inducer compounds, salt compounds and bioagent under artificial inoculation conditions.

Materials and Methods

1. Bacteria and inoculum preparation:

A virulent isolate of *E. carotovora* subsp. *carotovora* was isolated from potato (*Solanum tuberosum* L.) tubers showing bacterial soft rot and identified according to Fahy and Persley (1983). Two isolates of *Pseudomonas fluorescens* (Pf1 and Pf5), two isolate of *Serratia marcescens* (Sm1 and Sm2), isolate of *P. aeruginosa* (Pa1) and isolate of *B. subtilis* (Bs3) as bioagents were selected from the stock culture collection maintained in bacterial diseases laboratory, Department of Plant Pathology, Faculty of Agriculture, Ain Shams University. These isolates were evaluated against several diseases (Abd El-Ghaffar and Abd El-Sayed, 1997; Abd El-Ghaffar and Mosa, 2001; Abd El-Sayed *et al.*, 2003 and 2006).These isolates were grown on yeast extract peptone agar (YEPA) medium for 48 hrs at 28°C. The bacterial cells were suspended in sterilized distilled water (SDW) and adjusted to 10^7 colony forming units (cfu/ml) for the pathogen (Wang *et al.*, 1994) and to 10^8 cfu/ml for bioagents, spectrophotometrically (Abd El-Ghaffar *et al.*, 2006).

2. Treatments:

BION (acibenzolar S-methyl) was applied at 0.1, 0.3 and 0.5g/l and salicylic acid (2-hydroxybenzoic acid, $C_7H_6O_3$) was applied at 0.3, 0.6 and 0.9 mM as resistance inducing factors. Potassium sulfate, ammonium phosphate and calcium chloride as salt compounds were applied at 0.1, 0.3 and 0.5 g/l. Antagonistic bacteria mentioned previously, were applied as bioagents. These factors were applied at the same time of inoculation with the pathogen and before or after 24 hours. Certain tubers were only inoculated with the pathogen as a control. Entire potato tubers were weighted and surface sterilized by dipping in 70% ethanol fore one min. followed by two successive rinses in SDW and dried. Sterilized cork borer (0.5-cm-diam.) was used to make a hole (1cm, depth) in the middle of each sterilized tuber.

The pathogen (0.2 ml) and the tested factors (0.2 ml) were piptted into the hole. The holes were closed again with the same removed cylinders of tubers. Treated tubers were placed in sterilized plastic boxes (12X 9 X 8 cm). These boxes were closed with a lid as chamber room and incubated at 28 C'for five days (Abd El-Ghaffar and Abd El-Sayed, 1997).Three boxes of tubers were used as replicates per each treatment and each box containing three tubers

3. Disease assessment:

Disease severity was estimated as percentage of rotted tissue weight according to change weight of tuber before and after treatment divided on weight of tuber before treatment (Schober and Vermeulen, 1999). Percentage of disease reduction (PDR) was calculated according to percentage of rotted tissue weight (Saettler *et al.*, 1989) as the following formula:

$$PDR = \frac{Ack - Atr}{Ack} \times 100$$

Whereas: Ack = disease severity in control and Atr = disease severity in treatment.

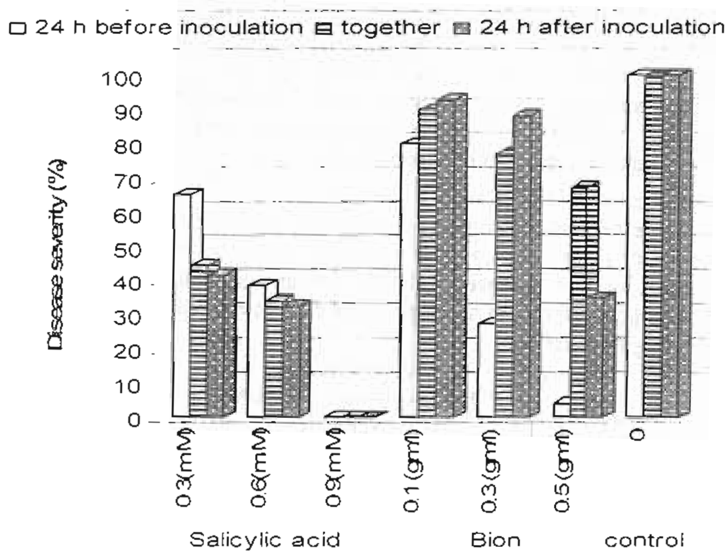
Results and Discussion

1. Effect of some resistance inducing factors against bacterial soft rot of potato:

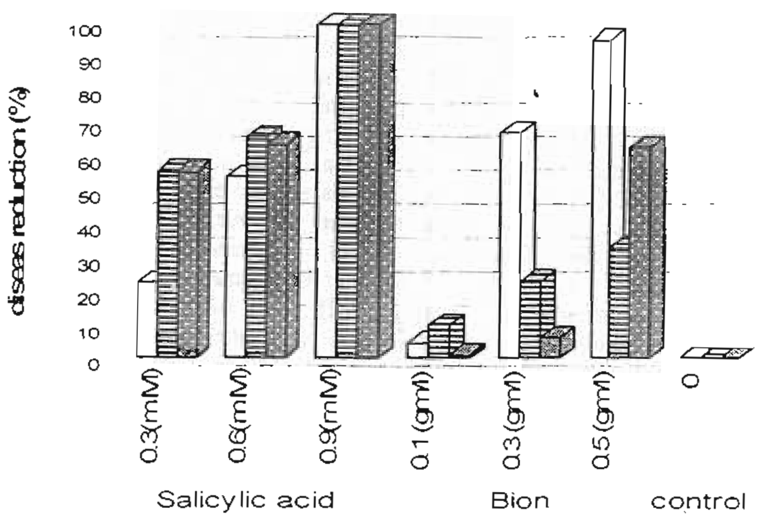
Application of salicylic acid and BION as resistance inducing factors has significantly decreased severity of bacterial soft rot disease of potato compared with the control (Fig.1 A&B). Percentage of disease reduction was increased by increasing the rate of salicylic acid and BION. Efficiency of salicylic was more effective against the disease than BION. Also, salicylic acid was the most effective to reduce the disease, at 9 mM, where percentage of disease reduction was 100%. Applications of salicylic acid lead to decrease the disease, when this compound was applied at the same time or after inoculation with the pathogen. Meanwhile, application of BION tends to reduce the disease, when it was applied before inoculation with the pathogen. These results are in agreement with Abd El-Sayed *et al.* (2003). The effect of salicylic acid was not caused by direct action on the growth of pathogen, but the effect of was rather a consequence of induction of plant defence response (Malamy and Klessing, 1992). Many Biochemical changes occur during systemic acquired resistance (SAR), *i.e.* pathogenesis- related (PR) proteins. Acidic PR-proteins including acidic B-1.3 glucanase and chitinase are secreted in the intercellular spaces, where they would act against fungal and or bacterial pathogens at early stage of infection process. Basic B,1,3-glucanase and chitinase accumulate in the vacuole, may interact with pathogens, at a later stage of infection, during host cell deterioration (Ye *et al.*, 1995 and Kuc, 1995).

2. Effect of some salt compounds against bacterial soft rot of potato:

Application of potassium sulfate, ammonium phosphate and calcium chloride as salt compounds significantly decreased severity of bacterial soft rot disease of potato compared with the control (Fig. 2A&B). Efficiency of salt compounds was increased against the disease by increasing their rate. Severity of bacterial soft rot of potato was completely reduced, when potassium sulphate and calcium chloride were applied at 0.5 g/l and at the same time of inoculation with the pathogen. Tested

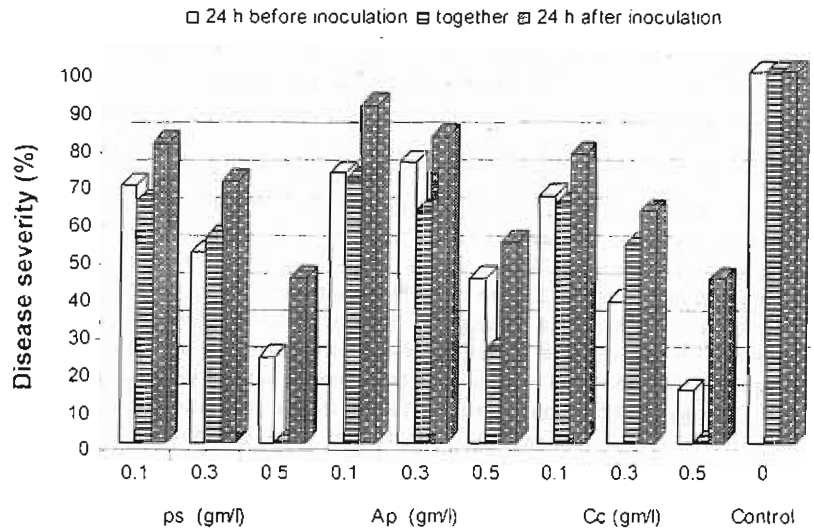


(A)

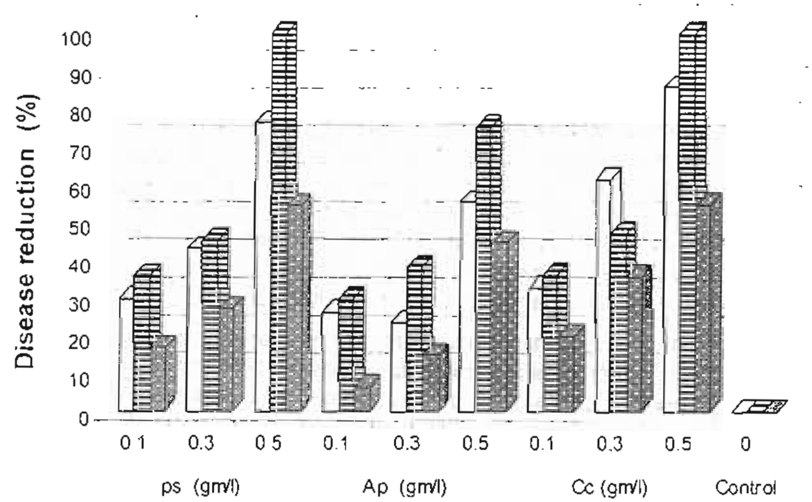


(B)

Fig. 1. Influence of salicylic acid and BION at different rates as inducer agents on percentage of rotted tissue weight (A) and percentage of disease reduction (B), using three treatments under artificial inoculation conditions.



(A)



(B)

Fig. 2. Influence of potassium sulfate (PS), ammonium phosphate (AP) and calcium chloride (Cc), at different rats as salts on percentage of rotted tissue weight (A) and percentage of disease reduction (B), using three treatments under artificial inoculation conditions.

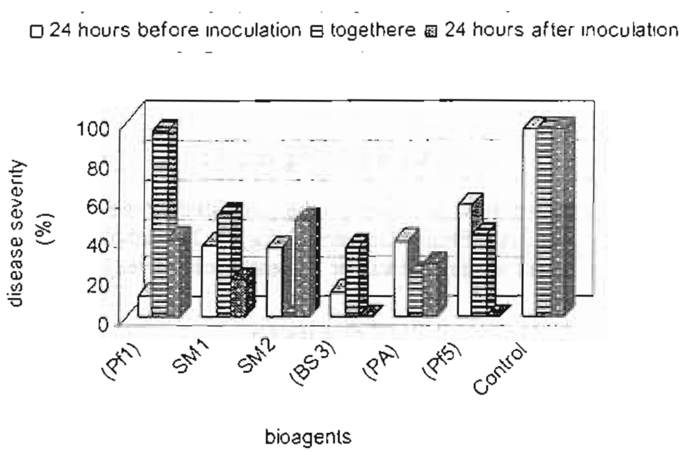
salts were most effective against the disease when they were applied at the same time of inoculation with the pathogen. Applications of salts before inoculation with the pathogen were moderately effective against the disease and were less effective when they were applied after inoculation with the pathogen.

Free calcium ions in tissue stimulate pectate lyase (PL) activity but cause inhibition of polygalacturonase (PG) activity for virulent isolates of soft rot bacteria (Alghisi and Fravaron, 1995). The nitrogen content of the plant is positively correlated with the amount of bacterial soft rot (Wright, 1993). The ratio of chloride to potassium in plant tissues was found to be highly correlated with visual symptom development (Schneider, 1985). Increased chloride uptake can reduce the synthesis of malate in potato plant (Luttage and Higinbotham, 1979), a carbon substrate that might be utilized by plant pathogens. Schober and Vermeulen (1999) found no differences in the enzyme activity between the nitrogen and calcium treatment. Nitrogen levels in the crop were known to influence soft rot severity (Carballo *et al.*, 1994). Nitrogen fertilization increases the dry matter content of broccoli (Everaarts, 1994) and has a direct effect on the cell wall composition, especially the esterification of pectate in cell walls. Nitrogen fertilization interacts with the production of plant defence substance as phenols and inhibited calcium uptake (Reerink, 1993). Perombelon and Salmond (1995) found a correlation between the resistance of potatoes against bacterial soft rot and the composition of the plant cell walls. Resistant potato cultivars had consistently higher amount of calcium in cell wall preparations than susceptible cultivars and they had increased levels of galacturonic acid in cell walls.

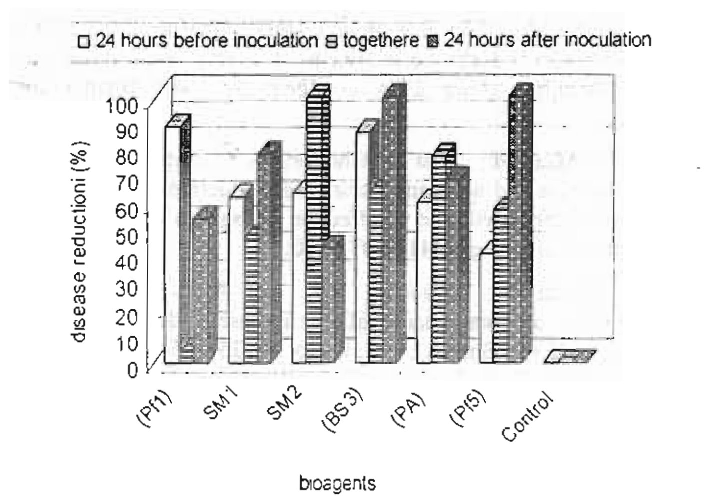
3. Effect of some bioagents against bacterial soft rot of potato:

All tested bioagents have significantly reduced severity of bacterial soft rot disease of potato compared with the control (Fig. 3). Severity of disease completely decreased when isolates of *P. fluorescens* (Pf5) and *B. subtilis* (Bs3) were applied after inoculation with the pathogen and when the isolate of *Serratia marcescens* (Sm2) was applied at the same time inoculation with the pathogen. Meanwhile, disease severity was decreased when *P. fluorescens* (Pf1) was applied before inoculation with the pathogen, *S. marcescens* (Sm1) after inoculation with the pathogen and *P. aeruginosa* (Pa1) at the same time inoculation with the pathogen.

Fluorescent pseudomonads are aggressive rhizosphere colonizes and produce a wide range of antimicrobial compounds (Kloepper, 1983 and Gross, 1988). Certain fluorescent pseudomonads can protect plants from diseases caused by root pathogens and often this biocontrol effect involves antimicrobial compounds such as siderophores (Bakker *et al.*, 1987), hydrogen cyanide (Voisard *et al.* 1989) and antibiotics (Raaijmakers *et al.*, 2002) like phenazine-1-carboxylate, pyroluteorin and 2,4-diacetyl phoroglucinol. *P. aeruginosa* produces a plethoro of extracellular lytic enzyme and secondary metabolites of low molecular weight known as auto-inducers (Pedersen, 1992), where auto-inducers are extracellular N-acyl-homoserine lactones synthesized by various Gram-negative bacteria. The blue phenazine pigment together with Ferripyochellin (a sidrophore of *P. aeruginosa)*, and a reducing agent such as NADH, which catalyses the reaction of hydroxyl and superoxide radicals (Britigan, 1993).



(A)



(B)

Fig. 3. Influence of *Pseudomonas fluorescens* (Pf1 and Pf5), *P. aeruginosa* (PA1), *Serratia marcescens* (Sm1 and Sm2) and *Bacillus subtilis* (Bs3) as bioagents, on percentage of rotted tissue weight (A) and percentage of disease reduction (B), using three treatments under artificial inoculation conditions.

Experiments screening of *Bacillus* spp. had elicited induced systemic resistance (ISR) on other crops (Jetiyanon and Kloepper 2002), where two strains of *B. pumilus* and a strain of *B. mycoides* enhanced peroxidase activity and increased production of one chitinase isozyme and two isozymes of B-1,3 glucanase (Bargabus *et al.*, 2002 and 2004). Another strain of *B. pumilus* had greatly increased levels of salicylic acid (Zhang *et al.*, 2002).

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مكافحة مرض العفن الطري البكتيري في البطاطس

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يسبب مرض العفن الطري البكتيري خسائر فادحة في مصر والعالم، وعلى عوائل نباتية عديدة من أهمها محصول البطاطس يتسبب هذا المرض عن البكتريا *Erwinia carotovora* subsp. *carotovora* .

جرت هذة الدراسة بهدف مكافحة هذا المرض باستخدام وسائل مكافحة عديدة تتضمن كل من حمض الساليسيليك والبيون كعومل حث كيمائية ، وكبريتات البوتاسيوم وفوسفات الأمونيوم وكلوريد البوتاسيوم بالتركيز (٠,١ ، ٠,٣ و ٠,٥ جم / ليتر) كأملاح ، وعزلات من بكتيريا مضادة كعوامل مكافحة حيوية من كل من الأنواع *Pseudomonas fluorescens* (Pf1 and Pf5) *Serratia marcescens* (Sm1 and Sm2), *Bacillus subtilis* (Bs3) *P. aeruginosa,* وذلك تحت ظروف العدوى الصناعية للدرنات لإختبار مدى **فعالية هذه العوامل في قدرتها على خفض شدة المرض في ثلاثة مواقيت للمعاملة على النحو التالي : (١) قبل ٢٤ ساعة من العدوى بالبكتريا الممرضة. (٢) المعاملة في نفس الوقت مع العدوى بالبكتريا الممرضة. (٣) المعاملة بعد مرور ٢٤ ساعة على المعاملة بالبكتريا الممرضة. ومن أبرز النتائج التي تم الحصول عليها في هذه الدراسة :**

١- خفضت جميع المعاملات الشدة المرضية للعفن الطري بشكل معنوي إحصائيا بالمقارنة بمعاملة المقارنة.
٢- إنخفضت الشدة المرضية للمرض كليا عند المعاملة بحمض الساليسيليك بالتركيز ٠,٩ مللي مول/ ليتر بالتواقيت الثلاثة للمعاملة ، وكذلك عند المعاملة بكل من كبريتات البوتاسيوم وكلوريد الكالسيوم بالتركيز ٠,٥ جم / ليتر عند تطبيقها بنفس وقت العدوى بالبكتريا الممرضة.
٣- خفضت كل من *B. subtilis* (Bs3) *S. marcescens* (Sm2) *P. fluorescens* (Pf5) الشدة المرضية للعفن كليا في المواقيت الثلاثة للمعاملة.
٤- تزداد فعالية كل من عوامل الحث الكيمائية (حمض السليسيلك ، البيون) والأملاح الثلاثة في تخفيض الشدة المرضية مع زيادة التركيز المستخدم لكل منها .
٥- حمض الساليسيلك وكلوريد الكالسيوم و كبريتات البوتاسيوم كانت أكثر العوامل فعالية ضد المرض بالمقارنة بكل من البيون وفوسفات الأمونيوم .
٦- باقي العزلات الحيوية المستخدمة خفضت المرض بشكل معتدل مقارنة بالعزلات الأكثر فعالية وذلك في المواقيت الثلاثة للمعاملة.

وعلى ضوء هذه النتائج لابد من إختبار وتقييم العوامل الأكثر فعالية في هذا البحث تحت الظروف الطبيعية للمرض للإستفادة منها في مكافحة المرض.