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Bioactivity of certain monoterpenoids against potato soft-rot bacteria

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ABSTRACT

Evaluation the inhibitor activity of five monoterpenoides namely carvacrol, p-cymene, eugenol, isoeugenol and thymol against growth of potato soft rot pathogenic bacteria (*Pectobacterium carotovorum* subsp. *carotovorum*) studied in different concentrations under laboratory and field conditions. Carvacrol and thymol showed the strongest effect against *P. c.* subsp. *carotovorum* isolates. The growth of tested bacteria decreased significantly by increasing the concentrations of carvacrol and thymol. Eugenol+Thymol and carvacrol+thymol mixtures showed equal significance against *P. c*. subsp. *carotovorum* isolates. Experiments to enhance the antimicrobial efficacy by mixing with the four synergists: Triphenyl phosphate, Dimethyl Meleate, Piperonyl butoxide and Triton X-100 reached. Thymol+Triton X-100 in 1:2 a ratio showed the highest effect against Pcc160 isolate with MIC 125 µg/mL. Scanning electron microscope (SEM) showed distinct inhibitory effect of carvacrol, (eugenol+ thymol) mixture and (carvacrol + Triton X-100 in 1:1 (v/v) ratio) mixture on the growth and survival of *P.c.* subsp. *carotovorum* (Pcc 160). Under field conditions, tested monoterpenoides, their combinations and joint action effects of the most active monoterpenoids, carvacrol, eugenol and thymol with Triphenyl phosphate and Triton X100 had significant reduced the percentage of soft rot infection on potato plants. The combination eugenol with Triphenyl phosphate (TPP) was the most effective, where it caused a reduction of the relative disease control (RDC) with 90. 54 % in season1and 89% RDC in season 2.

Keywords: Monoterpenoids, soft rot, *Pectobacterium* sp., scanning electron microscopy and synergists.

INTRODUCTION

Scientists have worked to minimize the usage of synthetic pesticides to manage weeds, insects, acari, and plant infections over the past 20 years in an effort to lessen the risks of environmental pollution. In addition to killing the intended pathogen. pesticides can also destroy a number of helpful species. There is also reason for grave concern regarding the growing prevalence of disease resistance to synthetic pesticides (Heydari *et al.,* 2010; Kaur *et. al.,* 2024). To find novel physiologically active terpenoids that might be used as a source of agrochemicals, conduct a thorough search. Essential oils are mostly composed of monoterpenoids, sesquiterpenes, and diterpenes. Aldehydes and phenols, which constitute monoterpenoids, have been shown to have stronger antibacterial properties than alcohols, ketones, esters, and hydrocarbons. Lipophilicity, partition coefficient, and H-bonding parameters are among their physico-chemical characteristics that are closely linked to the mechanisms behind their antimicrobial actions (Marinelli *et al.,* 2018; Stephane *et al.,* 2020). According to Zielińska-Błajet *et al.* (2020), phenolic monoterpene components like carvacrol and thymol have demonstrated the greatest antibacterial activity in several *in vitro* experiments. Due to their functional hydroxyl group, which is why monoterpenoids are known to have agrochemical effects, they make an excellent basis for creating synthetic derivatives that may be utilised as agrochemicals (Kaur *et al.,* 2014).

Potatoes are a widely consumed food in Egypt. About 409535 Fadden are farmed for potato plants in several governorates, including Elbehera, Elsharkea, Ismailia, Giza, Benisouf, and New Valley. Plant diseases resulting from plant pathogens are complex processes involving multiple components. Nonetheless, it has been documented that the pathogen's peptic and cellulitic enzymes directly contribute to pathogenesis (Gaber *et al.,* 1990; Walker *et al.,* 1994). Among the most frequent plant infections that cause losses in agricultural crops, whether during planting or after harvest were fungi and bacteria. The expense of treating chronic illnesses has increased, particularly in recent years, thus it has become vital to look for safe, effective substitutes. The soft rot caused by *Erwinia carotovora* subsp. *carotovora* is the most significant and widespread bacterial disease that affects a wide range of plants in the field or during storage (Cetinkaya *et al.,* 2004). Since soft rot is a prevalent

and major potato problem, our work is just one of many attempts to identify safe, natural alternatives that are eco-friendly and biodegradable to manage a serious pest (bacteria) that is attacking crop plants.

MATERIALS & METHODS

Bacterial strains:

Three pathogenic *Pectobacterium* strains were used in this study, *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc160 (accession no. LN811442) and Pcc5K (accession no. LT592254)) (Ashmawy et al., 2019) and *Pectobacterium carotovorum* subsp. *carotovorum* Pcc accession no. OP565055 (Youssef and Selim 2024).

2. Bioassay tests:

2.1. Bactericidal effect of the tested monoterpenoids:

Five monoterpenoids namely carvacrol (98%), p-cymene (99%), eugenol (99%), isoeugenol (98%) and thymol (99%) were screened for their ability to inhibit the growth of 3 isolates of *Pectobacterium* sp. (Pcc160, Pcc5K and Pcc) at the concentrations 250, 500, 1000, 2000 and 4000 µg/mL. Testing effect of monoterpenoids on growth of the bacterial isolates was studied by determination of inhibition by agar disc diffusion method (NCCLS, 1997).

2.2. Inhibitory activities of selected monoterpenoids combinations against soft rot bacteria:

Three combinations of selected monoterpenoids ((carvacrol + thymol), (eugenol + carvacrol) and (eugenol + thymol)) were tested for their antibacterial activity toward *Pectobacterium* sp. (Pcc160, Pcc5K and Pcc) isolates at the concentrations 1000, 2000 and 4000 µg/mL. The bioassay of previous combinations was carried out by agar diffusion method (NCCLS, 1997).

2.3. Joint action effects of the most active monoterpenoids with Triphenyl phosphate, dimethyl meleate, piperonyl butoxide and Triton X100 against *Pectobacterium* sp:

The most active monoterpenoids, carvacrol, eugenol and thymol were mixed with triphenyl phosphate, dimethyl meleate, piperonyl butoxide and triton X-100 as synergist components in 1:1 and 1:2(v/v) for Joint action effects against *Pectobacterium* sp .(Pcc160, Pcc5K and Pcc) isolates at the concentrations 250, 1000 and 4000 µg/mL. The bioassay of previous combinations was carried out by agar diffusion method (NCCLS, 1997).

2.4. Bacteriostatic/bactericidal aspect after exposure to tested monoterpenoides, their combinations and joint action effects of the most active monoterpenoids with Triphenyl phosphate dimethyl meleate, piperonyl butoxide and Triton X100:

The effects of monoterpenoids, their combinations and the mixtures of selected monoterpenoids with synergist components were tested to determine if they were bacteriostatic (BS) or bactericidal (BC) in nature according to Shoeib and Alkufeidy (2014).

3. Determination of minimum inhibitory concentration (MIC):

Tested compounds were dissolved in dimethyl sulfoxide (DMSO). Appropriate volumes of the stock solution (100, 125, 150, 200, 250, 300, 500, 750,1000, 1500, 1750 and 2000µg/mL) recommended by European Society of Clinical Microbiology and infection Disease (ESCMID, 2000).

4. Scanning electron microscopy (SEM) examination:

4.1. Examination of *Pectobacterium* cells treated with tested monoterpenoides, their combinations and joint action effects of the most active monoterpenoids with Triton X100:

The best result obtained by carvacrol, (eugenol+thymol) mixture and(carvacrol+triton X100 in 1:1 a ratio) treatments resulted in highest bacterial growth inhibition was selected for further study by SEM examination. *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc160) was treated with previous treatments at a concentration 4000 µg/ml. The scanning electron microscopy analysis was according to Tahmasebi et al., (2015).

5. Field Experiments:

-Efficacy of tested monoterpenoides, their combinations and joint action effects of the most active monoterpenoids with Triphenyl phosphate and Triton X100 on Relative Disease Control (RDC) against potato soft rot disease:

Field experiments were carried out at the El Subhia research station in Alexandria during the successive growing seasons of 2022–2023, with the aim of examining the effectiveness of carvacrol, (eugenol+thymol), (eugenol+ triphenyl phosphate in 1:2 a ratio), (carvacrol+triton X100 in 1:1 a ratio), and (thymol+ triton X100 in 1:2 a ratio) for the control of soft rot disease in potatoes (*Solanum tuberosum*, L.). September 2022 saw the planting of potato tubers. The soil type of the field was sandy clay and Spunta was the variety trial, In a fully randomized block design, a split-plot design with four replications used. Six days before to inoculation, the five treatments—which had MIC concentrations of 200, 750, 200, 2000, and 125 µg/ml, respectively—were sprayed onto the plants. The positive control in this experiment was potato plants that had been inoculated with *Pectobacterium* sp. (Pcc160). The two negative controls, water and blanc (organic solvents and surfactant). Each treatment contained three plant duplicates and the bactericide standard, streptomycin (streptomycin sulfate). The severity of infection was recorded three weeks after inoculation.

6. Statistical analysis:

The Statistical analysis system (SAS Institute Inc., 2004) was used to apply analysis of variance (ANOVA) to the data. The L.S.D. test was used to compare means at P<.05 .

RESULTS

1. Bioassay tests:

1.1 Bactericidal effect of the tested monoterpenoids:

Antibacterial effect of the monoterpenoids, carvacrol, p-cymene, eugenol, isoeugenol and thymol using disc diffusion method were showed in Table (1) the best of main effect of treatments on growth of *P. carotovorum* sub sp. *carotovorum* were carvacrol and thymol followed by eugenol, isoeugenol and p-cymene compared with DMSO as control treatment. The best of main effect of bacterial isolates were Pcc160 isolate followed by Pcc5K and Pcc isolates. No significant differences in the main effect between isolates of *P. c*. subsp. *carotovorum* (Pcc5K and Pcc) in their affected by the previous compounds were recorded. Both tested isolates (Pcc5K and Pcc) were resistant to p-cymene at 250µg/ml, 500µg/ml, 1000µg/ml, 2000µg/ml and 4000µg/ml previous compound concentrations. Pcc160 isolate was sensitive to all previous tested compound. The best main effect of the concentrations of the previous compounds were 4000µg/ml and 2000µg/ml followed by 1000µg/ml, 500µg/ml, 250µg/ml and control treatment. No significant differences in the main effect between the concentrations 2000µg/ml and 4000µg/ml and between 500µg/ml and 1000µg/ml of the previous compounds. Data in Tables (2) showed bacteriostatic (BS) and bactericidal (BC) action of carvacrol, p-cymene, eugenol, isoeugenol and thymol on growth of P.c.subsp. carotovorum (Pcc160, Pcc5K and Pcc) isolates. Results in Table (2) indicated that carvacrol and thymol had bacteriostatic (Bs) effect for Pcc160 isolate at different concentrations. Data in Table (2) showed that eugenol and isoeugenol had bacteriostatic (Bs) effect for Pcc5K and Pcc160 isolates at different concentrations. Data in Table (2) showed that p-cymene had bacteriostatic (Bs) effect for Pcc160 isolate at concentrations of 1000µg/ml, 2000µg/ml and 4000µg/ml while, Pcc5K and Pcc isolates had resistance to the compound at all concentrations.

Table 1. Antibacterial activity of some monoterpenoid on growth of *Pectobacterium carotovorum* subsp. *carotovorum* isolates at different concentrations *in vitro*.

Bacterial isolates / Treatments		Inhibition zone (mm)			The main effect of concentration	The main effect of treatments
		Pcc160	Pcc5K	Pcc		
Control	Conc. µg/ml 0	0.0000 o	0.0000 o	0.0000 o	**0µg/ml (0.0) C**	**0.39815 A**
Carvacrol	250	1.3333 mn	0.0000 o	0.0000 o	**250µg/ml (0.03333) C**	
	500	2.3333 jkl	1.6667 lmn	1.3333 mn		
	1000	10.0 e	2.3333 jkl	1.6667 lmn		
	2000	20.0 c	2.3333 jkl	2.0 klm		
	4000	22.3333 a	2.3333 jkl	2.0 klm		
Cymene	250	0.0000 o	0.0000 o	0.0000 o	**500µg/ml (0.09556) BC**	**0.2222 C**
	500	0.0000 o	0.0000 o	0.0000 o		
	1000	1.0000 n	0.0000 o	0.0000 o		
	2000	1.3333 mn	0.0000 o	0.0000 o		
	4000	1.6667 lmn	0.0000 o	0.0000 o		
Eugenol	250	0.0000 o	1.3333 mn	0.0000 o	**1000µg/ml (0.22222) B**	**0.19815 B**
	500	1.6667 lmn	1.6667 lmn	0.0000 o		
	1000	2.6667 jk	2.0 klm	1.3333 mn		
	2000	7.0 g	2.0 klm	2.0 klm		
	4000	8.6667 f	2.6667 jk	2.6667 jk		
Isoeugenol	250	1.0000 n	0.0000 o	0.0000 o	**2000µg/ml (0.42889) A**	**0.12778 BC**
	500	1.6667 lmn	0.0000 o	0.0000 o		
	1000	2.6667 jk	0.0000 o	0.0000 o		
	2000	4.0 i	2. klm	2.0 klm		
	4000	5.6667 h	2.0 klm	2.0 klm		
Thymol	250	1.3333 mn	0.0000 o	0.0000 o	**4000µg/ml (0.52) A**	**0.33704 A**
	500	3.0 j	1.0000 n	0.0000 o		
	1000	7.6667 g	1.0000 n	1.0000 n		
	2000	16.6667 d	1.6667 lmn	1.3333 mn		
	4000	21.3333 b	2.0 klm	2.6667 jk		
The main effect of bacterial isolates		**0.48333 A**	**0.09333 B**	**0.07333 B**		

LSD (P=0.05), Treatments = 0.1198, Concentration = 0.1313, Bacterial isolates = 0.0928, Interaction of all = 0.0772.

Table 2. Bacteriostatic / bactericidal (BS/BC) action of five monoterpenoids on growth of *Pectobacterium carotovorum* subsp. *carotovorum* isolates at different concentrations *in vitro*.

Monoterpens	Conc. %	Pcc160	Pcc5K	Pcc
Carvacrol	2.5	BS	NT	NT
	5	BS	BS	BS
	10	BS	BS	BS
	20	BS	BS	BS
	40	BS	BS	BS
Cymene	2.5	NT	NT	NT
	5	NT	NT	NT
	10	BS	NT	NT
	20	BS	NT	NT
	40	BS	NT	NT
Eugenol	2.5	NT	BS	NT
	5	BS	BS	NT
	10	BS	BS	BS
	20	BS	BS	BS
	40	BS	BS	BS
Isoeugenol	2.5	BS	NT	NT
	5	BS	NT	NT
	10	BS	NT	NT
	20	BS	BS	BS
	40	BS	BS	BS
Thymol	2.5	BS	BS	BS
	5	BS	BS	BS
	10	BS	BS	BS
	20	BS	BS	BS
	40	BS	BS	BS

(BS) Bacteriostatic , NT: not tested to detect BS/BC, where the isolates exhibited resistance to monoterpenoids , then no inhibition zone appeared

1. 2. Inhibitory activities of selected monoterpenoids combinations against soft rot bacteria:

Carvacrol, eugenol and thymol which gave highly significant antibacterial effect in vitro were selected to study their combined effect on growth of *P.c*.subsp. *carotovorum* (Pcc160, Pcc5K and Pcc) isolates based on agar diffusion method. Data presented in Table (3) showed that the best main effect of treatments on growth of tested isolates was (eugenol+ thymol) and (carvacrol+thymol) mixtures which revealed equal significance followed by (carvacrol+eugenol) mixture compared with the control treatment. The main effect of tested isolates showed that Pcc160 was more sensitive isolate than Pcc5K and Pcc isolates for all treatments. The best main effect of the concentrations of the previous mixtures were 4000µg/ml followed by 2000µg/ml and 1000µg/ml. Data in Tables (4) showed bacteriostatic (BS) and bactericidal (BC) action of (carvacrol+thymol) (carvacrol+eugenol) and (eugenol+ thymol) mixtures on growth of P.c.subsp. carotovorum (Pcc160, Pcc5K and Pcc) isolates. Results in Table (4) indicated that, (carvacrol+thymol) mixture had bacteriostatic (Bs) effect for Pcc160 and Pcc5K isolates at different concentrations while, Pcc isolate had resistance to the compound at all concentrations. Data in Table (4) showed that (carvacrol+eugenol) and (eugenol+ thymol) mixtures had bactericidal (Bc) effect for Pcc160 isolate at concentration of 4000 µg/mL.

Table 3. Antibacterial activity of selected monoterpenoids combinations on growth of *Pectobacterium carotovorum* subsp. *carotovorum* isolates at different concentrations *in vitro*.

Bacterial isolates / Treatments		Inhibition zone (mm)			The main effect of concentrations	The main effect of treatments
		Pcc160	Pcc5K	Pcc		
Control	Conc. µg/m 0	0.0 k	0.0 k	0.0 k	**0µg/ml (0.0) C**	**0.41111 A**
Carvacrol +Thymol	1000	7.6667 e	1.0 jk	0.0 k	**10µg/ml (0.2111) C**	
	2000	15.3333 c	1.6667 ij	0.0 k		
	4000	21.6667 b	2.0 hij	0.0 k		
Carvacrol+Eugenol	1000	2.0 hij	0.0 k	1.0 jk	**20µg/ml (0.4519) B**	**0.2 B**
	2000	5.0 f	1.6667 ij	1.6667 ij		
	4000	8.6667 e	2.0 hij	2.0 hij		
Eugenol + Thymol	1000	4.0 fg	1.6667 ij	1.6667 ij	**40µg/ml (0.7593) A**	**0.45556 A**
	2000	11.6667 d	1.6667 ij	2.0 hij		
	4000	25.6667 a	3.0 ghi	3.3333 gh		
The main effect of bacterial isolates		**0.84722 A**	**0.12222 B**	**0.09722 B**		

LSD (P=0.05),Treatments = 0.187,Concentrations = 0.2159,Bacterial isolates = 0.187,Interaction of all = 0.159.

Table 4. Bacteriostatic (BS) and bactericidal (BC) action of monoterpenoids mixtures on growth of *Pectobacterium carotovorum* subsp. *carotovorum* isolates at different concentrations *in vitro*.

Monoterpenoids mixture	Conc (µg/ml)	Pcc160	Pcc5K	Pcc
Carvacrol +Thymol	1000	BS	BS	NT
	2000	BS	BS	NT
	4000	BS	BS	NT
Carvacrol+Eugenol	1000	BS	NT	BS
	2000	BS	BS	BS
	4000	BC	BS	BS
Eugenol + Thymol	1000	BS	BS	BS
	2000	BS	BS	BS
	4000	BC	BS	BS

(BS) Bacteriostatic (BC) Bactericidal, NT: not tested to detect BS/BC, where the isolates exhibited resistance to monoterpenoids mixtures, then no inhibition zone appeared.

1.3. Joint action effects of the most active monoterpenoids with Triphenyl phosphate, dimethyl meleate, piperonyl butoxide and Triton X100 against *Pectobacterium* sp:

The joint action effect of the active monoterpenoids, carvacrol, eugenol and thymol with Triphenyl phosphate, Dimethyl Meleate,Piperonyl butoxide and Triton X100 in the ratios 1:1 and 1:2 (v/v) against *Pectobacterium carotovorum* subsp. *carotovorum* isolates is presented in Table (5). The results revealed that all tested monoterpenoids were synergized by Triphenyl phosphate, Dimethyl Meleate, piperonyl butoxide and Triton X-100 Particularly, the highest synergistic action was noted in the case of (carvacrol + Triton X100 in 1:1 (v/v) ratio) mixture at 4000µg/ml conc. followed by (eugenol+ Triphenyl phosphate in 1:2 (v/v) ratio) mixture at the highest concentration(4000µg/ml) against *P. c.*subsp. *carotovorum* Pcc160 isolate. Data in Tables 6,7,8) showed bacteriostatic (BS) and bactericidal (BC) action of tested monoterpenoids were synergized by Triphenyl phosphate, Dimethyl Meleate, piperonyl butoxide and Triton X-100 on growth of P.c.subsp. carotovorum (Pcc160, Pcc5K and Pcc) isolates, Results indicated that (carvacrol+ Dimethyl Meleate in 1:1 (v/v) ratio) mixture had bactericidal (Bc) effect for Pcc160 isolate at 40µg/ml conc. while, Pcc isolate had resistance to (carvacrol + piperonyl butoxide in 1:1 and 1:2 (v/v) ratio) mixture at all concentration. (carvacrol+ Triton X100 in 1:1 and 1:2 (v/v) ratio) mixture had bacteriostatic (Bs) effect for Pcc160, Pcc5K and Pcc isolates at 40µg/ml conc. (eugenol+ Dimethyl Meleate in 1:1and 1:2 (v/v) ratio) mixture had bacteriostatic(Bs) effect for Pcc160, Pcc5K and Pcc isolates at 4000µg/ml conc. (thymol+ Dimethyl Meleate in 1:2 (v/v) ratio) mixture had bactericidal (Bc) effect for Pcc5K isolate at 40µg/ml conc. (thymol+ Triton X100 in 1:2 (v/v) ratio) mixture bactericidal (Bc) effect for Pcc160 isolate at 4000µg/ml conc

Table 5. Antibacterial activity of joint action effects of the most active monoterpenoids with (Triphenyl phosphate (TPP), Dimethyl Meleate (DE), Piperonyl butoxide (PBo) and Triton X100 (TX)) against *Pectobacterium* sp.isolates at different concentrations *in vitro*.

Treatments \ Bacterial isolates			Inhibition zone (mm)		
	Ratio	**Conc. µg/ml**	**Pcc5K**	**Pcc160**	**Pcc**
Control (Carvacrol + Tpp)	0	250	0.00000 y	1.3333 vwx	0.00000 y
		1000	2.3333 stu	10.0000 f	1.6667 uvw
		4000	2.3333 stu	22.3333 a	2.0000 tuv
Carvacrol + TPP	1:1	250	0.00000 y	0.00000 y	0.00000 y
		1000	0.00000 y	0.00000 y	0.00000 y
		4000	3.333 qr	9.6667 fg	3.000 qrs
	1:2	250	0.00000 y	0.00000 y	0.00000 y
		1000	6.6667 jk	0.00000 y	0.00000 y
		4000	13.3333 d	0.00000 y	0.00000 y
Control (Carvacrol +De)	0	250	0.00000 y	1.3333 vwx	0.00000 y
		1000	2.3333 stu	10.0000 f	1.6667 uvw
		4000	2.3333 stu	22.3333 a	2.0000 tuv
Carvacrol + De	1:1	250	0.00000 y	0.00000 y	0.00000 y
		1000	0.00000 y	1.3333vwx	1.3333 vwx
		4000	2.6667 rst	9.0000 gh	3.3333 qr
	1:2	250	0.00000 y	0.00000 y	0.00000 y
		1000	1.0000 wx	2.0000 ty	0.00000 y
		4000	1.6667 uvw	6.3333 kl	3.6667 pq
Control (Carvacrol + PBo)	0	250	0.00000 y	1.3333 vwx	0.00000 y
		1000	2.3333 stu	10.0000 f	1.6667 uvw
		4000	2.3333 stu	22.3333 a	2.0000 tuv
Carvacrol + PBo	1:1	250	0.00000 y	0.00000 y	0.00000 y
		1000	0.00000 y	0.00000 y	0.00000 y
		4000	0.00000 y	1.6667 uvw	0.00000 y
	1:2	250	0.00000 y	0.00000 y	0.00000 y
		1000	0.00000 y	3.3333 qr	0.00000 y
		4000	2.3333 stu	4.3333 op	0.00000 y
Control (Carvacrol + TX)	0	250	0.00000 y	1.3333 vwx	0.00000 y
		1000	2.3333 stu	10.0000 f	1.6667 uvw
		4000	2.3333 stu	22.3333 a	2.0000 tuv
Carvacrol + TX	1:1	250	0.00000 y	0.00000 y	0.00000 y
		1000	1.6667 uvw	0.00000 y	1.3333 vwx
		4000	13.3333 d	22.3333 a	4.3333 op
	1:2	250	0.00000 y	0.00000 y	0.00000 y
		1000	3.3333 qr	1.3333 vwx	1.6667 uvw
		4000	7.6667 i	13.3333 d	2.3333 stu
Control (Eugenol +TPP)	0	250	1.3333 vwx	0.00000 y	0.00000 y
		1000	2.0000 tuv	2.6667 rst	1.3333 vwx
		4000	2.6667 rst	8.6667 h	2.6667 rst
Eugenol + TPP	1:1	250	0.00000 y	0.00000 y	0.00000 y
		1000	0.00000 y	0.00000 y	0.00000 y
		4000	0.00000 y	0.00000 y	0.00000 y
	1:2	250	0.00000 y	0.00000 y	0.00000 y
		1000	0.00000 y	0.00000 y	0.00000 y
		4000	12.0000 e	14.3333 c	3.6667 pq
Control (Eugenol + De)	0	250	1.3333 vwx	0.00000 y	0.00000 y
		1000	2.0000 tuv	2.6667 rst	1.3333 vwx
		4000	2.6667 rst	8.6667 h	2.6667 rst
Eugenol + De	1:1	250	0.00000 y	0.00000 y	0.00000 y
		1000	0.00000 y	2.6667 rst	0.00000 y
		4000	3.333 qr	7.3333 ij	5.3333 mn
	1:2	250	0.00000 y	0.00000 y	0.00000 y
		1000	0.00000 y	0.00000 y	2.6667 rst
		4000	3.333 qr	4.6667 no	5.0000 mno
Control (Eugenol + PBO)	0	250	1.3333 vwx	0.00000 y	0.00000 y
		1000	2.0000 tuv	2.6667 rst	1.3333 vwx
		4000	2.6667 rst	8.6667 h	2.6667 rst

Eugenol + PBo	1:1	250	0.00000 y	0.00000 y	0.00000 y
		1000	0.00000 y	0.00000 y	0.00000 y
		4000	2.3333 stu	0.00000 y	0.00000 y
	1:2	250	0.00000 y	0.00000 y	0.00000 y
		1000	0.00000 y	0.00000 y	0.00000 y
		4000	0.00000 y	1.3333 vwx	3.6667 pq
Control (Eugenol +TX)	0	250	1.3333 vwx	0.00000 y	0.00000 y
		1000	2.0000 tuv	2.6667 rst	1.3333 vwx
		4000	2.6667 rst	8.6667 h	2.6667 rst
Eugenol + TX	1:1	250	0.00000 y	0.00000 y	0.00000 y
		1000	6.6667 jk	0.00000 y	0.00000 y
		4000	9.6667 fg	16.667 uvw	0.00000 y
	1:2	250	0.00000 y	0.00000 y	0.00000 y
		1000	5.6667 lm	0.00000 y	0.00000 y
		4000	11.6667 e	0.00000 y	10.000 f
Control (Thymol+TPP)	0	250	0.00000 y	1.3333 vwx	0.00000 y
		1000	1.0000 wx	7.6667 i	1.0000 wx
		4000	2.0000 tuv	21.3333 b	2.6667 rst
Thymol + TPP	1:1	250	0.00000 y	0.00000 y	0.00000 y
		1000	0.00000 y	0.00000 y	0.00000 y
		4000	4.3333 op	3.6667 pq	0.00000 y
	1:2	250	0.00000 y	0.00000 y	0.00000 y
		1000	0.00000 y	0.00000 y	0.00000 y
		4000	0.00000 y	2.3333stu	1.6667 uvw
Control (Thymol + De)	0	250	0.00000 y	1.3333 vwx	0.00000 y
		1000	1.0000 wx	7.6667 i	1.0000 wx
		4000	2.0000 tuv	21.3333 b	2.6667 rst
Thymol + De	1:1	250	0.00000 y	0.00000 y	0.00000 y
		1000	1.3333 vwx	1.0000 wx	0.00000 y
		4000	1.6667uvw	1.6667 uvw	0.00000 y
	1:2	250	0.00000 y	0.00000 y	0.00000 y
		1000	4.6667 no	0.00000 y	0.00000 y
		4000	9.3333 fgh	2.3333 stu	0.00000 y
Control (Thymol +PBo)	0	250	0.00000 y	1.3333 vwx	0.00000 y
		1000	1.0000 wx	7.6667 i	1.0000 wx
		4000	2.0000 tuv	21.3333 b	2.6667 rst
Thymol + PBo	1:1	250	0.00000 y	0.00000 y	0.00000 y
		1000	0.00000 y	0.00000 y	0.00000 y
		4000	1.6667 uvw	1.6667 uvw	1.3333 vwx
	1:2	250	0.00000 y	0.00000 y	0.00000 y
		1000	0.00000 y	0.00000 y	0.00000 y
		4000	1.0000 wx	2.0000 tuv	0.00000 y
Control (Thymol + TX)	0	250	0.00000 y	1.3333 vwx	0.00000 y
		1000	1.0000 wx	7.6667 i	1.0000 wx
		4000	2.0000 tuv	21.3333 b	2.6667 rst
Thymol + TX	1:1	250	0.00000 y	0.00000 y	0.00000 y
		1000	0.00000 y	0.00000 y	2.3333 stu
		4000	0.00000 y	1.3333 vwx	5.3333 mn
	1:2	250	0.00000 y	0.00000 y	0.00000 y
		1000	0.00000 y	2.3333 stu	0.00000 y
		4000	0.00000 y	9.6667 fg	1.6667 uvw
The main effect of bacterial isolates			**0.16605 B**	**0.40370 A**	**0.10093 C**
The main effect of conc			**(250 µg/ml) 0.01574 C**	**(1000µg/ml) 0.15988 B**	**(4000µg/ml) 0.49506 A**
The main effect of ratio			**(0) (0.3707 A)**	**1:1 (0.1349 B)**	**1:2 (0.1651 B)**
The main effect of Treatments			**Carvacrol (0.29568 A)**	**Eugenol (0.19660 B)**	**Thymol (0.17840 B)**
The main effect of Synergist		**TPP(0.2975A)**	**De (0.2333 B)**	**PBo(0.20058 BC)**	**TX (0.1568 C)**

LSD (0.05), treatments = 0.0521, synergist = 0.0602, ratio = 0.0521, concentrations = 0.0521, bacterial isolates = 0.0521, interaction of all = 0.0862.

Table 6. Bacteriostatic (BS) and bactericidal (BC) action of (Carvacrol + Triphenyl phosphate (TPP), Dimethyl meleate (DE), Piperonyl butoxide (PBO) and Triton X-100 (TX)) mixtures on growth of *Pectobacterium carotovorum* subsp. *carotovorum* isolates at different concentrations *in vitro*.

Treatment	Ratio	concentrations (µg/ml)	Pcc160	Pcc5 K	Pcc
Carvacrol + TPP	1:1	2.5	NT	NT	NT
		10	NT	NT	NT
		40	BS	BS	BS
	1:2	2.5	NT	NT	NT
		10	NT	BS	NT
		40	NT	BS	NT
Carvacrol + DE	1:1	2.5	NT	NT	NT
		10	BS	NT	BS
		40	BC	BS	BS
	1:2	2.5	NT	NT	NT
		10	BS	BS	NT
		40	BS	BS	BS
Carvacrol + PBO	1:1	2.5	NT	NT	NT
		10	NT	NT	NT
		40	BS	NT	NT
	1:2	2.5	NT	NT	NT
		10	BS	NT	NT
		40	BS	BS	NT
(Carvacrol + TX	1:1	2.5	NT	NT	NT
		10	NT	BS	BS
		40	BS	BS	BS
	1:2	2.5	NT	NT	NT
		10	BS	BS	BS
		40	BS	BS	BS

(BS) Bacteriostatic, NT: not tested to detect BS/BC, where the isolates exhibited resistance to Carvacrol mixtures, then no inhibition zone appeared.

Table 7. Bacteriostatic (BS) and bactericidal (BC) action of (Eugenol Triphenyl phosphate (TPP), Dimethyl meleate (DE), Piperonyl butoxide (PBO) and Triton X-100 (TX)) mixtures on growth of *Pectobacterium carotovorum* subsp. *carotovorum* isolates at different concentrations *in vitro*.

Treatment	Ratio	concentrations (µg/ml)	Pcc160	Pcc5 K	Pcc
Eugenol + TPP	1:1	2.5	NT	NT	NT
		10	NT	NT	NT
		40	NT	NT	NT
	1:2	2.5	NT	NT	NT
		10	NT	NT	NT
		40	BS	BS	BS
(Eugenol + (De)	1:1	2.5	NT	NT	NT
		10	BS	NT	NT
		40	BS	BS	BS
	1:2	2.5	NT	NT	NT
		10	NT	NT	BS
		40	BS	BS	BS
Eugenol + PBO	1:1	2.5	NT	NT	NT
		10	NT	NT	NT
		40	NT	BS	NT
	1:2	2.5	NT	NT	NT
		10	NT	NT	NT
		40	BS	NT	BS
Eugenol + TX	1:1	2.5	NT	NT	NT
		10	NT	BS	NT
		40	BS	BS	NT
	1:2	2.5	NT	NT	NT
		10	NT	BS	NT
		40	NT	BS	BS

(BS) Bacteriostatic, NT: not tested to detect BS/BC, where the isolates exhibited resistance to Eugenol mixtures, then no inhibition zone appeared.

Table 8. Bacteriostatic (BS) and bactericidal (BC) action of (Thymol + Triphenyl phosphate (TPP), Dimethyl meleate (DE), Piperonyl butoxide (PBO) and Triton X-100 (TX)) mixtures on growth of *Pectobacterium carotovorum* subsp. *carotovorum* isolates at different concentrations *in vitro.*

Treatment	Ratio	concentrations (µg/ml)	Pcc160	Pcc5 K	Pcc
Thymol + TPP	1:1	2.5	NT	NT	NT
		10	NT	NT	NT
		40	BS	BS	NT
	1:2	2.5	NT	NT	NT
		10	NT	NT	NT
		40	BS	NT	BS
Thymol + De	1:1	2.5	NT	NT	NT
		10	BS	BS	NT
		40	BS	BS	NT
	1:2	2.5	NT	NT	NT
		10	NT	BS	NT
		40	BS	BC	NT
Thymol + PBO	1:1	2.5	NT	NT	NT
		10	NT	NT	NT
		40	BS	BS	BS
	1:2	2.5	NT	NT	NT
		10	NT	NT	NT
		40	BS	BS	NT
Thymol + TX	1:1	2.5	NT	NT	NT
		10	NT	NT	BS
		40	BS	NT	BS
	1:2	2.5	NT	NT	NT
		10	BS	NT	NT
		40	BC	NT	BS

(BS): Bacteriostatic , NT: not tested to detect BS/BC, where the isolates exhibited resistance to Thymol mixtures, then no inhibition zone appeared.

2. Determination of minimum inhibitory concentration (MIC):

Table (9) shows the effect of different carvacrol, (carvacrol+thymol), (carvacrol+Triton100X (TX) in 1:1 a ratio), (eugenol+Triphenyl phosphate (TPP) in 1:2 a ratio) and (thymol+Triton100X (TX) in 1:2 a ratio) concentrations (100, 200, 250, 300, 500, 1000, 1500, 1750 and 2000 µg/mL) compared with control to determine (MIC) of these compounds on the growth of *P.c*. subsp. *carotovorum* Pcc160 isolate. All concentrations of the previous compounds had variable inhibitory effect on the bacterial isolate. The MIC of carvacrol, (carvacrol+thymol). (carvacrol+Triton100X(TX)), (eugenol+Triphenyl phosphate (TPP)) and (thymol+Triton100X(TX)) was reached at concentrations of 200, 750, 200, 2000 and 125 µg/mL respectively that completely (100µg/ml) inhibited Pcc160 isolate.

Table 9. Antibacterial activity of tested monoterpenoides, their combinations and joint action effects of the most active monoterpenoids with Triphenyl phosphate(TPP) and Triton100X(TX) against *Pectobacterium carotovorum* subsp. *carotovorum* isolate using minimum inhibitory concentration (MIC) method**.**

Treatments	Bacterial isolates
	Pcc160
Carvacrol	**200**
Carvacrol+Thymol	**750**
Carvacrol+Triton100X(TX) in 1:1 a ratio	**200**
Eugenol+Triphenyl phosphate(TPP) in 1:2	**2000**
Thymol+Triton100X(TX) in 1:2	**125**

MIC: Values given as µg/ml

3. Scanning electron microscopy (SEM) examination:

3.1.Examination of *Pectobacterium* cells treated with tested monoterpenoides, their combinations and joint action effects of the most active monoterpenoids with Triton X100:

Analyze the cell morphology of *P. c*. subsp. *carotovorum* (Pcc160) using scanning electron microscope (SEM) after treatment with carvacrol, (eugenol+ thymol) mixture and (carvacrol + Triton X100 (TX) in 1:1 (v/v) ratio) mixture at 40µg/ml conc. control cells were not treated with previous compounds (Fig.1) in which damage of the cells and deformations of cell wall were detected (Fig.1d) . The present study demonstrates the remarkable 4000µg/ml of previous compounds that cause alterations in the surface of the treated *P. c*. subsp. *carotovorum*. It is clear from the images that the treated bacterial cell forms showed significant structural changes compared

to untreated bacterial cells, When cells were treated with carvacrol structural changes showed roughness on the cell surface and the treated cells were explode and showed deep surface cracks (Fig. 1a). The (eugenol+ thymol) mixture treated bacterial cells of *P. c*. subsp. *carotovorum*, appeared enlarged in size, explode and showed deep surface cracks (Fig. 1b). The (carvacrol + Triton X100 (TX)) mixture treated bacterial cells of *P. c*. subsp. *carotovorum*, appeared explode, corrugating and showed deep surface cracks (Fig. 1c). The surface of the membrane caused by the control, which showed a bright and smooth surface without any apparent irregularities (Fig.1d).

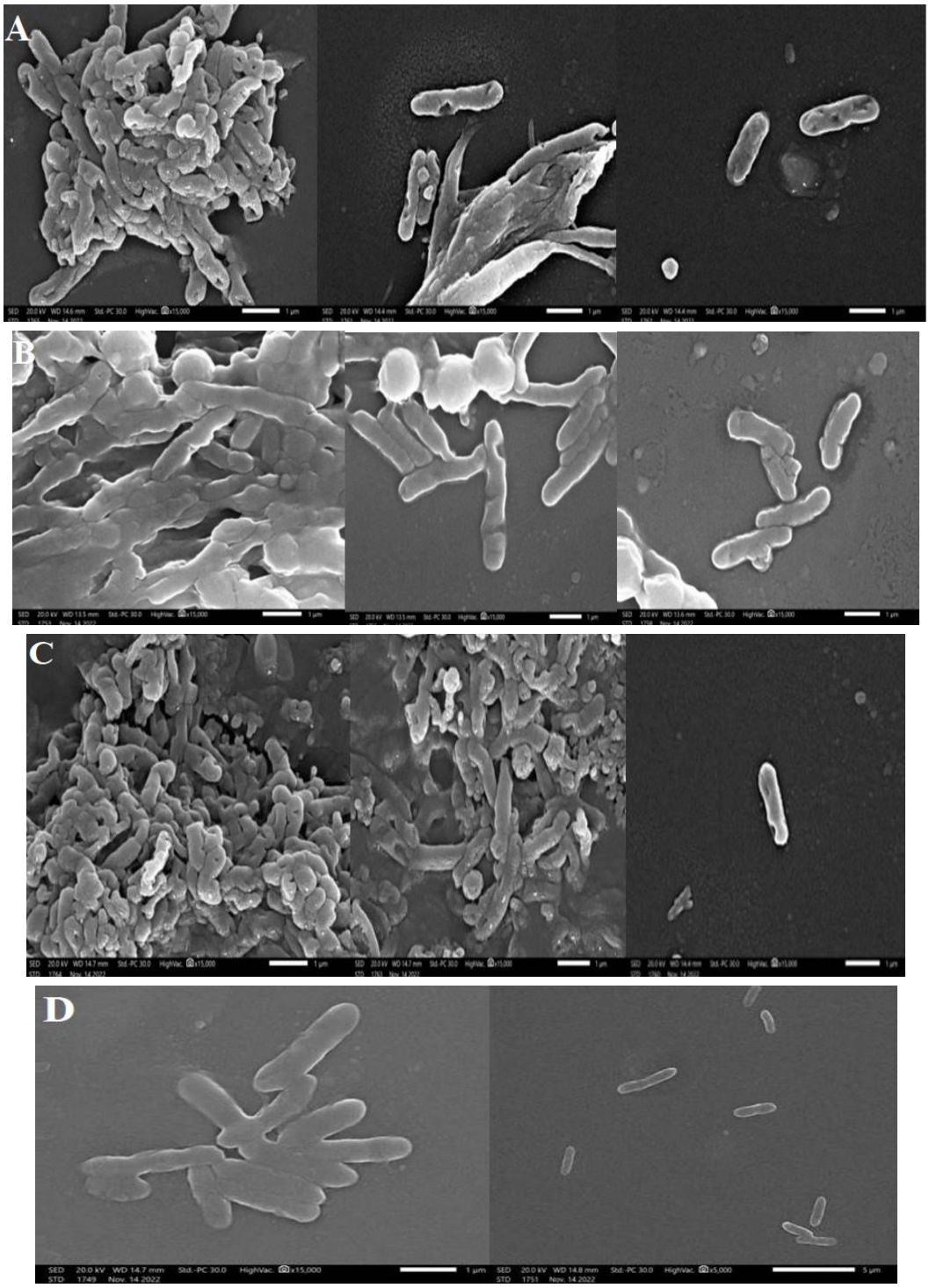

Fig. 1. Scanning electron microscopic micrographs of *P. carotovorum* subsp. *carotovorum* Pcc160 isolate treated with carvacrol, (eugenol+ thymol) mixture and (carvacrol + Triton X100 (TX) in 1:1 (v/v) ratio) mixture at 40µg/ml conc. (a) Bacteria treated with carvacrol were surface roughening, explode and showed deep surface cracks, (b) Bacteria treated with (eugenol+ thymol) mixture were enlarged in size, explode and showed deep surface cracks, (c) Bacteria treated with (carvacrol + Triton X100 (TX)) mixture were explode, corrugating and showed deep surface cracks and (d) non treated standard cell morphology (control).

4. Field Experiments:

Efficacy of tested monoterpenoides, their combinations and joint action effects of the most active monoterpenoids with Triphenyl phosphate and Triton X100 on Relative Disease Control (RDC) against potato soft rot disease.

Table (10) showed that the most treatments had significant reduced the percentage of soft rot infection on potato plants on seseason1, and this was clearly shown by an increase in the relative disease control (RDC) percentage compared to the positive control. The combination (eugenol+ triphenyl phosphate (TPP) in 1:2 a ratio) ranked first in this context with a RDC of 90.54 with disease percentage 5.62%in season (1) with significant increase between treatments in season (1) followed by (carvacrol+triton X100 (TX) in 1:1 a ratio) with RDC 84.89% and (eugenol+thymol) with RDC 79.04% in season1,the same result was recorded (eugenol+ TPP) 89% RDC in season (2) with non-significant increase between (Eugenol +thymol), (carvacrol+TX) and carvacrol. It is promising in these results that it exceeded the control rate that was reached using the commercial bactericide treatment, Streptomycine, which gave RDC 73.06%. In season1 and 55.32 % RDC in season2.

Table 10. Efficacy of tested monoterpenoids, their combinations and joint action effects of the most active monoterpenoids with Triphenyl phosphate and Triton X100 against soft rot disease on potato plants expressed as Relative Disease Control (RDC).

	Season1		Season2	
TRT	**DISEASE%**	**RDC%**	**DISEASE%**	**RDC%**
CONTROL (-)	0.00g	100a	0.00d	100a
CONTROL(+)	59.52a	0g	58.09a	0d
BLANC	59.14a	0g	58.96a	0d
(eugenol+ triphenyl phosphate TPP) 1:2	5.62a	90.54g	6.51cd	89.83ab
(Eugenol +thymol)	12.34f	79.04b	9.31cd	84.1b
(carvacrol+triton X100 TX) in 1:1	8.98de	84.89c	9.89cd	82.96b
(Thymol+ triton X100 TX) in 1:2	38.74ef	34.55b	26.12b	54.96c
CARVACROL	25.25c	57.15e	11.99c	79.57b
Streptomycine	15.86d	73.06d	25.79b	55.32c
LSD 0.05	**3.68**	**5.74**	**11.45**	**15.28**

DISCUSSION

Bactericidal effect of the tested monoterpenoids, carvacrol, p-cymene, eugenol, isoeugenol and thymol on growth of *P. c*. subsp. *carotovorum* Pcc160, Pcc5K and Pcc isolates revealed that carvacrol and thymol followed by eugenol were highly effective against *P. c.* subsp. *carotovorum in vitro* to inhibit growth of *P. c.* subsp. *carotovorum* at concentration 4000µg/ml, these findings were in line with (El-Zemity *et al.,* 2008; Ahmed *et al.,* 2023) when observed that carvacrol, thymol and eugenol were effective against *Erwinia carotovora*, Zhang *et al.* (2018) cleared that thymol showed the best inhibitory effect against *Erwinia carotovora* and Kotan *et al.* (2009) They demonstrated that thymol and carvacrol had strong antibacterial effects against 25 strains of phytopathogenic bacteria and might be employed as possible disinfectants against bacteria that spread through seeds. In contrast Víchov´a *et al.* (2024) found that thymol was the least effective against *Pectobacterium carotovorum* subsp. *carotovorum*. The antibacterial activity of monoterpenoids stems from their potential to interact with bacterial cell membranes. This inhibition results from interaction with the cell membrane's phospholipid bilayer, which increases permeability and causes cellular constituents to be lost (Sikkema *et al.,* 1994; Ultee *et al.,* 1999). Furthermore, it has been documented that a number of enzyme systems, including those involved in the synthesis of structural components and the production of energy, are impaired (Beuchat 1994). Increased monoterpenoids have the ability to absorb into the lipid bilayer. These lipophilic substances' partitioning inside the membrane can impact the activity of proteins and enzymes embedded in the membrane as well as cause a loss of membrane integrity and ion gradient dissipation, which can ultimately result in cell death (Sikkema *et al.,* 1995). Di Pasqua *et al.* (2006) provided illustrations of how the addition of thymol, carvacrol, and eugenol to the growth media altered the composition of fatty acids in the membranes of microbial cells.

Inhibitory activities of selected monoterpenoids combinations on growth of *P. c*. subsp. *carotovorum* Pcc160, Pcc5K and Pcc isolates achieved that (eugenol+ thymol) and (carvacrol+thymol) mixtures were effective against tested isolates, These findings were approved with Oluoch *et al.* (2021) who cleared that the most effective combination for inhibition the growth of *Ralstonia solanacearum* was found when thymol combined with eugenol. Join action effects of the most active monoterpenoids with Triphenyl phosphate, dimethyl meleate, piperonyl butoxide and Triton X100 against *Pectobacterium* sp, achieved that the mixture (carvacrol + Triton X100 in 1:1 (v/v) ratio) had the highest synergistic action against *P.c*. subsp. *carotovorum* Pcc160 isolate at 4000µg/ml conc. these findings were in agreement with El-Zemity *et al.* (2008), who cleared that the joint action effect of the active monoterpenoid carvacrol with Triton X-100 against *E. carotovora* gave the highest synergistic action. Carvacrol and thymol had bacteriostatic (Bs) effect for *P.c*. subsp. *carotovorum* Pcc160 isolate

at different concentrations, results were in contrast with Zamuner *et al.* (2023) who cleared that carvacrol and thymol had Bactericidal effects against *Xanthomonas citri* subsp. *citri* (*X. citri*), the causal agent of citrus canker disease at 200 ug.mL^{-1}and Pcc5K isolates at different concentrations, (Carvacrol+eugenol) and (eugenol+ thymol) mixtures had bactericidal (Bc) effect for *P.c*. subsp. *carotovorum* Pcc160 isolate at concentration of 4000 µg/mL, these effects were conformity with Shoeib and Alkufeidy (2014), which cleared that some natural antibacterial agents had bacteriostatic or bactericidal action against G-ve bacteria. (Carvacrol+thymol) mixture had bacteriostatic (Bs) effect for *P.c*. subsp. *carotovorum* Pcc160 . In the present study, soft rot bacteria *P. c*. subsp. *carotovorum* Pcc160 isolate was found to be inhibited in variable degrees by the compounds carvacrol, (carvacrol+thymol), (carvacrol+Triton100X (TX) in 1:1 a ratio), (eugenol+Triphenyl phosphate (TPP) in 1:2 a ratio) and (thymol+Triton100X (TX) in 1:2 a ratio) used, (Thymol+Triton100X (TX) in 1:2 a ratio) mixture was found most effective followed by carvacrol and (carvacrol+Triton100X (TX) in 1:1 a ratio) mixture, Ahmed *et al.* (2023) cleared that *Erwinia carotovora* recorded sensitivity towards tested monoterpenoids such as carvacrol, thymol and eugenol with values of 50, 300 and >300 µg mL-1, respectively.

According to the results obtained by Scanning Electron Microscope (SEM) showed that carvacrol, (eugenol+ thymol) mixture and (carvacrol + Triton X100 (TX) in 1:1 (v/v) ratio) mixture caused damage of the cells of *P. c.* subsp. *carotovorum* (Pcc 160 isolate) and deformations of cell wall which lead to the bacterial cells were apparent corrugating, deep surface cracks, enlarged in size, surface roughening and explode, These findings were approved with Jiang *et al.* (2021) who cleared that Scanning electron microscopy (SEM) micrographs confirmed that Dickeya zeae cell membranes were damaged by carvacrol. Pretreatment of potato plants with (eugenol+ triphenyl phosphate (TPP) in 1:2 a ratio), (Eugenol +thymol), (carvacrol+triton X100 (TX) in 1:1 a ratio),(Thymol+ triton X100 (TX) in 1:2 a ratio) and carvacrol before inoculation with *P. c.* subsp. *carotovorum* gave an excellent control rate (El-Zemity *et al.,* 2008). The combination (eugenol+ triphenyl phosphate (TPP) in 1:2 a ratio) was the most effective to increase the relative disease control (RDC) percentage where, the RDC was 90.54 with disease percentage 5.62% in season (1) and 89% RDC with disease percentage 6.51% in season (2), Marei and Abdelgaleil (2019) found that Eugenol had a moderate effect of inhibition on the dehydrogenases activity of *Erwinia carotovora* var. *carotovora*, this proved that the heigh effect of the combination (eugenol+ triphenyl phosphate (TPP) in 1:2 a ratio) against *P. c*. subsp. *carotovorum* was referred to triphenyl phosphate (TPP) when combined with eugenol.

CONCLUSION

Monoterpenoids, their combinations and joint action effects of the most active monoterpenoids with Triphenyl phosphate, dimethyl meleate, piperonyl butoxide and Triton X100 in the present study efficiently reduced the growth of soft rot bacteria, *P. c*. subsp. *carotovorum* in an *in vitro* assay, Moreover, they significantly reduced the percentage of soft rot infection on potato plants and increased the relative disease control (RDC) percentage in an *in vivo* assay. Thus, our results indicated that the previous compounds have the potential to be used in soft rot disease management.

Conflict of interests:

There are no conflicts of interest, according to the authors.

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النشاط الحيوى لبعض التربينات الآحادية ضد بكتيريا العفن الطرى على البطاطس

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تقييم النشاط التثبيطى لخمس من التربينات الآحادية وهى كارفاكرول و بى- سيمين و ايزوايجينول و ثيمول ضد نمو البكتيريا المسببة العفن الطرى للبطاطس(*Pectobacterium carotovorum* subsp. *carotovorum*) قد تم دراسته بتركيزات مختلفة تحت الظروف المعملية والحقلية. أظهر الكارفاكرول و الثيمول أقوى تأثير ضد عزلات *P. c*. subsp. *carotovorum* . يتناقص نمو البكتيريا المختبرة معنويا بزيادة تركيزات الكارفاكرول و الثيمول. أظهرت المخاليط (carvacrol+thymol), (Eugenol+ Thymol)معنوية متساوية ضد عزلات *P. c.* subsp. *carotovorum* . تجارب تعزيز كفاءة التضاد الميكروبى بواسطة الخلط مع أربع متآزرين تراى فينيل فوسفات و داى ميثيل مليت و ببرونيل بتوكسيد و تريتون أكس-100 قد أدركت. أظهر(Thymol+Triton X-100 in 1:2 (v/v) ratio) أعلى تأثير ضد العزلة Pcc160 بأقل تركيز مثبط MIC 125 µg/mL. أظهر المجهر الألكترونى الماسح تأثير تثبيطى واضح للكارفاكرول ومخلوط (eugenol+ thymol) ومخلوط (carvacrol + Triton X-100 in 1:1 (v/v) ratio) على نمو وبقاء (Pcc *P.c*. subsp. *carotovorum* (160. فى ظروف الحقل التربينات الآحادية المختبرة ومجموعاتهم وتأثيرات الفعل المشترك للتربينات الآحادية الأكثر نشاط كارفاكرول وايجينول وثيمول مع تراى فينيل فوسفات وتريتون أكس-100 قد قللت معنويا نسبة الأصابة بالعفن الطرى على نباتات البطاطس. الجمع ايجينول مع تراى فينيل فوسفات كان الأكثر تأثيرآ حيث أنه سبب إنخفاض فى مقاومة المرض النسبية (RDC) ب 90.54% فى موسم النمو الأول و 89% فى موسم النمو الثانى.

الكلمات المفتاحية: التربينات الآحادية، العفن الطرى، بكتوباكتريم، المجهر الألكترونى الماسح، المتآزرين.