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Postharvest application of acetic acid vapours and chitosan solution for controlling gray and blue moulds of apple fruits

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ABSTRACT

Efficiency of acetic acid vapours either alone, or in combination with chitosan coating for the controlling gray and blue moulds of apple fruits during storage was investigated . In vitro test results indicated that four concentration of acetic acid vapours i.e. 00, 2.0, 4.0 ,6.0 and 8.0 µl/L were tested against linear growth and spore germination of Botrytis cinerea and Penicillium sp. Results revealed that all concentrations significantly reduced the linear growth and spore germination of B. cinerea and Penicillium sp. Complete reduction in linear growth and spore germination of B. cinerea and Penicillium sp. was obtained with acetic acid vapours at 8.0 and 6.0 µl/L respectively. Complete reduction in linear growth and spore germination of B. cinerea and Penicillium sp. was obtained with chitosan solution at 6.0 and 5.0 g / L respectively. The highest reduction was obtained with chitosan solution at 5.0 and 4.0 g / L which reduced the linear growth and spore germination of both fungi more than 77.8 and 74.2 % respectively. Moreover, In vivo, test, all concentrations of acetic acid vapours significantly reduced the gray and blue moulds as disease incidence or rotted part tissues of apple fruits. The highest reduction was obtained with acetic acid vapour at 6.0 and 8.0 µl/L which reduced the both diseases and rotted part tissues more than 84.0 and 86.0 % respectively. Also, all concentrations of chitosan solutions significantly reduced the gray and blue moulds as disease incidence or rotted part tissues of apple fruits. The highest reduction was obtained with chitosan solutions at 5.0 and 6.0 g /L which reduced the both diseases and rotted part tissues more than 84.0 %. Acetic acid vapour and chitosan solutions alone or in combination significantly reduced the gray and blue moulds as disease incidence or rotted part tissues of apple fruits. The most effective treatment was combined treatment between acetic acid vapour at 8.0 µl/L followed by chitosan solutions at 5.0 or 6.0 g/ L which reduced the both diseases and rotted part tissues more than 84.0 and 87.0 % respectively.

Key words: Acetic acid vapours, Chitosan coating, Gray mould, Blue mould, Apple fruits

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INTRODUCTION

Worldwide gray mould caused by *Botrytis cinerea* Pers; Fr. and blue mould caused by *Penicillium* sp. are the most important diseases attacking apple fruit during storage [1], [2], [3], [4]. Using of chemical fungicides gave satisfactory control against mould infection , but have residual harmful effect to human and environment [5]. Moreover, successive use of fungicides could lead to develop some significant fungal isolates resistant to used fungicides. Therefore, alternative fungicide treatments are needed for the management of postharvest diseases of fruits [6], [7]. Acetic acid (AA) is a universal metabolic intermediary and occurs in plants and animals [8]. It was commonly used by food manufactures as antimicrobial preservative or acidulates in a variety of food products [9]. Vapours of acetic acid were extremely effective for killing spores of postharvest fungi which cause diseases to various fruits [10], [11]. Fumigation with acetic acid vapours prevented postharvest decay of apple , kiwifruit , pear ,

tomato , citrus and stone fruits [12], [13], [14], [10], [15]. [11] reported that, acetic acid vapours cussed complete inhibition of linear growth of *Botrytis cinerea* and reduced gray mould incidence of table grapes more than 84.6 as compared with control fruits. Chitosan is theoretically be used as a preservative for coating fruit. The coating is non-toxic and safe [16], and exhibits antifungal activity against several fungi [17], [18], [19], [20], [21]. A chitosan coating is known to have the potential to prolong the storage life and control the decay of strawberries, tomatoes, peaches, pears, kiwifruit, litchi, apples, longan and citrus fruits, as examined by [22], [23], [24], [25],[26], [27], [28]. The aim of this study was to evaluate the efficacy of acetic acid vapours either alone, or in combination with chitosan coating for the control of gray and blue moulds of apple fruits during storage .

EXPERIMENTAL SECTION

Source of pathogenic fungi and apple fruits

Pathogenic isolates of *Botrytis cinerea* and *Penicillium* sp the causal agent of gray and blue mould diseases were kindly obtained from Plant Pathology Dept., National Research Centre , Giza , Egypt. Meanwhile, apple fruits cv. Anna were obtained from commercial market in Egypt.

Fumigation: Acetic acid fumigation (v/v in air) was carried out in fumigation chamber according to the methods which were described by [12].

Testing of acetic acid fumigation on linear growth of pathogenic fungi

Four concentration of acetic acid vapours *i.e.* 00, 2.0, 4.0 ,6.0 and 8.0 µl/l were tested against linear growth of *Botrytis cinerea* and *Penicillium* sp.

Disks (6-mm-diameter) of 10 days old cultures of fungi were fumigated with acetic acid vapours at previous concentrations for 30 min in fumigation chamber, then transferred to plates containing PDA medium. Linear growth of fungi was measured when the control plates reached full growth and the average diameter was calculated. five replicates were used for each treatment.

Testing of acetic acid fumigation on spore germination of pathogenic fungi

Drops of spore suspension of *B. cinerea* and *Penicillium* sp. were placed on PDA medium at six equidistant points on Petri-plates containing 10 ml of medium. Inoculated plates were uncovered and fumigated with acetic acid vapours at 00, 2.0, 4.0, 6.0 and 8.0 µl/l for 30 min in fumigation chamber. Fumigated plates were covered and incubated for 24h at 25 ˚C. Percent germination of spores was determined by counting 100 spores five times in each drop microscopically [12].

Evaluation of the inhibitory effect of chitosan on the linear growth of and spore germination of pathogenic fungi

The inhibition by chitosan toward the linear mycelial growth of *B. cinerea* and *Penicillium* sp. were determined on potato dextrose agar (PDA) at 25 ± 2°C according to [29]. The prepared PDA medium was dispersed in 100 ml quantities into 250 ml Erlenmyer flasks and sterilized by autoclaving at 121°C for 15 min. Chitosan were prepared as described previously and then added to PDA medium before its solidification to obtain the final concentrations of 0, 2, 4 and 6 g/L (w/v) and mixed gently with 0.1% Tween 80 (Sigma) to enhance solubility. Each flask was then disbanded in sterilized Petri- plates (9- cm diameter) before its solidification. Plates were individually inoculated with equal disks (6- mm diameter) taken from 7-days old cultures of each *B. cinerea* and *Penicillium* sp., then incubated at 25 ± 2°C. Linear mycelial growth of fungus was measured, when the control plates reached full growth and the average growth diameter was calculated. Each treatment was represented by 5 plates as replicates.

Meanwhile, the inhibition by chitosan toward the spores germination of both *B.cinerea* and *Penicillium* sp were determined on potato dextrose broth (PDB) at 25 ± 2°C according to [29]. The prepared potato dextrose broth (PDB) was dispersed in 5 ml quantities into 10 ml test tube and sterilized by autoclaving at 121°C for 15 min. Chitosan were prepared as described previously and then added to PDB to obtain the concentrations of 0, 2, 4 and 6 g/L (w/v) and mixed gently with 0.1% Tween 80 (Sigma) to enhance solubility. Each tube was then inoculated with 1.0 ml of the spore suspension at a concentration of 10^6 spores /ml. Inoculated test tubes were incubated at25 ± 2°C for 20 hours on rotary shaker (200 rpm). Germinated spores were examined microscopically to determine the germination rate [30]**.** Experiment was represented by one handred spores per replicate and five replicates per treatment were used.

In vivo: **Testing of different concentrations of acetic acid vapours on gray and blue mould diseases of apple fruits.**

Different concentrations of acetic acid vapours *i.e* 0 , 2 , 4, 6 and 8 µl/L were tested to study their effect against gray and blue mould diseases of apple fruits. Fresh apple fruits (c.v. Anna) apparently free from physical damage and diseases were artificially wounded using sterilized scalpel. Fruits were fumigated with acetic acid vapours at previous concentrations for 30 min in fumigation chamber . Inoculation of treated fruits was carried out by spraying fruits with spore suspension (10^6 spores/ml) of *B. cinerea* and *Penicillium* sp then air dried . All treated or un-treated fruits were placed into carton boxes at the rate of 10 fruits/box. Each particular concentration as well as control treatment was represented by three carton box. All boxes were stored at 20±2C° for 15 days. Percentage of infected fruits (disease incidence) and rotted part tissues of fruits (disease severity) were recorded after 15 days.

In vivo: **Testing of different concentrations of chitosan solutions on gray and blue mould diseases of apple fruits.**

Chitosan solutions at different concentrations *i.e.* 0.0,1.0, 2.0 3.0 ,4.0, 5.0 and 6.0 g / L were tested to study their effect against gray and blue moulds incidence on apple fruits. Fresh apple fruits apparently free from physical damage and diseases were artificially wounded using sterilized scalpel. Fruits were dipped in chitosan solutions previous concentrations for 3 min, then air dried . Inoculation of treated fruits was carried out by spraying fruits with spore suspension (10^6 spores/ml) of *B. cinerea* and *Penicillium* sp. then air dried . All treated or un-treated fruits were placed into carton boxes at the rate of 10 fruits/box. Each particular concentration as well as control treatment was represented by three carton box. All boxes were stored at 20±2C° for 20 days. Percentage of infected fruits (disease incidence) and rotted parts of fruits (disease severity) were recorded after 20 days.

$$\% \text{ of rotted part of fruit} = \frac{\text{Rotted part weight}}{\text{fruit weight}} \times 100$$

***In vivo*: Evaluation of acetic acid vapours and chitosan solutions alone or in combination on gray and blue mould diseases of apple fruits.**

Acetic acid vapours at 8.0 µl/L and chitosan solutions at 5.0 or 6.0 g/ L were applied alone or in combination for controlling gray and blue mould diseases of apple fruits. . Fresh apple fruits apparently free from physical damage and diseases were artificially wounded using sterilized scalpel. Fruits were fumigated with acetic acid vapours at 8.0 µl/L for 30 min in fumigation chamber then were dipped in chitosan solutions 5.0 or 6.0 g/ L for 3 min, then air dried . Inoculation of treated fruits was carried out by spraying fruits with spore suspension (10^6 spores/ml) of *B. cinerea* and *Penicillium* sp. then air dried . All treated or un-treated fruits were placed into carton boxes at the rate of 10 fruits/box. Each particular concentration as well as control treatment was represented by three carton box. All boxes were stored at 20±2C° for 20 days. Percentage of infected fruits (disease incidence) and rotted parts of fruits (disease severity) were recorded after 20 days.

Statistical analysis **:**

Tukey test for multiple comparison among means was utilized [31].

RESULTS

***In vitro*: Effect of acetic acid vapours on linear growth and spore germination of pathogenic fungi**

Four concentration of acetic acid vapours *i.e.* 00, 2.0, 4.0 ,6.0 and 8.0 µl/L were tested against linear growth and spore germination of *Botrytis cinerea* and *Penicillium* sp. Results in Table (1and 2) reveal that all concentrations significantly reduced the linear growth and spore germination of *B. cinerea* and *Penicillium* sp. Complete reduction in linear growth and spore germination of *B. cinerea* and *Penicillium* sp. was obtained with acetic acid vapours at 8.0 and 6.0 µl/L respectively. The highest reduction was obtained with acetic acid vapour at 6.0 and 4.0 µl/L which reduced the linear growth and spore germination of both fungi more than 76.7 and 74.8 % respectively. Meanwhile, other concentrations were less effective.

Table (1) Linear growth (mm) of *B. cinerea* and *Penicillium* sp. as affected with different concentrations of acetic acid vapours

Acetic acid vapours (µl/l)	*B. cinerea*		*Penicillium* sp.	
	Linear growth (mm)	Reduction %	Linear growth (mm)	Reduction %
0.0	90.0 a	00.0	90.0 a	00.0
0.2	44.0 b	51.1	46.0 b	48.9
0.4	31.5 c	65.0	32.0 c	64.4
0.6	18.0 d	80.0	21.0 d	76.7
0.8	00.0 e	100.0	00.0 e	100.0

Figures with the same litter are not significantly different (P =0.05)

Table (2) Average percent of spore germination of pathogenic fungi as affected with different concentrations of acetic acid vapours

Acetic acid vapours (µl/l)	*B. cinerea*		*Penicillium* sp.	
	Spore germination	Reduction %	Spore germination	Reduction %
0.0	94.0 a	00.0	93.0 a	00.0
0.2	42.0 b	53.3	44.0 b	52.7
0.4	22.0 c	76.6	23.4 c	74.8
0.6	00.0 d	100.0	00.0 d	100.0
0.8	00.0 d	100.0	00.0 d	100.0

Figures with the same litter are not significantly different (P =0.05)

In vitro*: *Evaluation of the inhibitory effect of chitosan on the linear growth and spore germination of pathogenic fungi

The inhibition by chitosan toward the linear mycelial growth and spore germination of *B. cinerea* and *Penicillium* sp. were determined. Results in Table (3 and 4) indicate that all concentrations significantly reduced the linear growth and spore germination of *B. cinerea* and *Penicillium* sp. Complete reduction in linear growth and spore germination of *B. cinerea* and *Penicillium* sp. was obtained with chitosan solution at 6.0 and 5.0 g / L respectively. The highest reduction was obtained with chitosan solution at 5.0 and 4.0 g / L which reduced the linear growth and spore germination of both fungi more than 77.8 and 74.2 % respectively. Meanwhile, other concentrations were less effective.

Table (3) Linear growth (mm) of *B. cinerea* and *Penicillium* sp. as affected with different concentrations of chitosan solutions

Chitosan (g/L)	*B. cinerea*		*Penicillium* sp.	
	Linear growth (mm)	Reduction %	Linear growth (mm)	Reduction %
0.0	90.0 a	00.0	90.0 a	00.0
1.0	55.0 b	38.9	56.0 b	37.8
2.0	41.0 c	54.4	47.0 c	47.8
3.0	33.0 d	63.3	35.4 d	60.6
4.0	22.0 e	75.6	23.4 e	73.9
5.0	17.0 e	81.1	20.0 e	77.8
6.0	00.0 f	100.0	00.0 f	100.0

Figures with the same litter are not significantly different (P =0.05)

Table (4) Average percent of spore germination of pathogenic fungi as affected with different concentrations of chitosan solutions

Chitosan (g/L)	*B. cinerea*		*Penicillium* sp.	
	Spore germination	Reduction %	Spore germination	Reduction %
0.0	94.0 a	00.0	93.0 a	00.0
1.0	51.0 b	45.7	53.0 b	43.0
2.0	41.0 c	56.4	42.3 c	54.5
3.0	32.0 d	66.0	33.0 d	64.6
4.0	22.0 e	76.6	24.0 d	74.2
5.0	00.0 f	100.0	00.0 e	100.0
6.0	00.0 f	100.0	00.0 e	100.0

Figures with the same litter are not significantly different (P =0.05)

***In vivo:* Effect of different concentrations of acetic acid vapours on gray and blue mould diseases of apple fruits.**

Different concentrations of acetic acid vapours *i.e* 0 , 2 , 4, 6 and 8 µl/L were tested to study their effect against gray and blue mould diseases of apple fruits. Results in Table (5 and 6) indicate that all concentrations of acetic acid vapours significantly reduced the gray and blue moulds as disease incidence or rotted part tissues of apple fruits. The highest reduction was obtained with acetic acid vapour at 6.0 and 8.0 µl/L which reduced the both diseases and rotted part tissues more than 84.0 and 86.0 % respectively. Acid vapour at 6.0 µl/L reduced the both diseases and rotted part tissues more than 75.0 and 80.0 % respectively. Meanwhile, AA at concentration of 6.0 µl/L showed moderate effect.

Table (5) Percent of gray and blue moulds incidence on apple fruits as affected with different concentrations of acetic acid vapours

Acetic acid vapours (µl/l)	Disease incidence %			
	Gray mould	Reduction %	Blue mould	Reduction %
0.0	100.0 a	00.0	100.0 a	00.0
0.2	42.0 b	58.0	44.0 b	66.0
0.4	34.7 c	65.3	37.5 c	62.5
0.6	23.0 d	77.0	25.0 d	75.0
0.8	14.4 e	85.6	16.0 e	84.0

Figures with the same litter are not significantly different (P =0.05)

Table (6) Percent of rotted part tissues caused by gray and blue moulds on apple fruits as affected with different concentrations of of acetic acid vapours

Acetic acid vapours (µl/l)	Rotted part tissues %			
	Gray mould	Reduction %	Blue mould	Reduction %
0.0	100.0 a	00.0	100.0 a	00.0
0.2	39.0 b	61.0	41.0 b	59.0
0.4	31.0 c	69.0	31.0 c	69.0
0.6	19.0 d	81.0	20.0 d	80.0
0.8	12.0 e	88.0	14.0 e	86.0

Figures with the same litter are not significantly different (P =0.05)

***In vivo:* Effect of different concentrations of chitosan solutions on gray and blue mould diseases of apple fruits.**

Chitosan solutions at different concentrations *i.e.* 0.0,1.0, 2.0 3.0 ,4.0, 5.0 and 6.0 g / L were tested to study their effect against gray and blue moulds incidence on apple fruits. Results in Table (7 and 8) reveal that that all concentrations of chitosan solutions significantly reduced the gray and blue moulds as disease incidence or rotted part tissues of apple fruits. The highest reduction was obtained with chitosan solutions at 5.0 and 6.0 g /L which reduced the both diseases and rotted part tissues more than 84.0 % . Chitosan solutions at 4.0 gl/L reduced the both diseases and rotted part tissues more than 74.0 and 76.0 % respectively. Meanwhile, chitosan solutions at concentration of 3.0 g / L showed moderate effect.

Table (7) Percent of gray and blue moulds incidence on apple fruits as affected with different concentrations of chitosan solutions

Chitosan (g/L)	Disease incidence %			
	Gray mould	Reduction %	Blue mould	Reduction %
0.0	100.0 a	000.0	100.0 a	00.0
1.0	51.0 b	49.0	52.0 b	48.0
2.0	41.2 c	58.8	41.0 c	59.0
3.0	33.0 d	67.0	34.0 d	66.0
4.0	26.0 e	74.0	25.0 e	75.0
5.0	15.0 f	85.0	16.0 f	84.0
6.0	14.5 f	85.5	15.0 f	85.0

Figures with the same litter are not significantly different (P =0.05)

Table (8) Percent of rotted part tissues caused by gray and blue moulds on apple fruits as affected with different concentrations of chitosan solutions

Chitosan (g/L)	Rotted part tissues %			
	Gray mould	Reduction %	Blue mould	Reduction %
0.0	100.0 a	00.0	100.0 a	00.0
1.0	48.0 b	52.0	50.0 b	50.0
2.0	40.0 c	60.0	40.0 c	60.0
3.0	30.0 d	70.0	31.0 d	69.0
4.0	22.0 e	78.0	24.0 e	76.0
5.0	14.0 f	86.0	16.0 f	84.0
6.0	14.0 f	86.0	16.0 f	84.0

Figures with the same litter are not significantly different (P =0.05)

In vivo: Evaluation of acetic acid vapours and chitosan solutions alone or in combination on gray and blue mould diseases of apple fruits.

Acetic acid vapours at 8.0 µl/L and chitosan solutions at 5.0 or 6.0 g/ L were applied alone or in combination for controlling gray and blue mould diseases of apple fruits. Results in table (9 and 10) indicate that acetic acid vapours and chitosan solutions alone or in combination significantly reduced the gray and blue moulds as disease incidence or rotted part tissues of apple fruits. The most effective treatment was combined treatment between acetic acid vapour at 8.0 µl/L followed by chitosan solutions at 5.0 or 6.0 g/ L which reduced the both diseases and rotted part tissues more than 84.0 and 87.0 % respectively.

Table (9) Percent of gray and blue moulds incidence on apple fruits as affected with acetic acid vapours and chitosan solutions alone or in combination

Application	Disease incidence %			
	Gray mould	Reduction %	Blue mould	Reduction %
Single treatment				
AA at 8(µl/L)	13.0 b	87.0	15.0 b	85.0
Chitosan at 5 g / L	14.0 b	86.0	16.0 b	84.0
Chitosan at 6 g / L	14.0 b	86.0	15.0 b	85.0
Combined treatment				
AA + Chitosan at 5 g /L	6.0 c	94.0	8.0 c	92.0
AA + Chitosan at 6 g /L	6.0 c	94.0	7.0 c	93.0
Control	100.0 a	00.0	100.0 a	00.0

Figures with the same litter are not significantly different (P =0.05)

Table (10) Percent of rotted part tissues caused by gray and blue moulds on apple fruits as affected with acetic acid vapours and chitosan solutions alone or in combination

Application	**Rotted part tissues %**			
	Gray mould	Reduction %	Blue mould	Reduction %
Single treatment				
AA at 8(µl/L)	11.0 b	89.0	12.0 b	88.0
Chitosan at 5 g / L	12.0 b	88.0	13.0 b	87.0
Chitosan at 6 g / L	12.0 b	88.0	12.5 b	87.5
Combined treatment				
AA + Chitosan at 5 g /L	5.0 c	95.0	5.5 c	94.5
AA + Chitosan at 6 g /L	5.0 c	95.0	6.0 c	94.0
Control	100.0 a	00.0	100.0 a	00.0

Figures with the same litter are not significantly different (P =0.05)

DISCUSSION

Worldwide gray mould caused by *Botrytis cinerea* Pers; Fr. and blue mould caused by *Penicillium* sp are the most important diseases attacking apple fruit during storage [1], [2], [3]. Acetic acid (AA) was commonly used by food manufactures as antimicrobial preservative or acidulates in a variety of food products [9]. In present study acetic acid vapour at 6.0 and 5.0 µl/L completely inhibited growth and spore germination of *Botrytis cinerea* and *Penicillium* sp. In this respect, the inhibitory effect of acetic acid vapours on microorganisms is greater than due to

pH alone and it can penetrate the microbial cell to exert its toxic effect [32]. The mechanisms of acetic acid inhibition to microorganisms apparently that it may affect the cell membrane interfering with the transport of metabolites and maintenance of membrane potential [9]. Vapours of acetic acid were extremely effective for killing spores of postharvest fungi which cause diseases to various fruits [10], [111].

Fumigation with acetic acid vapours prevented postharvest decay of apple , kiwifruit , pear , tomato , citrus and stone fruits [12], [13], [14], [10], [15]. In the present study results revealed that the highest reduction of gray and blue mould incidence was obtained with AA at 8.0 µl/L . In this regard, [11] reported that, acetic acid vapours cussed complete inhibition of linear growth of *Botrytis cinerea* and reduced gray mould incidence of table grapes more than 84.6 as compared with control fruits. Acetic acid vapours at low concentrations, as that used, has many qualities that make then an excellent biocide: first it kills spores, second it does not injure the fumigated fruits surface third, it is effective at low temperatures which means that fruit in 1C$^{\circ}$ cold storage could be effectively treated with acetic acid vapours. Forth, it is not flammable at the low concentrations that are required to kill fungal spores [12].

There are several advantages of using acetic acid fumigation to control postharvest 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, and it can be used to treat products in airtight storage rooms or containers without requiring handling of the products, [10]. Chitosan is theoretically be used as a preservative for coating fruit. The coating is non-toxic and safe [16], and exhibits antifungal activity against several fungi [17], [18]. In the present study results revealed that complete reduction in linear growth and spore germination of *B. cinerea* and *Penicillium* sp. was obtained with chitosan solution at 6.0 and 5.0 g / L respectively. There are several hypotheses have been postulated by which chitosan affects the growth of pathogenic fungi [33]**,** first: by its polycationic nature, it is believed that chitosan interferes with negatively charged residues of macromolecules exposed on the fungal cell surface. This interaction leads to the leakage of intracellular electrolytes and proteinaceous constituents. Second the interaction of diffused hydrolysis products with microbial DNA, which leads to the inhibition of mRNA and protein synthesis, third : the interaction of chitosan with fungal DNA and RNA. Fourth : malformation of fungal mycelial. Moreover, chitosan coating is known to have the potential to prolong the storage life and control the decay of strawberries, tomatoes, peaches, pears, kiwifruit, litchi, apples, longan and citrus fruits, as examined by [22], [23], [24], [25],[26], [27], [28].

In the present study, results indicated that that all concentrations of chitosan solutions significantly reduced the gray and blue moulds as disease incidence or rotted part tissues of apple fruits. The highest reduction was obtained with chitosan solutions at 5.0 and 6.0 g /L which reduced the both diseases and rotted part tissues more than 84.0 %.However, in the present study, protective effect of acetic acid vapours were evaluated alone or in combination with chitosan coating. Results revealed that acetic acid vapours and chitosan solutions alone or in combination significantly reduced the gray and blue moulds as disease incidence or rotted part tissues of apple fruits. The most effective treatment was combined treatment between acetic acid vapour at 8.0 µl/L followed by chitosan solutions at 5.0 or 6.0 g/ L which reduced the both diseases and rotted part tissues more than 84.0 and 87.0 % respectively. This results due to acetic acid vapour at 8.0 µl/L completely inhibited linear growth and spore germination of pathogenic fungi followed by chitosan coating as protective material against any infection with postharvest diseases. It could be suggested from the present study that combination treatment between acid vapours and chitosan as fruit coating considered as one of applicable safely for controlling postharvest diseases of apple fruits.

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