J. Agric. Sci. Mansoura Univ., 32 (5): 3515 - 3522, 2007

EFFECT OF EXPOSURE OF POTATO TUBERS TO VOLATILE COMPOUNDS ON *Rhizoctonia solani* AND *Ralstonia solanacearum* TRANSMITTED BY TUBERS

Abd El-Aziz, M. E.; N. Y.Abd El-Ghafar; F. M. Abo El-Abbas and M. H. Mostafa

Dept. of Plant Pathology, Fac. of Agric. Ain Shams Univ. Cairo, Egypt

ABSTRACT

Potato tubers infected with *Rhizoctonia solani* and *Ralstonia solanacearm*, the causal organisms of black scurf and bacterial wilt diseases were obtained from store-houses and fields at different areas in Egypt. The pathogens were isolated from infected potato tubers and identified. Tubers were treated with the volatile compounds (methanol , ethanol , amyl alcohol , formic acid , acetic acid , propionic acid , gasmonic acid , formaldehyde , acetaldehyde , chloroform , acetone) in fumigation chamber (2.3 L) at different levels. Results indicated that formaldehyde , formic acid , acetic acid and propionic acid showed high reductive effect on mycelial growth of *Rhizoctonia solani* from treated sclerotia on water agar medium**.** Formaldehyde and acetic acid showed eradicative effect on latent infection of *Ralstonia solanacearm* .However, these compounds showed no harmful effect on potato tubers sprouting when potato tubers were fumigated by 2.5 µl / l air space for 60 min.

Keywords: Black scurf , Bacterial wilt , Formaldehyde , Acetic acid , Vapor

INTRODUCTION

Potato plants are attacked by a wide range of fungal , bacterial and viral pathogens, where they cause serious losses in crop production and / or tuber quality . Potatoes are a propagated vegetatively, and potato seed tubers can be an important source of disease inocula. Tuber carrying *Rhizoctonia solani* that cause black scurf (Hollins *et al*, 1983) and *Ralstonia solancerum* that cause bacterial wilt (Overbeek *et al*, 2002). *Rhizoctonia solani* causes both stem canker and black scurf of potato (*Solanum tuberosum* L.), which lead to tuber yield reductions and losses in tuber quality. Stem canker consists of stem lesions that can reduce tuber yield by reducing the transport of nutrients throughout the plant. *Ralstonia solanacearum* is mainly transmitted through tuber seed , and under cool conditions the plant can be infected without exhibiting visible symptoms , resulting in latent infection in vascular tissues of progeny tubers and causes yield losses as high as 100% (Ciampi *et al*, 1980 and Hayward , 1991). Fumigation of stored potatoes by 2-aminobutane gave excellent control of gangrene and skin spot , and partial control of silver scurf under a wide range of environments (Graham *et al* , 1973). Ozone applied to freshly harvested seed-grade potatoes up to 0-20 mg O_3/kg/hr for 1, 7 or 21 days had little significant effect on the incidence or severity of a range of diseases or tuber color, but did increase tuber weight loss in a dosage-dependant manner, and when tubers were inoculated with a range of pathogens (*Fusarium sambucinum, F. solani., Phytophthora infestans, Helminthosporium solani*), introduced at wound depths appropriate to each disease, disease levels typically increased, however ozone treatment (20 mg O_3/kg/hr) did not reduce development of any of these diseases. (Spencer , 2003). Fumigation with 2-

aminobutane did not have any substantial effect on the growth pattern or the yield of the crop obtained from treated seed , although in most cases it was probable that there were small increases in the number of sprouting, percentage of emergence , stem number and weight and number of seed tubers at harvest. (Quinn *et al*, 1976). The present investigation was aimed to study the effect of exposure of potato tubers to different vapors of organic volatile compounds on the persistence of *Rhizoctonia solani* and *Ralstonia solanacearum* transmitted by naturally infected tubers and also on tubers sprouting

MATERIALS AND METHODS

All experiments were conducted in the laboratories of Department Plant Pathology , Faculty of Agriculture, Ain Shams University, Cairo, during 2004 - 2006 .

Plant material:

Samples of potato tubers with typical symptoms either black scurf or infected with bacterial wilt , were obtained from store-houses(Agrofood company) and fields (Nubaria region) at different areas , and stored in the refrigerator at 4ºC until used

Isolation of *Rhizoctonia solani*

Black or dark brown sclerotia developed on surfaces of mature tubers resembling soil will not wash off were used as source of *R.solani*.(Frank,1981)

Potato tubers were surface sterilized with 70% alcohol and flamed. then black sclerotia from the surface of the tubers were transferred to Petri dishes containing water agar (W.A) medium. After incubation at 25 C for 48 hr, small portions of the edges of developing colonies were transferred to Petri dishes containing potato dexstrose agar (PDA) medium and incubated at 25 ºC for 7 days.

Isolation of *Ralstonia solanacearm*

Potato tubers were surface sterilized with 70% alcohol and flamed then basal part was removed , and pieces of paranchymatous containing vascular bundles were creshed with solution (0.85 Nacl).Bacterial suspension was streaked in transferred to Petri dishes contained Kelman،s medium (tetrazollium chloride ,TZC containing only 2.5 g dextrose) . After 48 h of incubation at 30ºc , the typically fluid,slightly red-tinted colonies of virulent *R. solanacearum* are easily distinguished from other saprophytic bacteria (round shaped , uniformly dark red-colored colonies). *R.. solanacearum* was identified according to French *et al* (2002).

Volatile compounds:

The following vapors of different organic compounds (Table 1)were used

Method of treatment:

Naturally infected potato tubers were placed into cylindrical containers (2.3 L.) with lid (stainless steel material). Two concentration , 2.5 µ l / liter air space or 5 µ l / liter air space of volatile compounds were applied onto a filter paper while were placed into the lid of the containers then tightly closed for 30 or 60 min.

Table (1) : Volatile compounds used in this study

Compounds	Chemical formula
Methanol (absolute)	CH_3OH
Ethanol (absolute)	C_2H_6O
Amyl alcohol (absolute)	$C_5H_{11}OH$
Formic acid (absolute)	CH_2O_2
Acetic acid (absolute)	CH_3COOH
Propionic acid (absolute)	CH_3CH_2COOH
Gasmonic acid (absolute)	$C_{12}H_{18}O_3$
Formaldehyde (absolute)	CH_2O
Acetaldehyde (absolute)	C_2H_4O
Chloroform (absolute)	CHC_{13}
Acetone (absolute)	CH_3COCH_3

Producer company {Silver star for chemicals (Mark,LTD,USA).}

Effect of volatile compounds on mycelial growth (from sclerotia) of *Rhizoctonia solani .*

Potato tubers showed sclerotia (5-8) of *R.. solani* were classified to three groups with three infected tubers in each . First group was exposed to volatile compound produced from evaporation by 2.5 µ l / liter air space (5.75 µ l / container) for 30 min, second group was treated the same concentration but for 60 min , in addition third group was treated by 5 µ l / liter air space (11.5 µ l / container) for 60 min. Three infected tuber were left as a control . After exposure, *R. solani* sclerotia from all treated tubers were collected and cultured on W.A. and incubated at 24°c for one week. Diameter of fungal growth for each treatment was recorded and compared with the diameter of mycelia growth in control.

Effect of volatile compounds on growth of *Ralstonia solanacearm*

Infected potato tubers with bacterial wilt **(***R. solanacearm*) were treated by 5 µ l / liter air space for 60 min by using three infected tubers for each treatment. After exposure a small portion of vascular bundles of tubers was cultured on TZC media and growth of bacterial was recorded.

Effect of volatile compounds vapors on potato tuber sprouting

Infected potato tubers were Classified into two groups . One group was treated by 2.5 µ l / liter air space for 30 min by using five infected tubers for each volatile compound , second group was treated by 2.5 µ l / liter air space for 60 min by using five infected tubers for each volatile compounds. after 25 days , the sprouts were removed from each tuber and their number and weight were determined. The total weight of sprouts and the sprout mean number were calculated for each potato tuber. A completely randomized experimental design including five replication was used.

Statistical analysis

All experiments were set up in a complete randomized design. Data were subjected to analysis of variance (ANOVA) using the Statistical Analysis System (SPSS statistical software for windows .v.10). Means were separated by Duncan's multiple range test at $P< 0.05$ level

RESULTS

Isolation and identification of *Rhizoctonia solani*.

Sclerotia of *Rhizoctonia solani* were collected from diseased potato tubers with typical symptoms of black scurf collected from many different location in Egypt . Sclerotia were left to germinate on water ager and hyphal tips were transferred to PDA medium .

Isolation and identification of *Ralstonia solanacearum*

Ralstonia solanacearum were isolated from diseased potato tubers with typical symptoms of bacterial wilt collected from many different locations in Egypt. Results of cultural characters showed that colonies were whitish fluidal white with pink center on tetrazollium chloride (TZC) medium

Effect of volatile compounds vapor on mycelial from germinated sclerotia growth of *Rhizoctonia solani*

Potato tubers showed black scurf symptoms were exposed to vapor of different volatile compounds . Results in table (2) indicate that all treatments with volatile compounds vapor by 2.5 µl / l air space for 30 min or 60 min or 5 µl / l air space for 60 min in fumigation chamber (2.3 L) significantly reduced (P≤ 0.05) sclerotial viability of *R. solani* .

Table (2) : Effect of vapor of volatile compounds at different periods of exposure on diameter of Rhizoctonia solani mycelia produced from treated sclerotia .

Volatile compound	Period of exposure 30 min by 2.5 µl / l air space		Period of exposure 60 min by 2.5 µl / l air space		Period of exposure 60 min by 5 µl / l air space	
	Diameter of growth	% Reduction of growth	Diameter of growth	% Reduction of growth	Diameter of growth	% Reduction of growth
Methanol	4.5^{de}	47.0	3.8^{bc}	56.8	5.8^{e}	30.1
Ethanol	6.9^{g}	18.8	4.6^{c}	47.7	5.8^{e}	30.1
Amyl alcohol	5.1^{ef}	40.0	4.0^{bc}	54.5	4.8^{d}	42.1
Formic acid	4.3^{cde}	49.4	3.5^{bc}	60.2	0.0^{a}	100
Acetic acid	6.1^{fg}	28.2	4.2^{bc}	52.2	0.0^{a}	100
Propionic acid	3.7^{bcd}	56.4	3.0^{b}	65.9	2.0^{b}	75.9
Gasmonic acid	4.8^{de}	43.5	6.5^{d}	26.1	4.2^{cd}	49.3
Formaldehyde	2.0^{a}	76.4	0.0^{a}	100.0	0.0^{a}	100
Acetaldehyde	3.0^{ab}	64.7	3.6^{bc}	59.0	5.0^{de}	39.7
Chloroform	2.3^{a}	72.9	6.3^{d}	28.4	3.7^{c}	55.4
Acetone	3.2^{abc}	62.3	2.9^{b}	67.0	3.6^{cd}	56.6
Control	8.5^{h}	-	8.8^{e}	-	8.3^{f}	-

*** Values followed by the same latter in the same column are not significantly different at P ≤ 0.05 according to Duncan's multiple range tests.**

Treatment by 2.5 µl / l air space for 30 min by formaldehyde vapor gave the highest reductive effect on sclerotial viability by 76.4-100 % . Formic acid , acetic acid , propionic acid and gasmonic acid give less reductive effect on sclerotia viability 49.4 % , 28.2% , 56.4% , 43.5%, respectively , treatment by 2.5 µl / l air space for 60 min by formaldehyde , formic acid , acetic acid , propionic acid and acetone give high reductive effect on sclerotia viability 100 % , 60.2% , 52.2% , 65.9%, 67% , and gasmonic acid give less reductive effect by 26.1% and when treated by 5 µl / l air space for 60 min

formaldehyde , formic acid , acetic acid , propionic acid , give high reductive effect on sclerotia viability by 100% , 100% , 100% , 75.9% respectively .The effect of formaldehyde , formic acid , acetic acid and propionic acid increase when doses of volatile compounds and fumigation period increased.

Effect of vapor of volatile compounds on *Ralstonia solanacearum.*

Result in table (3) indicated that when naturally infected potato tuber by *Ralstonia solanacearm* treated by 5µl / l for 60 min of volatile compounds vapors in fumigation chamber, and cultivation of vascular areas on TZC media, complete eradication of bacterium was observed when tuber exposed to acetic acid or formaldehyde vapors and the other volatile compounds do not affect *Ralstonia solanacearm.*

Table (3): Effect of exposure of infected potato tubers to volatile compounds by 5ul \ L air space for 60 min in fumigation chamber on *Ralstonia solanacearm* growth on TZC medium

Volatile compounds	Methanol	Ethanol	Amyl alcohol	Formic acid	Acetic acid	Propionic acid	Gasmonic acid	Formaldeh-yde	Acetaldeh-yde	Chloroform	Acetone	Control (water)
growth	+	+	+	+	-	+	+	-	+	+	+	+

+ = Bacterial cell growth didn't affected by volatile compounds when compared with control.

- = Bacterial cell growth was eradicate when compared with control.

Effect of volatile compounds vapors on potato tuber sprouting

Result in table (4) indicate that vapors have not harmful effect on potato tubers sprouting when treated by volatile compounds vapor with 2.5 µl / l air space for 60 min.

Table (4): Effect of potato tubers treatment by volatile compounds vapor with 2.5ul \ L for 60 min on tuber sprouting

Volatile compound	Treatment for 60 min by 2.5 µl / l air space			
	sprout No.	% Sprouting as compared to control	sprout weight(g)	% Sprout weight
Methanol	4.0^{abc}	105.2	6.10^{ab}	84.4
Ethanol	2.4^{a}	63.1	4.84^{ab}	67.0
Amyl alcohol	6.8^{c}	178.9	7.74^{b}	107.2
Formic acid	2.8^{ab}	73.6	5.68^{ab}	78.6
Acetic acid	4.0^{abc}	105.2	6.84^{ab}	94.7
Propionic acid	5.2^{abc}	136.8	5.36^{ab}	47.5
Gasmonic acid	4.2^{abc}	110.5	6.78^{ab}	93.9
Formaldehyde	4.0^{abc}	105.2	6.56^{ab}	90.8
Acetaldehyde	2.8^{ab}	73.6	5.96^{ab}	82.5
Chloroform	6.0^{bc}	157.2	5.54^{ab}	76.7
Acetone	3.2^{ab}	84.2	4.50^{a}	62.3
Control	3.8^{abc}	100	7.22^{ab}	100

***Values followed by the same latter in the same column are not significantly different at P ≤ 0.05 according to Duncan's multiple range tests.**

While some of volatile compounds such as methanol , amyl alcohol , acetic acid , propionic acid ,Gasmonic acid , formaldehyde and chloroform led to increase the sprout number by 5.2% , 78.9 % , 5.2 % , 36.8 % , 10.5% , 5.2% , 57.8% respectively

DISCUSSION

Fumigation with volatile compounds to control many of plant pathogens was applied in different locations of the world . 2-aminobutane was used as a fumigant to control gangrene skin spot and silver scurf on potato tubers and gave excellent control (Graham *et al* , 1973) but this compound caused damage to potato tubers (Hims and Fletcher 1980).

Many of pathogens transmitted by potato tubers such as *Rhizoctonia solani* and *Ralstonia solanacearm* and transmitted to growing plant when they used as vegetatively propagations (Tsror *et al* , 1999). Control of these pathogens by using vapors of some alcohols , acids , aldehydes and ketones as a potato tuber treatment didn't studied . Vapors of organic acid (acetic acid , formic acid and propionic acid) were used as control agent to postharvest decay (Sholberg, 1998). In this study these compounds were tested on seed borne pathogens that transmitted by potato tuber . Formaldehyde , acetic acid , formic acid and propionic acid gave high reduction effect on sclerotia viability of *Rhizoctonia solani* and acetic acid eradicate of *Ralstonia solanacearum* from vascular tissues.

Fumigation for 1 hr in a 50 cu. ft (1.42 cu. m) chamber with a rate eqmvalent to 0.75 lb potassium permanganate/3 pt formaldehyde/ 6,000 cu. ft (0.34 kg/1.70 1./169.90 cu. m) killed spores of *Botrytis cinerea*, *Fulvia fulva* and *Fusarium culmorum* , though in a series of 1 hr fumigations based on this rate the mycelium of *B. cinerea* was not killed. Fumigations for 24 hr in a 50 cu. ft (1.42 cu. m) chamber and a commercial glasshouse with 0.75 lb potassium permanganate/3 pt formaldehyde/6,000 cu. ft (0.34 kg/1.70 1./169.90 cu. m) and 0.8 lb/ 1.25pt/6,000cu. ft (0.36 kg/0.71 1./169–90 cu. m) killed both spores and mycelium of *B. cinerea*. (White and Fletcher, 1971) formaldehyde used to control *Rhizoctonia solani* by tuber treatment for 20 min dipped in 2 % formaldehyde (Wicks *et al* , 1996).

In this study formaldehyde was used as a fumigant agent to control *Rhizoctonia solani* and *Ralstonia solanacearm* that transmitted by potato tuber and given high reduction affect on sclerotia viability of *Rhizoctonia solani* and eradicate the cell growth of *Ralstonia solanacearum.*

Many of compounds were tested on tuber sprouting such as chlorine (Tweddell *et al,* 2003) . Ethyl salicylic acid (Mostafa and Gado , 2007) . Ozone (Spencer , 2003) . In this study expose tuber to vapor of some alcohols , acids , aldehydes and ketones by using 5µl / l for 60 min in fumigation chamber had not harmful effect on potato tubers sprouting, however some of compounds gave small increases in the number of sprouting eyes.

REFERENCES

Balali, G. R.; Neate, S. M. ; Scott, E. S. ; Whisson, D. L. and Wicks, T. J. (1995). Anastomosis group and pathogenicity of isolates of *Rhizoctonia solani* from potato crops in South Australia. Plant Pathology 44 : 1050–1057.

Ciampi, L. ; Sequeira, L. and French, E. R. (1980) . Latent infection of potato tubers by *Pseudomonas solanacearum* . Am. Potato J. 57 : 337 – 386

Frank,J.A.(1981) Rhizoctonia canker (black scurf). In : Compendium of Potato Diseases.Hooker,W.J.(ed), pp52-54, The American Phytopatholgical Society,USA

French, E.B.; Gutarra, L.; Aley, P. and Elphinstone, J. (1995). Culture media for *Ralstonia solanacearum* isolation, identification, and maintenance. Fitopatologia 30:126-130.

Graham, D. C. ; Hamilton1, G. A.; Nash, M. J. and Lennard, J. H. (1973) . Fumigation of bulk-stored potatoes with 2-aminobutane for control of gangrene , skin spot and silver scurf diseases. Potato Research 16: 234-243

Hayward, A. C. (1991). Biology and epidemiology of a bacterial wilt caused by *Pseudomonas Solanacearum*, Ann. Rev. Phytopathol. 29 : 65 – 87

Hims, M. J. and Fletcher, J. T. (1980) . Damage to potato tubers caused by fumigation with 2-aminobutane . Potato Research . 23 : 365-369

Hollins, T. W. ; Jellis, G. J. and Scott, P. R. (1983) . Infection of potato and wheat by isolates of *Rhizoctonia solani* and *R. cerealis*. Plant Pathology 32 : 303–310.

Mostafa , M.H. and Gado, E.A.M. (2007). Effect of ethyl salicylic acid on sprouting and rots incidence of potato tubers under long-term storage. Arab Univ.J.Agric.Sci.,Ain Shams Univ., Cairo, 15 : 169-176

Overbeek, L. S. ; Mike, C. ; Jacek, K. ; Jack, T. and Jan, D. (2002). A polyphasic approach for studying the interaction between *Ralstonia solanacearum* and potential control agents in the tomato phytosphere . J. of Microbiological Methods 48 : 69-86

Quinn, C. E. ; Harper, P. C. and Graham, D. C. (1976) Effect of fumigation of potato seed tubers with 2-aminobutane on plant growth and yield . Potato Research . 19 : 147-155

Sholberg, P. L. (1998). Fumigation of fruit with short-chain organic acids to reduce the potential of postharvest decay. Plant Diseases 82:689-693.

Spencer, R. C. J. (2003) . Ozone As A Post-Harvest Treatment For Potatoes . Master of Science, The Department of Plant Sciences, University of Saskatchewan Saskatoon, Sk, Canada, pp 139 (www.library2.usask.ca)

Tsror, L. ; M. Aharon and O. Erlich (1999). Survey of bacterial and fungal diseases in imported and domestic potato seed tubers. Phytoparasitica 27:1-12

Tweddell, R. J ; Boulanger, R. and Arul, J. (2003). Effect of chlorine atmospheres on sprouting and development of dry rot, soft rot and silver scurf on potato tubers. Postharvest Biology and Technology 28: 445-454

White, J. G. and Fletcher, J. T. (1971) . Glasshouse fumigation with formaldehyde . Plant Pathology .20 : 191-194

Wicks, T. J. ; B. Morgan and B. Hall (1996) . Influence of soil fumigation and seed tuber treatment on the control of *Rhizoctonia solani* on potato . Australian journal of experimental agriculture 36 : 339-345

تأثير تبخير درنات البطاطس بالمواد الطيارة على فطر *Rhizoctonia solani* و بكتريا *Ralstonia solanacearm* المنقولة بالدرنات

محمد السيد عبد العزيز , ناجى يسن عبد الغفار , فوزى مرسى ابو العباس و مصطفى حلمى مصطفى

قسم أمراض النبات, كلية الزراعة, جامعة عين شمس , مصر

تنقل درنات البطاطس العديد من الإصابات مثل فطر *Rhizoctonia solani* المسبب لمرض القشرة السوداء وبكتريا *Ralstonia solanacearm* المسببة لمرض الذبول البكتيري . ويهدف هذا البحث إلى دراسة تأثير تعريض الدرنات الحاملة لهذه المسببات المرضية لأبخرة بعض المواد الطيارة (الميثانول ،الايثانول ، الأميل الكحول ، حمض الفورمك ، حمض الخليك ، حمض البروبيونك ، حمض الجاسمونك ، الفورمالدهيد ، الأسيتالدهيد ، الكلوروفورم ، الأسيتون) على هذه المسببات المرضية ، قد تم تعريض الدرنات المصابة لأبخرة هذه المواد داخل غرفة تبخير حجمها ٢,٣ لتر فراغ باستخدام ٢,٥ ميكروليتر لمدة ٣٠ دقيقة أو ٦٠ دقيقة وأيضا استخدام ٥ ميكروليتر لمدة ٦٠ دقيقة . ووجد أن الفورمالدهيد و حمض الفورمك و حمض الاسيتك و حمض البروبيونك من أفضل المواد تأثيرا على حيوية الأجسام الحجرية لفطر *Rhizoctonia solani* وذلك عند زراعة الأجسام الحجرية على أجار مائي بعد المعاملة . ووجد أن هذه المواد عند استخدامها بهذه الجرعات لا يوجد لها اى تأثير سيئ على إنبات الدرنات ، عند تعريض الدرنات المصابة بالبكتريا المسببة للذبول البكتيري لأبخرة المواد الطيارة وجد أن أبخرة الفورمالدهيد و حمض الخليك تعمل على إيقاف خلايا البكتريا عن النمو عند حقنها على بيئة TZC بعد المعاملة , مما يؤكد على انها اثرت على البكتريا الكامنة فى الاوعية الخشبية للدرنات .