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Chemical Antibacterial Agents Used to Disinfect Cultivation Tools against the Crown Gall Disease of Stone Fruits

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Abstract
Several chemicals were tested and evaluated as antibacterial agents against the Palestinian isolate of *Agrobacterium tumefaciens*, the causal agent of the crown gall disease. Based on the disk diffusion method on nutrient agar, formaldehyde appeared to be the strongest followed by sulfuric acid, hydrochloric acid, sodium hydroxide, Hypex and Dettol. On the other hand, those chemicals revealed 90-100% inhibition against the bacterial cell contaminating common pins. The other chemicals showed either an intermediate or weak bacterial inhibition of the bacterium on agar nutrient media.
Keywords: *Agrobacterium tumefaciens*, Crown gall, Antibacterial disinfectants, Palestine

1. Introduction

Crown gall is a bacterial disease that infects hundreds of plant species, both woody and herbaceous. ***The*** disease has a wide range of dicotyledonous (broad-leaved) plant hosts, especially members of the rosaceae family such as apple, pear, peach, cherry, almond, raspberry and roses. In addition, a strain, termed biovar 3, causes crown gall of grapevine (Agrios, 1997; Trigiano *et. al*. 2004).

The disease which is caused by *Agrobacterium tumefaciens* gains its name from the large tumor-like swellings (galls) that typically occur at the crown of the plant, just above soil level. Although the disease lowers the marketability of nursery stock, it usually does not cause serious damage to older plants. Galls vary considerably in size. The goal ranges from 1/4 inch to a foot or more in diameter with the majority being a few inches across. Young galls are soft at the surface and have a light, tan-colored, frosty appearance. As the galls grow older, they become darker, turning almost into black, and usually are hard and woody (Agrios, 1997).

There is often no visible effect on the plant other than the galls, but when galls are numerous or when a large gall has girdled the stem, the plant may become stunted and sickly with small red or yellow leaves. Top symptoms alone are inconclusive, but the presence of galls confirms the identity of the disease (Agrios, 1997; Streets, 1979).

The bacterium is capable of surviving in the soil for at least a year and possibly stays alive longer. It is easily spread in soil water or rain splash but can penetrate plants only through fresh wounds. Such wounds might be made during pruning, cultivating, transplanting, budding or grafting, or feeding by insects or other pests. The wondering of animals through the planted fields is sufficient for making wounds which let the pathogen enter (Agrios, 1997; Trigiano *et. al*. 2004).

.As the mechanical injuries of plants by cultivation tools are the major entry sits for the pathogen, the current project aims at finding out the effective chemical disinfectants that can be used by farmers in practical ways to disinfect the agricultural tools, particularly, those used regularly for pruning and grafting (Agrios, 1997; Trigiano *et. al*. 2004).

2. Material and Methods

2.1 Sample collection

Fifteen woody samples were collected from the galls of the almond trees showing the crown gall symptoms. The samples were collected from 6 year-old trees planted in Asira-El-Shamaleih, seven kilometers to the north of Nablus city.

2.2 Bacterial isolation

The collected samples were cut and grinded and then cultured in nutrient agar Petri plates. After 48 hrs incubation period at 37 degree Celsius, the bacterial colonies were sub-cultured and maintained into nutrient broth and nutrient agar Petri plates. The bacterial isolate was stored in the microbiology laboratory of the Arab American University.

2.3 Biological identification

The bacterium was tentatively identified as *A. tumefaciens* based on results obtained from several biochemical tests. Bacterial stains including the simple and gram one was done according to Johnson and Case (2001) and Brooks *et. al.*(2001) respectively. Also, the growth of the bacterial isolate on the selective and differential media including MacConkey Agar (MAC), Eosin Methylene Blue (EMB) Agar and Mannitol Salt Agar (MSA) was

done according to Tortora *et. al.* 2002. The shape and the color of the colonies and the pattern of bacterial growth were recorded for each medium (Strohi, *et. al.* 2001). To test its oxygen requirements, the isolate was streaked on nutrient agar plates and then kept inside an anaerobic GasPak jar for 48 hrs at 37 degree Celsius. The chemical pocket containing sodium bicarbonate and sodium borohydride was used (Tortora *et. al.* 2002). In addition, the motility of the bacterial isolate was tested in semisolid agar medium using the stab technique. Cloudiness in the stabbed areas was checked 48 hrs after incubation at 37 degree Celsius (Forbes *et. al.* 2002). On the other hand, the isolate was tested for its ability to ferment carbohydrates in media containing single type of carbohydrates and a pH indicator. Therefore, the media of phenol red lactose, phenol red dextrose, and phenol red sucrose were used. The media were placed in test tubes equipped with Durham tubes (small inverted tubes to detect gas production). After 48hrs incubation at 37 degree Celsius, the inoculated media were checked for gas production and color change (Forbes *et. al.* 2002; Johnson and Case, 2001). Furthermore, to study its ability to hydrolyze starch, the isolate was streaked on starch agar media then incubated similarly (Johnson and Case, 2001). As a second step, iodine drops were added to the media around the bacterial colonies to see the possibility of coloration. Additionally, the ability of the isolate to make protein catabolism was tested in the media including nutrient gelatin, litmus milk and urea agar. The media were inoculated with the bacterial isolate and incubated as usual (Forbes *et. al.* 2002; Johnson and Case, 2001).

2.4 Serological identification of the *Agrobacterium*

Precise identification of the bacterial isolate was done serologically using the standard bacterium-specific antibodies. Thus, indirect enzyme-linked immunosorbant assay (I-ELISA) was used as adopted by Clark *et. al* (1986). The *Agrobacterium* specific-polyclonal antibodies and the goat anti-rabbit conjugate were purchased from Bioreba, Inc. The results of the ELISA tests were recorded one hour after the substrate incubation took place using an automated ELISA-Reader. The light absorbance was measured for ELISA wells at 405 nanometres (Sawalha 2009).

2.5 Preparation of bacterial inocula

Three loopfuls of the bacterial isolate growing on nutrient agar plates were added to 5 ml sterile nutrient broth then incubated at 37 degree Celsius for 48 hrs.

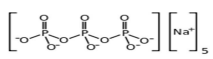
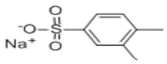
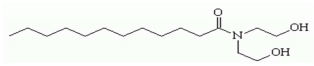
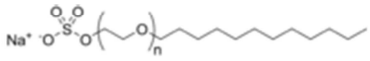
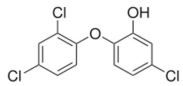
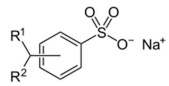
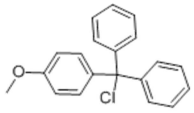
2.6 Preparation of the test chemicals

Concentrations of 10% and 5% of several chemicals were prepared using sterile distilled water (Fig 1). Some of these chemicals are detergents (indicated by their commercial names) as Modhesh and Bariq are used for cleaning flagstones, floors and bathrooms. Palmolive and Fairy are other detergents used to clean dishes and other kitchen instruments. Hypex which is the commercial name of a bleach material is used for sinks, tubs, drain boards, toilet bowls, garbage cans and kitchen instruments. In addition, Dettol and Septol are liquid disinfectants for laundry, floors, surfaces, lavatories, sinks and so on. The chemical concentrations were prepared aseptically in 50-ml falcon tubes.

Two categories of disinfectants were used. The first type (Table 1) was the common household chemicals available at the local market or at any consumer supermarkets. Some of these chemicals are detergents (named as their commercial names) as Modhesh (Sodium Tri Poly Phosphate) and Bariq (cocamide diethanolamine) that are used for cleaning tile floor and bathrooms. Palmolive and Fairy [sodium dodecylbenzenesulfonate, Ammonium C12-15 Pareth Sulfate, Magnesium Isododecylbenzenesulfonate, Lauramidopropylamine Oxide and Triclosan- 5-chloro-2-(2,4-dichlorophenoxy)phenol] which are other detergents used to clean dishes and other kitchen instruments. Hypex [calcium hypochlorite, $Ca(ClO)_2$] or Sodium hypochlorite [NaClO], which is the commercial name of a bleach material that is used for sinks, tubs, drain boards, toilet bowls, garbage cans and kitchen instruments. In addition, Dettol [5% of 4-Chloro-3,5-dimethylphenol (C_8H_9ClO)] and Septol [1.1% 6-chloro-hydroxy diphenyl methane] that are liquid disinfectants for laundry, floors, surfaces, lavatories, sinks and so on. The chemical concentrations were prepared aseptically in 50-ml falcon tubes.

The second disinfectant types were the chemicals one would obtain only at the chemistry or biology laboratories (i.e.: not readily available to consumers). These chemical were Formaldehyde, Phenol, Benzene, Kerosene, Sulfuric acid, Hydrochloric acid, and Sodium hydroxide. Concentrations of 10% and 5% of several chemicals were prepared using sterile distilled water.

Table 5: Common household chemicals available at the local markets

household chemical	Active ingredient	Chemical structure	concentration	comment
Modhesh	Sodium Tri Poly Phosphate		3%	Sodium Xylene Sulphonate, surfactant 
Bariq	cocamide diethanolamine	Detergent foaming Detergent thickening 	4%	Sodium Laureth Sulfate 70%, hydrotropes & surfactant 
Palmolive and Fairy	Triclosan = 5-chloro-2-(2,4dichlorophenoxy)phenol	 antibacterial & antifungal triclosan)	5%	sodium dodecylbenzenesulfonate, detergent  $R^1 + R^2 = C_{11}H_{24}$
Hypex	calcium hypochlorite	$Ca(ClO)_2$	5%	Oxidants
Dettol	4-Chloro-3,5-dimethylphenol		5%	antimicrobial
Septol	6-chloro-hydroxy diphenyl methane		1.10%	antiseptic

Seeding of Petri plates

Seeding was done on several Petri plates containing nutrient agar by transferring 0.5 ml of the bacterial suspension into the surface of each plate, and then spread evenly using a sterile hockey stick glass rod.

Antibacterial activities of chemicals against *A. tumefaciens*

The antibacterial activity of the tested chemicals was evaluated according to the agar disc-diffusion method (Tortora *et.al* 2002). So, sterile filter-paper discs (12.7 mm diameter) were dipped halfway in the chemical concentrations and placed on the center of the seeded Petri plates. Then, the plates were incubated at 37 C for 24 hrs and the zones of bacterial inhibition were measured (between disc edge and the bacterial growth) on the bottom of the plates. The chemicals that showed the largest zones were selected for the subsequent work.

In addition, the chemical disinfectants (the most efficient ones from the previous step) were evaluated for their abilities to prevent infection according to Aysan *et. al* (2003) using rustproof common pins. The seeded pins were exposed to the chemicals used in the test at 25 C for 5 minutes disinfection times then rinsed with sterile distilled water and transferred to nutrient broth media. After incubation at 37 C for 2 hrs, one milliliter of the media was added to the surface of each freshly cut carrot disk in Petri plate. The plates were maintained in a controlled climate room, for 20 days at 25°C and 70% RH. Positive control samples treated with bacterial suspension and negative ones covered with bacterial-free media were incubated similarly.

Statistical analysis

Analysis of the ELISA readings was made using the Two-Sample Tests of Proportions (TSTP) using a level of significance of 0.05 (Lind *et. al.* 2005). The data collected from the antibacterial actions of the tested materials were analyzed using the SPSS software. Also, one-way ANOVA was used to compare the studied treatments for any significance through the F-test. The significant difference was established using the Tukey's method at the 0.05 level of significance (Montgomery 2008).

3. Results

Biological identification of the *Agrobacterium*

The bacterial isolate was identified as *A. tumefaciens* according to the results obtained *In Vitro* together with its symptoms on the collected samples (Streets 1978). The results are displayed in table 2.

Table 2: Tests and results used in the bacterium identification

The Test		Result
Simple stain		Rod shape
Gram stain		Gram negative
Growing in selective media	MacConkey Agar	Positive
	Eosin Methylene Blue	Positive
	Mannitol Salt Agar	Negative
Growing in anaerobic jar		Aerobic
Motility Determination		Motile
Fermentation of Carbohydrate	Phenol Red Dextrose	Positive
	Phenol Red Lactose	Positive
	Phenol Red Sucrose	Positive
Starch Hydrolysis		Negative
Protein Catabolism	Nutrient Gelatin	Negative
	Litmus Milk	Negative
	Urea Agar	Negative

Serological identification of the *Agrobacterium*

ELISA readings recorded for the bacterial positive samples were at least two times greater than the readings recorded for the bacterial-free samples (Plate 1 and Fig 1).

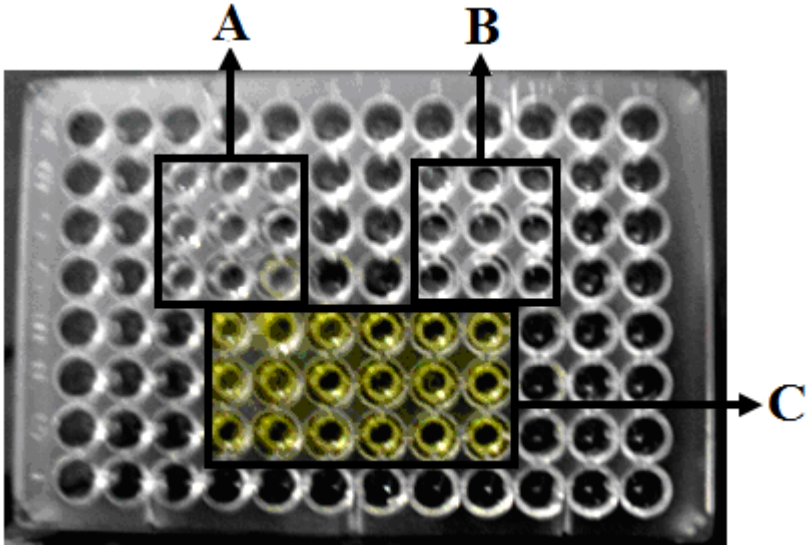
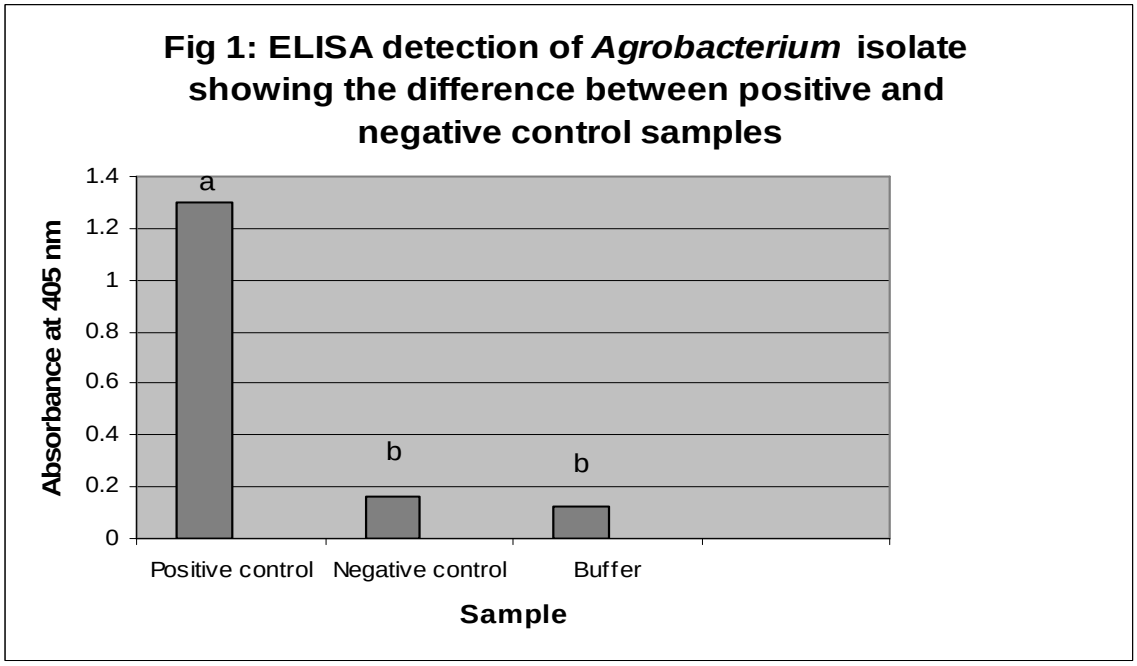


Plate 1: ELISA plate showing the antibody-antigen reaction of the studied *Agrobacterium* isolate. A: Negative control sample, B: Reaction with ELISA extraction buffer, C: Positive control sample.



***Letters above the column indicate the treatments with no significant difference

Antibacterial activities of chemicals against *A. tumefaciens*

The evaluation of the antimicrobial activities based on agar disc diffusion method revealed that formaldehyde has the maximum zone of inhibition (2.5 cm) followed by sulfuric acid (2.0 cm), hydrochloric acid (1.7 cm), sodium hydroxide (1.2 cm), Hypex (0.9 cm) and Dettol (0.8 cm). The other materials showed either an intermediate or a weak bacterial inhibition (Fig 2).

The statistical analysis using the one-way analysis of variance (ANOVA) showed a significant F test of 76.9 with a P-value of almost zero (Table 3).

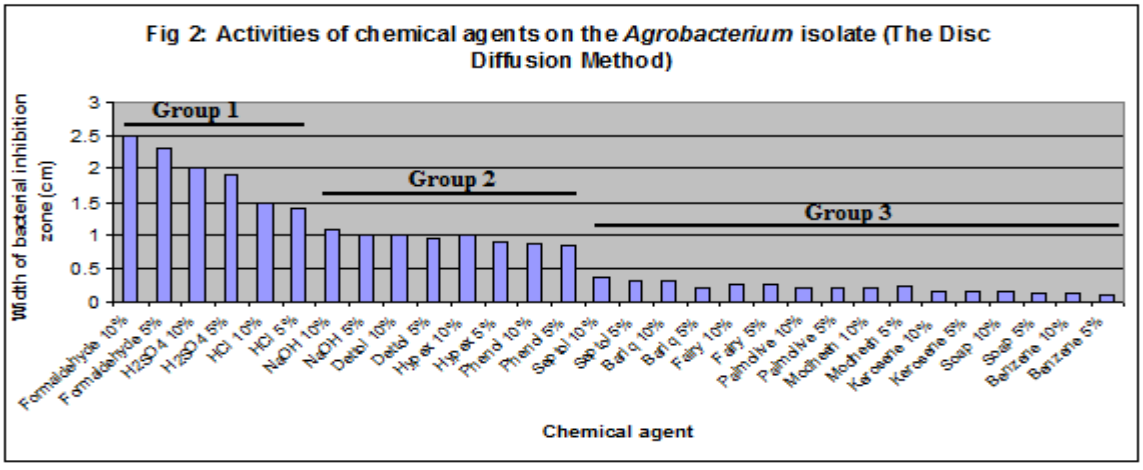


Table 3: One-way ANOVA

	Sum of Squares	DF	Mean Square	F	Sig.
Between Groups	58.504	29	2.017	76.954	.000
Within Groups	2.359	90	.026		
Total	60.863	119			

In addition, comparing the means using Tukey's method showed that only the pairs displayed in the table 3 have insignificant difference. The rest of the pairs have significant differences when compared at the 0.05 level of

significance.

For an illustration on how to use table 4, comparing treatments 1 and 2 shows the difference in their means is 0.2 with a common standard error of 0.11449 and the corresponding significance is 0.995.This observed significance is much bigger than the standard 0.05 indicating that there is no statistical difference between treatments 1 & 2. For the sake of convenience in the format of the table, the complete confidence intervals are not shown.

Table 4: Multiple comparisons using Tukey's HSD

(I)	(J)	Mean Difference	Sig.	(I)	(J)	Mean Difference	Sig.	(I)	(J)	Mean Difference	Sig.
1	2	.20000	.995	16	17	.00000	1.000	21	22	.00000	1.000
	3	.30000	.687		18	.10000	1.000		23	.00000	1.000
	4	.40000	.141		19	.05000	1.000		24	-.02000	1.000
3	2	-.30000	.687		20	.05000	1.000		25	.04000	1.000
	4	.10000	1.000		21	.10000	1.000		26	.06000	1.000
4	2	-.40000	.141		22	.10000	1.000		27	.07000	1.000
	5	.40000	.141		23	.10000	1.000		28	.09000	1.000
5	6	.10000	1.000		24	.08000	1.000		29	.09000	1.000
	7	.40000	.141		25	.14000	1.000		30	.10000	1.000
6	7	.30000	.687		26	.16000	1.000	22	23	.00000	1.000
	8	.40000	.141		27	.17000	1.000		24	-.02000	1.000
	9	.40000	.141		28	.19000	.998		25	.04000	1.000
	11	.40000	.141		29	.19000	.998		26	.06000	1.000
7	8	.10000	1.000		30	.20000	.995		27	.07000	1.000
	9	.10000	1.000	17	18	.10000	1.000		28	.09000	1.000
	10	.15000	1.000		19	.05000	1.000		29	.09000	1.000
	11	.10000	1.000		20	.05000	1.000		30	.10000	1.000
	12	.20000	.995		21	.10000	1.000	23	24	-.02000	1.000
	13	.25000	.927		22	.10000	1.000		25	.04000	1.000
	14	.27000	.853		23	.10000	1.000		26	.06000	1.000
8	9	.00000	1.000		24	.08000	1.000		27	.07000	1.000
	10	.05000	1.000		25	.14000	1.000		28	.09000	1.000
	11	.00000	1.000	18	19	-.05000	1.000		29	.09000	1.000
	12	.10000	1.000		20	-.05000	1.000		30	.10000	1.000
	13	.15000	1.000		21	.00000	1.000	24	25	.06000	1.000
	14	.17000	1.000		22	.00000	1.000		26	.08000	1.000
9	10	.05000	1.000		23	.00000	1.000		27	.09000	1.000
	11	.00000	1.000		24	-.02000	1.000		28	.11000	1.000
	12	.10000	1.000		25	.04000	1.000		29	.11000	1.000
	13	.15000	1.000		26	.06000	1.000		30	.12000	1.000
	14	.17000	1.000		27	.07000	1.000	25	26	.02000	1.000
10	11	-.05000	1.000		28	.09000	1.000		27	.03000	1.000
	12	.05000	1.000		29	.09000	1.000		28	.05000	1.000
	13	.10000	1.000		30	.10000	1.000		29	.05000	1.000
	14	.12000	1.000	19	20	.00000	1.000		30	.06000	1.000
11	12	.10000	1.000		21	.05000	1.000	26	27	.01000	1.000
	13	.15000	1.000		22	.05000	1.000		28	.03000	1.000
	14	.17000	1.000		23	.05000	1.000		29	.03000	1.000
12	13	.05000	1.000		24	.03000	1.000		30	.04000	1.000
	14	.07000	1.000		25	.09000	1.000	27	28	.02000	1.000
13	14	.02000	1.000		26	.11000	1.000		29	.02000	1.000
15	16	.05000	1.000		27	.12000	1.000		30	.03000	1.000
	17	.05000	1.000		28	.14000	1.000	28	29	.00000	1.000
	18	.15000	1.000		29	.14000	1.000		30	.01000	1.000
	19	.10000	1.000		30	.15000	1.000	29	30	.01000	1.000
	20	.10000	1.000	20	21	.05000	1.000				
	21	.15000	1.000		22	.05000	1.000				
	22	.15000	1.000		23	.05000	1.000				

	23	**.15000**	**1.000**		**24**	**.03000**	**1.000**				
	24	**.13000**	**1.000**		**25**	**.09000**	**1.000**				
	25	**.19000**	**.998**		**26**	**.11000**	**1.000**				
	26	**.21000**	**.991**		**27**	**.12000**	**1.000**				
	27	**.22000**	**.983**		**28**	**.14000**	**1.000**				
	28	**.24000**	**.952**		**29**	**.14000**	**1.000**				
	29	**.24000**	**.952**		**30**	**.15000**	**1.000**				
	30	**.25000**	**.927**								

Note: The mean difference is significant at the .05 level. **Mean Difference = I-J**

Furthermore, three groups of treatments can easily be formed from figure 3. The first group consists of the first 6 treatments with more than 1.4 cm width of bacterial inhibition zone. The second group includes treatments between the seventh and fourteenth one with inhibition zones range from 0.83 to 1.1 cm. The third group consists of the last 15 treatments (i.e. 15-30) with 0.35 cm or less zone of inhibition. When performing the same analysis on these three groups, it was found that the means of these groups are all statistically different as shown in the ANOVA table 5 with an F value of 476.39. Furthermore, the multiple comparison procedure emphasizes this as well. The results of such comparisons are illustrated in table 6.
On the other hand, the numbers which represent the studied treatments are displayed in table 7.

Table 5: One-way ANOVA

	Sum of Squares	DF	Mean Square	F	Sig.
Between Groups	54.207	2	27.103	476.386	.000
Within Groups	6.657	117	.057		
Total	60.863	119			

Table 6: Multiple comparisons of data using Tukey's HSD.

	(I) G	(J) G	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	.97958*	.06441	.000	.8267	1.1325
		3.00	1.73208*	.05709	.000	1.5966	1.8676
	2.00	1.00	-.97958*	.06441	.000	-1.1325	-.8267
		3.00	.75250*	.05164	.000	.6299	.8751
	3.00	1.00	-1.73208*	.05709	.000	-1.8676	-1.5966
		2.00	-.75250*	.05164	.000	-.8751	-.6299
LSD	1.00	2.00	.97958*	.06441	.000	.8520	1.1071
		3.00	1.73208*	.05709	.000	1.6190	1.8452
	2.00	1.00	-.97958*	.06441	.000	-1.1071	-.8520
		3.00	.75250*	.05164	.000	.6502	.8548
	3.00	1.00	-1.73208*	.05709	.000	-1.8452	-1.6190
		2.00	-.75250*	.05164	.000	-.8548	-.6502

*. The mean difference is significant at the 0.05 level.

Table 7: Treatment numbers used in the statistical analysis

Treatment	Number	Treatment	Number	Treatment	Number
Formaldehyde 10%	1	Hypex 10%	11	Palmolive 10%	21
Formaldehyde 5%	2	Hypex 5%	12	Palmolive 5%	22
H2SO4 10%	3	Phenol 10%	13	Modhesh 10%	23
H2SO4 5%	4	Phenol 5%	14	Modhesh 5%	24
HCl 10%	5	Septol 10%	15	Kerosene 10%	25
HCl 5%	6	Septol 5%	16	Kerosene 5%	26
NaOH 10%	7	Bariq 10%	17	Soap 10%	27
NaOH 5%	8	Bariq 5%	18	Soap 5%	28
Dettol 10%	9	Fairy 10%	19	Benzene 10%	29
Dettol 5%	10	Fairy 5%	20	Benzene 5%	30

In addition, studying the ability of disinfectants to prevent infection showed that formaldehyde, H2SO4, HCl, NaOH, Dettol and Hypex were strong anti-bacterial agents against the *Agrobacterium*. Dipping pins for 5 minutes in those chemical revealed 90 -100% inhibitions of the contaminating bacterial cells as no tumor symptoms on carrot slices developed (Plate 2, Table 8). With regard to the positive control samples, tumors developed, 7-8 days post inoculation. Furthermore, the bacterium was re-isolated from the carrot tumors and identified as *A. tumefaciens*.

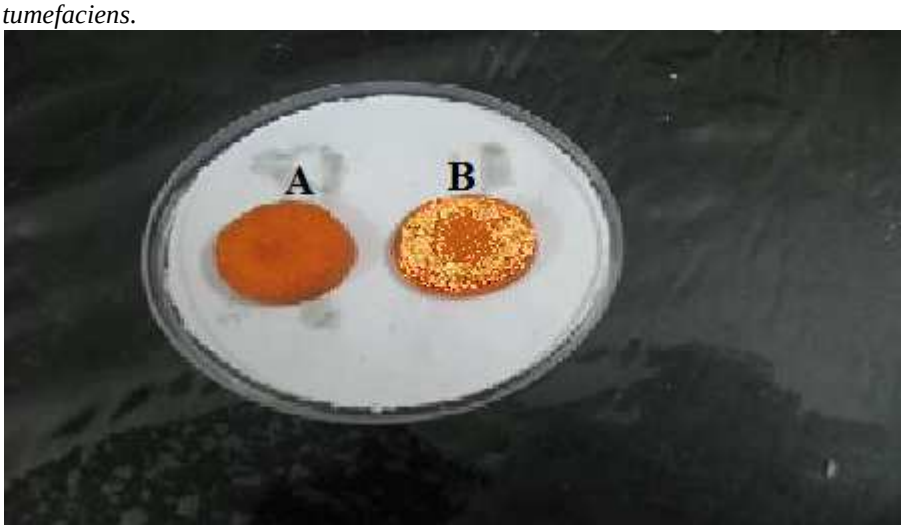


Plate 2: Tumors of *A. tumefaciens* on inoculated carrot slices. A: Healthy, B: Inoculated

Table 8: The ability of disinfectants (10%) to prevent infection of carrot slices

Disinfectant	Carrot infection	Infection rate
Formaldehyde	(-)	0/10
H2SO4	(-)	0/10
HCl	(-)	0/10
NaOH	(-)	0/10
Dettol	(-) & (+)	1/10
Hypex	(-) & (+)	1/10
Phenol	(-) & (+)	2/10
Septol	(-) & (+)	3/10
Bariq	(-) & (+)	4/10
Fairy	(-) & (+)	4/10
Palmolive	(-) & (+)	5/10

(-), (+): Negative and positive infections respectively

4. Discussion

Bacterial diseases of plants are very difficult to control because of the lack of effective chemicals. Antibiotics could be used but they are expensive and, in any case, the compounds that are valuable for human therapy are not allowed to be used in agriculture. Using alternatives such as copper compounds is not so much preferable as these compounds are potentially phytotoxic. Perhaps, the most applicable method of controlling these diseases is to prevent pathogen arrival and infection of the crops to get a considerable crop protection. Using sterile cultivation tools or disinfecting the contaminated ones is of prime importance to prevent pathogen inoculation and to achieve crop protection. In addition, controlling the bacterial diseases by prevention is much cheaper than controlling these diseases by chemicals that are usually expensive, especially, after infection and pathogen outbreak

Because *A. tumefaciens* is a mechanical transmissible pathogen that enters the plant easily through wounds, and much infection occurs through grafting and pruning (Agrios, 1997), tools used for grafting, budding or pruning should be disinfected before and after use to prevent or minimize the disease spread.

Our findings elucidated that several chemicals including formaldehyde, sulfuric acid, hydrochloric acid of group one, sodium hydroxide, Hypex and Dettol of groups two are effective antibacterial agents. Statistically, selecting any chemical from those groups gives reasonable control performance of the bacterium. So, dipping the cultivation tools in 5-10% solutions of these chemicals for at least 5 minutes is effective to kill the pathogen, and therefore prevents or minimizes the spread of the disease either in fields or in nurseries. Formaldehyde was among the most effective antimicrobials. It causes protein inactivation by forming covalent cross-links with several functional groups on proteins. However, it is more commonly available as formalin, a 37% aqueous solution of formaldehyde gas. Formalin is available in the market as it is used extensively to preserve biological specimens and to inactivate bacteria and viruses in vaccines. Also, it is used by morticians for embalming. (Tortora *et.al* 2002). The most important disadvantage of using this chemical appears in its bad odor, skin irritation and redness, and so its use may be not preferable.

Chemicals as the sulfuric acid, hydrochloric acid and sodium hydrochloride are also effective against the bacterium. But, although these chemicals are available in the markets, special care is needed during their use to avoid harm to human health and the adverse effect on environment. Also, washing tools with water after being dipped in these chemicals, especially the acids, is highly recommended to minimize the metal corrosion.

Special attention must be paid for using Hypex and Dettol as antibacterial agents. Although these materials when compared with others, have shown less effectiveness against *Agrobacterium* but they have several advantages as they are available and easily purchased from the market even from small shops and stores. Also, the householders use these materials extensively for laundry and to disinfect floors and hard surfaces as well as their use in kitchens against microbes contaminating tools, utensils and surfaces. These materials are less harmful to humans and the environment and the manufacturers recommend them as safe ones when used indoors.

Therefore, this study shows that Dettol and Hypex are recommended for the farmers and the workers in agriculture to be used as dipping chemicals for the cultivation tools to achieve a considerable disease control without harming humans and polluting the environment.

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