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Acetic Acid Vapours For Controlling Tomato Root Rot Disease Under Greenhouse Conditions

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ABSTRACT

Effect of acetic acid vapours on tomato root rot fungi under laboratory and greenhouse conditions was studied. Acetic acid vapours at four concentrations *i.e*.0.0, 2, 4 and 6 µl/L were tested against linear growth of *F. solani, R. solani* and *S rolfsii*. Results revealed that complete inhibition in linear growth was obtained with AA at 6 µl/L for all tested fungi. The most sensitive fungi to acetic acid vapours are *F. solani* and *R. solani* which inhibited at 4 µl/L, while *S. rolfsii* were more resistant to acetic acid vapours at 6 µl/L caused the complete inhibition of linear growth. Complete inhibition of chlamedospores of *F.solani* was obtained by AA vapour at 15 µl/L while, Sclerotia of *S.rolfsii* showed more resistance to acetic acid vapours as their germination was completely inhibited at 20 µl/L of acetic acid vapour. Under greenhouse conditions results indicated that complete reduction in total count of pathogenic fungi was obtained with AA at µl/L for *F. solani* & *R. solani* and at 100 µl/L for *S. rolfsii*. The highest reduction was achieved with AA at 25 µl/L which reduced the population by 74.0, 84.0 and 53.0 % for *F. solani, R. solani* and *S. rolsii* respectively. All tested concentrations of AA significantly reduced the percentage of root rot incidence of tomato plants . The most effective concentration of acetic acid vapour was 100 µl/L which reduced the disease incidence by 92.9, 94.2 and 93.9 % for *F. solani, R. solani* and *S. rolsii* respectively. It could be suggested that acetic acid vapours might be safely used for controlling tomato root rot disease under greenhouse conditions.

Keywords: Tomato plants - Acetic acid vapours- Root rot disease-

INTRODUCTION

Tomato plants is the one of most important vegetable crops in Egypt and other countries. Root rot disease caused by *Rhizoctonia solani* Kuhu.; *Fusarium solani* **(**Mart**)** Sacc. and *Sclerotium rolfsii* Sacc. is the most destructive disease of tomato [9,12,4,13]. Controlling such diseases mainly depend on fungicides treatments [19]. However, fungicidal applications cause hazards to human health and increase environmental pollution. Methyl bromide was extended to soil against fungi, nematodes, viruses and plant pathogenic bacteria, although too expensive for use in large field operation. It is used worldwide in most nurseries, greenhouse agriculture and other high –value plant production enterprises, [17]. This compound is expensive for use in large field conditions in spite of their hazards effect to the environment and human healthy [17]. Therefore, alternative treatments for control of plant diseases are needed [2,3,4].

Vapours of acetic acid (AA) were reported to be effective for controlling postharvest decay of apple, grapes, kiwifruit, pear, tomato, orange, apricot and stone fruit [20-24,14-16,3,5].

Abd-El-Kareem [5] reported that, acetic acid vapour at 75 µl/L caused complete reduction in population of cucumber root rot fungi and under greenhouse conditions it reduced the cucumber root rot disease more than 93.3 and 95.4 % for pre and post emergence respectively.

The present work aims to study the effect of acetic acid vapours on linear growth as well as spore germination tomato root rot fungi *In vitro*. It is effect on total count of fungi as well as its effect on tomato root rot disease under greenhouse conditions.

Materials and Methods

Effect of acetic acid fumigation on linear growth of tomato root rot fungi:

Fumigation:

Acetic acid fumigation was carried out in specially designed fumigation chamber 270 L in volume with fan to have closed circulated air current [14].

Soil-borne fungi:

All isolates were obtained from Plant Pathology Department, National Research Centre Egypt.

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Disks (6-mm-diameter) of 10 days old cultures of each pathogenic fungi *i.e. Fusarium solani* (Mart) Sacc; *Rhizoctonia solani* Kuhu. and *Sclerotium rolfsii* Sacc. were exposed to acetic acid vapours *i.e.0.0,* 2,4, and 6 µl/L (v/v in air) for 30 min in fumigation chamber. Fumigated disks were transferred to plates containing PDA medium, Unfumigated disks for each fungus served as control. Linear growth of fungi was measured when the control plates reached full growth and the average growth diameter was calculated. Twenty five disks as replicate were used for each particular treatment.

Effect of acetic acid fumigation on resting stage germination of tomato root rot fungi:

Acetic acid vapours at four concentrations *i.e*. 0.0, 5, 10, 15 and 20 µl/L against chlamedospores of *F.solani* and sclerotia germination of *R. solani* and *S. rolfsii* were tested.

Effect on sclerotia of R. solani and S. rolfsii:

Sclerotia of *R. solani* and *S. rolfsii* were harvested and surface disinfected with Ethanol alcohol (70 %) for 3 sec, then washed with sterilized water several times. Sclerotia were transferred to Petri plates containing 20 ml of PDA medium.

Effect on chlamydospores of F. solani:

Chlamydospores suspension were prepared by culturing *F, solani* fungus on Petri-plates containing water agar medium for 20 days at 25°C. Colony forming units (cfu) containing Chlamydospores were released in sterilized water using a needle and 1 ml of suspension was transferred to PDA medium at six equidistant points. While, 20 sclerotia were placed on Petri plates containing 20 ml of PDA medium. Inoculated plates were uncovered and fumigated with acetic acid vapours at previous concentrations for 30 min. Fumigated plates were covered and incubated at 20-25^{o} C. Determination of sclerotia germination was carried out according to the method described by Edmunds and Gleason, [10].

Chlamydospores was examined microscopy and percent of chlamydospores germination was calculated. The average percent of germination was calculated in ten replicates.

Effect of acetic vapours on tomato root rot disease under greenhouse conditions:

Mixed of peat-moss and vermiculite soil was artificially infested with tomato root rot fungi. Soils were mixed individually with the prepared inoculum of each *R.solani , F.solani* and *S.rolfsii* as follow:-

Preparation of fungal inocula:

Inocula of *R. solani , F. solani* and *S. rolfsii* were prepared by culturing each fungus on 50.0 ml potato dextrose broth (PDB) medium in 250 ml Erlenmeyer flasks for 15 days at 25° - 27 ^{o}C. and fungal inocula were prepared as follows :

Inoculum of *F. solani* was prepared as the upper solid layers that grew were washed and blended in sterilized water. Colonies forming units (cfu) were adjusted to 10^{6} cfu / ml using haemocytometers slide. Soil infestation was carried out at rate of 50 ml (10^{6} cfu / ml) / kg soil [11].

Inoculum of *S. rolfsii* and *R. solani* was prepared as the upper solid layers that grew were washed and air-dried with sterilized filter paper layers. The air-dry mycelium was blended in distilled water to obtain inocula pieces of 1-2 mm in diameter. Soil infestation was carried out at rate of 2.0g dry mycelium /kg soil, [7].

a- Effect on population reduction of tomato root rot fungi:

Artificially infested soils were fumigated with acetic acid vapours at concentrations 0.00, 12.5, 25,50 and 100 µl/L in air for 60 min in fumigation chamber. Unfumigated soil served as control. Total of tomato root rot fungi were counted using dilution methods. The reduction in percentage of total count of fungi referring to unfumigated soil was calculated.

b- Effect on root rot incidence of tomato plants:

Plastic pots (30 cm diameter) containing artificially infested soil with individuality tomato root rot fungus were fumigated for 60 min in fumigation chamber. Then air dried for 60 minutes. Tomato transplants cv. Kastel rock were planted at the rate of 6 transplants/pot and 6 pots as replicates for each particular treatment were used.

Percentage of root rot incidence after 35 or transplanting was recorded.

Statistical analysis:

Tukey test for multiple comparisons among means was ultized [18].

Results:

Effect of acetic acid fumigation on linear growth of tomato root rot fungi In vitro:

Acetic acid vapours at four concentrations *i.e*.0.0, 2, 4 and 6 µl/L were tested against linear growth of *F. solani*, *R. solani* and *S rolfsii.* Results in Table (1) indicate that all tested concentrations of acetic acid vapours inhibited the linear growth of all tested fungi. Complete inhibition in linear growth was obtained with AA at 6 µl/L for all tested fungi. The most sensitive fungi to acetic acid vapours are *F. solani* and *R. solani* which inhibited at 4 µl/L, while *S. rolfsii* were more resistant to acetic acid vapours as 6 µl/L caused the complete inhibition of linear growth.

Table 1: Linear growth (mm) of tomato root rot fungi as affected with different concentrations of acetic acid vapours.

	Tomato root rot fungi		
	Linear growth (mm)		
Acetic acid (µl/L)	*F.solani*	*R. solani*	*S.rolfsii*
0	90.0 a[(1)]	90.0 a	90.0 a
2	20.3 b	12.0 b	22.0 b
4	10.0 c	0.0 b	8.0 c
6	0.0 d	0.0 b	0.0 c

1- Figures with the same letter are not significantly different (P=0.05).

Effect of acetic acid fumigation on resting stage germination of tomato root rot fungi:

Acetic acid vapours at four concentrations *i.e*. 0.0, 5, 10, 15 and 20 µl/L against chlamedospores of *F.solani* and sclerotia germination of *R. solani* and *S. rolfsii* were tested. Results in Table (2) illustrate that all tested concentrations of acetic acid vapours significantly reduced germination of resting stages of all tested fungi. Complete inhibition of chlamedospores of *F.solani* was obtained by AA vapour at 15 µl/L Sclerotia of *S.rolfsii* showed more resistance to acetic acid vapours as their germination was completely inhibited at 20 µl/L of acetic acid vapour.

Table 2: Resting stage germination(%) of tomato root rot fungi as affected with different concentrations of acetic acid vapours.

Acetic acid (µl/L)	Chlamedospores	Sclerotia	
	F. solani	*R. solani*	*S. rolfsii*
0.0	90.0 a[(1)]	85.0 a	88.0 a
5.0	18.0 b	38.0 b	42.0 b
10	8.0 c	14.0 c	22.0 c
15	0.0 d	0.0	11.0 d
20	0.0 d	0.0	0.0 e

1- Figures with the same letter are not significantly different (P=0.05).

Effect of acetic acid vapours on total count of tomato root rot fungi:

Artificially infested soils were fumigated with acetic acid vapours at concentrations 0.00, 12.5, 25, 50 and 100 µl l^{-1} in air for 60 min in fumigation chamber. Results in Tables (3) indicate that, soil fungi response differently to acetic acid vapours. Complete reduction in total count of pathogenic fungi was obtained with AA at 50 µl/L for *F. solani* & *R. solani* and at 100 µl/L for *S. rolfsii* at µl/L. The highest reduction was achieved with AA at 25 µl/L which reduced the population by 74.0, 84.0 and 53.0 % for *F. solani*, *R. solani* and *S. rolsii* respectively.
Meanwhile, AA at 12.5 µl/L was less effective.

Table 3: Reduction in tomato root rot fungi population fumigated with acetic acid vapours.

Acetic acid (µl/L)	Reduction in tomato root rot fungi populations		
	F. solani	*R. solani*	*S. rolfsii*
12.5	65.0	77.0	42.0
25	74.0	84.0	53.0
50	100.0	100.0	85.0
100	100.0	100.0	100.0

Incidence of root rot disease in tomato plants:

Results in Table (4) indicate that all tested concentrations significantly reduced the percentage of root rot incidence of tomato plants. As the concentration increases the effect increases too. The most effective concentration of acetic acid vapour was 100 µl/L which reduced the disease incidence by 92.9, 94.2 and 93.9 % for *F. solani*, *R. solani* and *S. rolsii* respectively. Acetic acid vapour at 50 µl/L reduced the disease incidence more than 78.6 % for all tested fungi. Meanwhile, AA at 12.5 µl/L showed moderate effect.

Table 4: Percent of tomato root rot disease as affected with different concentrations of acetic acid vapours.

Acetic acid (µl/L)	Tomato root rot incidence %					
	F. solani		*R. solani*		*S.rolfsii*	
	Disease incidence	Reduction %	Disease incidence	Reduction %	Disease incidence	Reduction %
0.00	38.0 a[(1)]	—	52.0	—	66.0 a	—
12.5	22.0 b	42.0	20.0 b	61.5	47.0 b	40.4
25	15.0.0 c	60.5	12.0 c	76.9	33.0 c	50.0
50	9.0 c	78.6	8.0 d	84.6	12.0 d	81.8
100	3.0 d	92.9	3.0 e	94.2	4.0 e	93.9

1- Figures with the same letter are not significantly different (P=0.05).

Discussion:

Tomato plants is the one of most important vegetable crops in Egypt and other countries. Root rot disease caused by *Rhizoctonia solani* Kuhu.; *Fusarium solani* (Mart) Sacc. and *Sclerotium rolfsii* Sacc. is the most destructive disease of tomato [912,11]. Controlling such diseases mainly depend on fungicides treatments [19].

Soil treatment with specific chemical for reducing pathogen inoculum and disease incidence was first tried in the nineteen century

Munnecke and Van Gundy, [17]. Methyl bromide, chloropicrin, meta sodium, dizomet and dichloropropene are the most effective fumigants of soil for controlling soil-borne diseases.

These compounds are very expensive for use in large field conditions, in spite of their hazards effect to the environment and human health [17]. Alternatives, sheep, less toxic, more efficient of these compounds are needed [1,2].

Acetic acid vapours were extremely effective for controlling postharvest decay of several fruits, [20,21,14,15]. In the present study results revealed that complete inhibition in linear growth was obtained with AA at 6 µl/L for all tested fungi. The most sensitive fungi to acetic acid vapours are *F. solani* and *R. solani* which inhibited at 4 µl/L, while *S. rolfsii* were more resistant to acetic acid vapours at 6µl/l caused the complete inhibition of linear growth. On the other hand, acetic acidvapour at 20 µl/L caused complete inhibition of resting stages, complete inhibition of chlamedospores of *F.solani* was obtained by AA vapour at 15 µl/L while, Sclerotia of *S.rolfsii* showed more resistance to acetic acid vapours as their germination was completely inhibited at 20 µl/L of acetic acid vapour.

In this regard, killing of postharvest and common storage fungi with acetic acid vapours were reported by Sholberg and Gaunce [20]. In the present study , under greenhouse conditions results indicated that complete reduction in total count of pathogenic fungi was obtained with AA at 50 µl/L for *F. solani* & *R. solani* and at 100 µl/L for *S. rolfsii*. The highest reduction was achieved with AA at 25 µl/L which reduced the population by 74.0, 84.0 and 53.0 % for *F. solani, R. solani* and *S. rolsii* respectively. All tested concentrations of AA significantly reduced the percentage of root rot incidence of tomato plants. The most effective concentration of acetic acid vapour was 100 µl/L which reduced the disease incidence by 92.9, 94.2 and 93.9 % for *F. solani, R. solani* and *S. rolsii* respectively. These results open new area for the use of acetic acid in soil sterilization, avoiding other deleterious, dangerous effects of other fumigants. Acetic acid vapours is excellent biocide [20]. There are several advantages of using acetic acid vapours, it is a natural compound, had no residual hazard at low levels required to kill fungi, it is also generally-regarded-as-safe compound and it is inexpensive [24]. The inhibitory effect of acetic acid vapours on microorganisms is greater than that due to pH alone, and undissociated acetic acid as it can penetrate the microbial cell to exert its toxic effect [8]. The mechanisms of acetic acid inhibition of microorganisms is apparently to affect the cell membrane interfering with the transport of metabolites and maintenance of membrane potential [24].

On the other hand, there are several advantages of using acetic acid fumigation to control plant diseases: It is a natural compound found throughout the biosphere posing little or no residual hazard at low levels required to kill fungal spores; It is also generally - regarded - as - safe compound in the United States and does not require rigorous registration procedures; it is inexpensive, Sholberg *et al.,* [24]. It could be suggested that acetic acid vapours might be safely used commercially for controlling cucumber root rot disease under greenhouse conditions.

REFERENCES

1. Abdallah, M.A., N.S. El-Mougy, M.M. Abd-El-Kader, F. Abd-El-Karem, N.G. El-Gamal and R.S. El-Mohamedy, 2013. Aldehydes compounds for controlling black scurf disease of potato (*Solanum Tubrosum* L.) under field conditions. International Journal of Agriculture and Forestry, 3(2): 212- 224.
2. Abd-El-Kader, M.M., F. Abd-El-Karem, N.S. El-Mougy and R.S. El-Mohamedy, 2013. Integration between Compost, *Trichoderma harzianum* and Essential Oils for Controlling Peanut Crown Rot under Field Conditions. Journal of Mycology, 2013, Article ID 262130, 7 pages.
3. Abd-El-Kareem, F., 2001. Fumigation of table grapes with acetic acid vapors for controlling gray mould decay. *Egypt. J. Phytopathol.,* 29(1): 89-98.
4. Abd-El-Kareem, F., El-Mougy, S. Nehal, El-Gamal, G. Nadia and Y.O. Fotouh, 2006. Use of chitin and chitosan against tomato root rot disease under greenhouse conditions. *Research J. of Agricultural and Biological Science,* 2(4): 164- 169.
5. Abd-El-Kareem, F., 2009. Effect of acetic acid fumigation on soil-borne fungi and cucumber root rot disease under greenhouse conditions. *Archives of Phytopathology and Plant Protection,* 42: 213-220.
6. Abd-El-Kareem, F., N.S. El-Mougy and M.M. Abd-El-Kader, 2013. Application of compost and bio-agents as integrated soil treatment for controlling peanut crown rot disease under field conditions. Advances in Agriculture, Sciences and Engineering Research, 3(5): 858-866.

7. Al-Mahareeq, F.A.A., 2005. Biological control of Rhizoctonia solani and Sclerotium rolfsii by using local isolates of Trichoderma spp. M. Sc., Thesis, Fac. Graduate Studies, An-Najah National Univ., Nablus, Palestine, 93 pp.
8. Banwart, G.J., 1981. Basic Food microbiology-AVI westport conn. (c.f. Sholberg and Gaunce 1995).
9. Benhamou, N., P.J. Lafontaine and M. Nicole, 1994. Seed treatment with chitosan induces systemic resistance to Fusarium crown and root rot in tomato plants. *Phytopathology*, 84: 1432-1444.
10. Edmunds, B.A. and M.L. Gleason, 2003. Perennation of *Sclerotium rolfsii* var. *delphinii* in Iowa. Online. Plant Health Progress doi:10.1094/PHP-2003-1201-01-RS.
11. Elad, Y. and R. Baker, 1985. Influence of trace amount of cations and siderophore- producing Pseudomonads on Chlamydospores of *Fusarium oxysporum. Phytopathgology*, 75: 1047-1052.
12. El-Mougy, N.S., 1995. "Studies on wilt and root rot diseases of tomato in Egypt and their control by modern methods. M. Sc. Thesis, Faculty of Agriculture, Cairo University, Egypt.
13. El Mougy, N.S., N.G. El-Gamal, Y.O. Fotouh and F. Abd-El-Kareem, 2006. Evaluation of different application methods of chitin and chitosan for controlling tomato root rot disease under greenhouse and field conditions. Research J. of Agricultural and Biological Science, 2(4): 207-212.
14. Morsy, A.A., F. Abd-El-Kareem and M.A. Abd-Alla, 1999. Effect of acetic acid on postharvest decay of strawberry fruits. *Egypt. J. Phytopathol.*, 27: 117-126.
15. Morsy, A.A., F. Abd-El-Kareem and M.A. Abd-Alla, 2000 a. Effect of acetic acid fumigation common storage fungi of some grains. *Egypt J. phytopathol.*, 28: 95-106.
16. Morsy, A.A., M.A. Abd-Alla and F. Abd-El-Kareem, 2000b. Effect of acetic acid fumigation of wheat grains on fungal infection during storage. *Egypt. J. Phytopathol.*, 28: 107-117.
17. Munnecke, D.E. and S.D. Van Gundy, 1979. Movement of fumigants in soil. Dosage and differential effects. *Annual Review of Phytopathol*., 17: 405-429.
18. Neler, J., W. Wasserman and M.H. Kutner, 1985. *Applied linear statistical models*. Regression analysis of variance and experimental design: 2nd Ed. Richard, D. Irwin Inc. Home wood. Illinois.
19. Rauf, B.A., 2000. Seed-borne disease problems of legume crops in Pakistan. *Pakistan Journal of Scientific and Industrial Research*, 43: 249-254.
20. Sholberg, P.L. and A.P. Gaunce, 1995. Fumigation of fruit with acetic acid to prevent postharvest decay. *Hort Science*, 30: 1271-1275.
21. Sholberg, P.L. and A.P. Gaunce, 1996a. Fumigation of high moisture seed with acetic acid to control storage mold. *Canadian J. of Plant Science*, 76: 551-555.
22. Sholberg, P.L. and A.P. Gaunce, 1996b. Fumigation of stone fruit with acetic acid to control postharvest decay. *Crop. Prot*., 15: 681-686.
23. Sholberg, P.L., A.G. Regnolds and A.P. Gaunce, 1996. Fumigation of table grapes with acetic acid to prevent postharvest decay. *Plant Dis*. 80: 1425-1428.
24. Sholberg, P.L. P.J. Delaquis and A.L. Moyls, 1998. Use of acetic acid fumigation to reduce the potential for decay in harvest crops. Recent Res. Devel. In Plant Pathology, 2: 31-41.