EVALUATION OF TOXIC EFFECT OF EXTRACTED PLANT PHENOL OF *LAWSONIA INERMIS* AND *PUNICA GRANATUM* AND ITS COPOLYMER IN *TETRANYCHUS URTICAE* KOCH(ACARI : TETRANYCHIDAE)

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SUMMARY

Phenols were extracted from both leaves of Henna *Lawsonia inermis* and fruit husk of pomegranate *Punica granatum* and it was used in laboratory bioassays to assess their effectiveness to control the two spotted spider mite *Tetranychus urticae* Koch (Acari : Tetranychidae). Pomegranate phenol was more effective (mortality rates 60.55, 71.10 and 56.66%) compared with Henna Phenol (mortality rates , 58.33, 59.44 and 46.66%) at concentration 1.0% test with two spotted mite adult , nymph and egg respectively. The most promising phenol (Pomegranate) was prepared as Phenol-Ureaformaldehyde Copolymer. The copolymer appeared high toxic effect ,caused mortality of *T.urticae* adult, reached 61.66 % at the more effective concentration of 1.0% after 24h of spraying application, the mortality enhance with increase time of treatment , to give rate 75% after 72h of application. The mortality of nymph had increased, it ranged from 50 to 88.33% at concentration mentioned before. Egg mortality rates were reached 66.66% in copolymer treatment at concentration 1.0% after 72h of application. The toxic effect found in this study could be attributed to phenol. Polymerization method offers the possibility of use phenol in controlled drug release systems.

Introduction

Tow spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is a serious pest of numerous green house plants, nursery grown ornamentals and field crops. Two spotted spider mite damage includes webbing, fine stippling, leaf yellowing, leaf drop and even plant death (2, 10, and 6). A major problem in controlling these species is their ability to rapidly develop resistance to many acaricides (21). The negative effects of

pesticides on the beneficial entomofauna and the environment increasing public concern about pesticide applications and the widely occurring problems justifies the need for alternative strategies for mite control (9, 7). Phenols contain sufficient hydroxyl and other suitable groups (such as carboxyl) to form strong complexes with proteins and other macromolecules. Phenols have molecular weight ranging from 500 to over 20000 (16). For example, the most abundant of pomegranate poly phenols (Phenols) of fruit husk are called punicalagin, which have molecular weight of 1038 (23). They are plant secondary metabolites, produced by plants as a mechanism of defense against plant-feeding pests (20), could be useful as a biological control of pests (22, 13). Despite these most promising properties, problems related to their rapidly hydrolysis in the environment before they are used as an alternative pest control system (8). Controlled release formulations allowing smaller quantities of chemical materials to be used more effectively over a given time interval seem to be the best choice to meet these multiple demands of efficacy, suitability to mode of application and minimization of environmental damage (15). The aim of this study was assessed the activity of Phenol-ureaformaldehyde Copolymer as new product for *Tetranychus urticae* control.

Materials and Methods

Isolation of phenol from plant samples

leaves of Henna *Lowsonia inermis* and fruit husk of pomegranate *Punice granatum* were collected from different location in Basrah city Iraq ,the plant samples were dried in the dark at air temperatures between 25 and 35°C within 48h to constant weight , the material was ground to fine powder. A variable amount (10 to 25g) of dry powder was extracted in 100 ml of 80% of ethanol, stirred for 30 min and stored overnight at 4°C, the extracts were filtered through watman filter paper No.40 using slight vacuum. Condensed phenols were separated from low molecular weight phenol, other phenolics using 100g sephadex LH-20 dextrin resin in 80% of ethanol. After elution with 50% acetone, two extractions with ethylacetate, the purified condensed phenol powders were stored at 4°C in the dark (11).The phenol were characterized at Pharmacy college labs by Fourier Transform Infrared (FT. IR) spectrophotometer type Equinox 55, by shimed 24Bruker Company and Ultraviolet-visible (UV.) spectrophotomotry type spectro 40 by analytic Jena company and used in further studies (16).

Preparation of Phenol-Ureaformaldehyde Copolymer

Phenol of Pomegranate fruit husk was selected for this study, it was the most promising phenol on control of two spotted spider mite.

The Urea Formaldehyde Resins (UFR) was prepared according to the Mhoesn (18) method as following: the reaction vessel was charge with 6g (0.1 mole) of urea and 37% (0.8 mole) of formaldehyde solution. The reaction vessel was therimmersed in oil bath, strinning was contoued for 3h at 60°C, with addition of 10% (w/v) sodium-hydroxide in portions to maintain the pH at (10-11).The mixture was cooled and neutralized with 10% phosphoric acid, after the filtration, the aqueres solvent was evaporated and the resin was dissolved in 100 ml ethanol, filtered for desalting and dried with molecular sieves. The solvent was evaporated and dried under reducing pressure (0.1mm Hg) for 24 h at 30°C to give colorless product, which were the same properties at reference.

$$H_2NCO\ NH_2 + HCOH \xrightarrow{NaOH} (HOCH_2)_2\ NCON\ (CH_2OH)_2$$

Phenol-Ureaformaldehyde Copolymer was prepared by mixing 1.85g of phenol dissolve in 50ml of 95% ethanol and 1.85g urea formaldehyde dissolve in 50ml of 95% ethanol in a 250 ml two-necked flask, filted with mechanical stirrer and condenser at room temperature for 24h, The solvent was evaporated in vacuum- oven by Gallen Kambe company for 24h at (0.1mm Hg) and the product was characterized by U.V. and FT. IR analysis (16).

$$Ar - OH + HO - CH_2\ NRCONR_2 \longrightarrow Ar - O - CH_2\ NRCO\ NR_2 + H2O$$

Ar = Phenol

R = CH_2OH

Effects of Phenol on adult and nymph of two spotted spider mite *T. urticae*

Susceptible strains of two spotted spider mite *T.urticae* was reared on lima bean *Phaseolus* sp., mite adults were treated with tow concentration of both pomegranate and Henna phenol (0.5 and 1.0%) and a control (distilled water). Distilled water was used to dilute all phenol with an aqueous mucilage containing 1.0% Tween 80 using a turbo emulsifier (19).

Twenty female and male mites of the same age placed on 2cm diameter disks of lime bean leaves. Leaf disks were kept on moist soft cotton swabs placed in plastic Petri dishes (9-cm-diameter), were stored in a growth incubator at 24°C at photoperiod 16:8 (L:D)h. Three leaf disks were prepared for each concentration of phenol application. Phenol sprayed after 24h from transferring of mites to leaf disks, with 1ml of solution by using small hand sprayer. Mortality was assessed under a binocular microscope after 24, 48 and 72h of treatment. Mites were considered dead, if movement was imperceptible after repeated gentle probing with a single-hair brush. Correction of the mortality data was performed using Abbott's formula (1).This experimental procedure also used for nymphal stage.

Effects of Phenol on egg of Two spotted spider mite *T.urticae*

T.urticae adult females were transferred to 2cm-diameter leaf disks cut out of lima bean foliage and left for 24h for oviposition. The mites were removed when at least 20 eggs per disk were laid , leaf disk were sprayed with 1ml of concentrations of phenol which was used in the bioassay with adults and stored after treatment. Each treatment was replicated 3 times. The effect of treatment on percentage of egg mortality was assessed by counting eggs remaining on the leaf disks and egg hatched after 72h of treatment (17).

Effects of Phenol-Ureaformaldehyde Copolymer on two spotted spider mite *T.urticae*

Individual effect of Phenol-Ureaformaldehyde Copolymer (PUC) and Urea Formaldehyde Resins (UFR) (concentration 0.5 and 1.0%) was evaluated on adult, nymph and egg mortality of two spotted spider mite.

The methodology for both effects of PUC and UFR was same effects of phenol on mites stages under conditions mentioned before .

Statistical analyses

Percentage data were transformed by arcsine and statistically analyzed according to the complete randomized design with factorial experiment, means were separated using Revised Least Significant difference (R.L.S.d) test (3).

Results and Discussion

Characteristics of Extracted Phenol and Phenol-Ureaformaldehyde Copolymer

In Fourier transform infrared (FT.IR) spectra, characteristic the stretching vibrations of hydroxyl group in phenol and copolymer at 3300 – 3500 cm^{-1} .The presence of absorption band at 1700-1740 cm^{-1} related to carbonyl group. There are several other distinguished bands in the region 3000 cm^{-1} related to the stretching vibration for alkyl proton, and in region 1600 cm^{-1} related to the stretching vibration of the (C=C) aromatic ring. The bending vibration was distinguished for (N–H) in the region 1450 cm^{-1} , (C-O-C) in the region 1200 cm^{-1} , and (C-O) in the region 760 cm^{-1} as shown in Table (1) and (2).

The results of UV. Spectra of one gm /L of Phenol and Copolymer was recorded. UV. Spectra of the Phenol and Copolymer at region 200-375 nm in distilled water. The UV. Spectra of Phenol shown the λ max of two absorption bands at 214nm for $\pi \rightarrow \pi^*$ transition and at 272 nm for $\eta \rightarrow \pi^*$ transition. While the UV. Spectra of Copolymer shown blue η shift in both of $\pi \rightarrow \pi^*$ transition (λ max at 200 nm) and $\rightarrow \pi^*$ transition (λ max at 240nm).

Table (1) The stretching vibra

tion of the most important bands in the FT. IR spectra of extract Phenol

Remarks	Vibration	Position of bands/cm^{-1}
Stretching vibration of OH	O-H (br)	3300 (br)
Asymmetrical stretching vibration of CH	C-H (w)	٢٩٤٠
Symmetrical stretching vibration of CH	C-H (w)	٢٨٨٠
Stretching vibration of carbonyl	C=O (sh)	١٧٢٠

Remarks	Vibration	Position of bands/cm $^{-1}$
Bending vibration of CH	C-H (sh)	١٤٢٠
Vibration of C-O-C	C-O-C (br)	١١١٠
Stretching vibration of CO	C-O (br)	١٠٧٠
Stretching vibration of OH	O-H (br)	3300 (br)

Table (2) The FT.IR. characteristic bands and their position for the phenol
Ureaformaldehyde Copolymer.

Asymmetrical stretching vibration of CH	C-H (w)	2940
Symmetrical stretching vibration of CH	C-H (w)	2880
vibration of carbonyl	C=O (sh)	1720
Bending vibration of CH	C-H (sh)	1420
Bending vibration of CN	C-N (br)	1300
Vibration of C-O-C	C-O-C (br)	1200
Stretching vibration of CO	C-O (sh)	1080

When :
br = broad w = weak sh = sharp

Effects of Phenol on Two spotted mite *T.urticae*

Results indicated that Pomegranate Phenol (PP) were more effective at controlling the adult of two spotted mites than Henna Phenol (HP) as shown in table3 ,caused mortality 58.88 and 52.77% respectively at end the test , PP at 1.0% concentration had significant effective than HP (mortality 60.55 and 58.33% respectively) followed by PP and HP at 0.5% concentration (mortality 57.22 and 47.21% respectively). It was also showed that the mortality rates of adult mites ranged from 53.33 to 46.66% at concentration 1.0% PP and HP respectively after 24h of application. During the observations, adult mortality increased progressively until 72h after spraying, with average 70 and 68.33% respectively.

Table (3) Effect of Phenol on adult mortality of Two spotted spider mite *Tetranychus urticae*.

Phenol	Concentration %	Mortality rate % after			Mortality mean	mortality mean
		24h	48h	72h		
Henna	0.5	38.33	46.66	56.66	47.21	52.77
	1.0	46.66	60	68.33	58.33	
pomegranate	0.5	48.33	55	68.33	57.22	58.88

	1.0	53.33	58.33	70	60.55	
mortality mean		46.66	54.99	65.83		
R.L.S.d 0.01		1.33			1.09	1.33

R.L.S.d (0.01) interaction (Phenol , Concentration and Time) N.S
R.L.S.d (0.01) interaction (Phenol and Time) N.S.

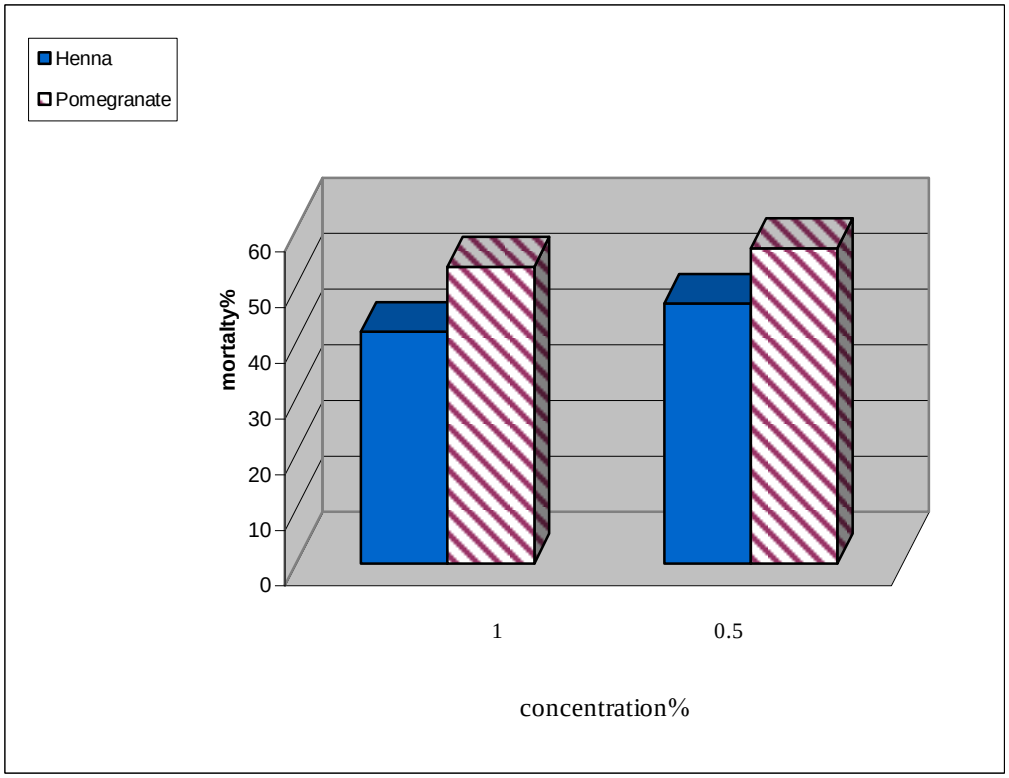
The results elucidated that the PP and PH affect on the mortality rates of nymph of *T.urticae* reached 68.04 and 56.66 % at end the test (table 4), the highest mortality rate percentage were 71.10 and 64.99 % at concentration 1.0 and 0.5% PP respectively, followed by 59.44 and 53.88% at concentration 1.0 and 0.5% HP respectively. Phenol revealed affect on mortality of nymph, reached 63.33 and 50% at concentration 1.0% PP and of HP respectively after 24h of application. Mortality continued in their increasing, with average 78.33 and 68.33% respectively at 72h after springing.

Table (4) Effect of Phenol on nymph mortality of two spotted spider mite *Tetranychus urticae*.

Phenol	**Concentration %**	**Mortality rate % after**			**mortality mean**	**mortality mean**
		24h	**48h**	**72h**		
Henna	0.5	45	53.33	63.33	53.88	56.66
	1.0	50	60	68.33	59.44	
Pomegranate	0.5	56.66	65	73.33	64.99	68.04
	1.0	63.33	71.66	78.33	71.10	
mortality mean		53.74	62.49	70.83		
R.L.S.d 0.01		1.194			0.974	1.194

R.L.S.d (0.01) interaction (Phenol, Concentration. and Time) N.S
R.L.S.d (0.01) interaction (Phenol and Time) N.S.

Egg mortality of the Two spotted spider mite was effected with all treatments after 72h of application (figure 1). PP was significantly more effective than HP (54.99 and 44.16% mortality respectively).Spraying with PP at concentration 1.0 and 0.5% had significant effect on egg mortality, with an average of 56.66 and 53.33% respectively, while the mortality was 46.66 and 41.66% at concentration 1.0 and 0.5% HP respectively.



R.L.S.d (0.01) Phenol 2.63
R.L.S.d (0.01) Concentration N.S
R.L.S.d (0.01) interaction (Phenol and Concentration) N.S

Figure (1) Effect of Phenol in egg mortality rate of two spotted spider mite *Tetranychus urticae* after 72h of application.

Several other studies found that plant phenol had an effect on two spotted spider mite stage. In this respect, Risen (22) recorded that phenols of *Melia azadrach* had an effect on different stages of *T.urticae* .Mehdi and Omran (17) also reported that phenol of *Citrallus colocynthis* caused increased adult and nymphal mortality, reached 53.3%. Phenol are generally characterized by their capacity to precipitate proteins (4 , 5), this trait can lead to the inhibition of enzymes in insects digestive tracts or to the formation of insoluble complexes with dietary proteins, thereby reducing the nutritive value of the ingested food through chemical degradation of essential amino acids (12).

Effects of Phenol-Ureaformaldehyde Copolymer on Two spotted spider mite *T.urticae*

Phenol of pomegranate was the most promising phenol, then formulated as Phenol-Ureaformaldehyde Copolymer (PUC), which was significantly more effective than Urea Formaldehyde Resin (UFR) on

mortality of *T.uricae* adults, reached 63.05 and 25.82% respectively, are represented in table (5). The highest mortality percentage was 68.33% at concentration 1.0% PUC, while the lowest was 23.88% at concentration 0.5% UFR. The mortality of adults on PUC and UFR at concentration 1.0 % after 24h of spraying application, reached 61.66 and 16.66% respectively, it increased when the time lengthened, until to 27h after application, reached 75 and 40% at concentrations mentioned before respectively.

Table (5) Effect of Phenol-Ureaformaldehyde Copolymer (PUC) on adult mortality of two spotted spider mite *Tetranychus urticae*

Treatment	Concentration %	Mortality rate % after			mortality mean	mortality mean
		24h	48h	72h		
PUC	0.5	43.33	56.66	73.33	57.77	63.05
	1.0	61.66	68.33	75	68.33	
UFR	0.5	13.33	23.33	35	23.88	25.82
	1.0	16.66	26.66	40	27.77	
mortality mean		33.74	43.74	55.83		
R.LS.d 0.01		3.46			N.S	3.46

R.L.S.D (0.01) interaction (Treatment ,Concentration and Time) N.S
R.L.S.D (0.01) interaction (Treatment and Time) 5.23
R.L.S.D (0.01) interaction (Treatment and Time) 6.36

The mortality rate of nymph was increased, reached 65.55% on PUC treatment compared with 31.38% on UFR treatment (table 6). PUC at 1.0 and 0.5% concentration were significantly more effective (73.33 and 57.77% of mortality respectively) than UFR 1.0 and 0.5% concentration (30.55 and 32.21% of mortality respectively). The results elucidated that the mortality percentage on nymph, reached 50 and 18.33% at concentration 1.0% PUC and UFR respectively after 24h of application, it continued increasing up to 72h after spraying, with average 88.33 and 41.66% respectively.

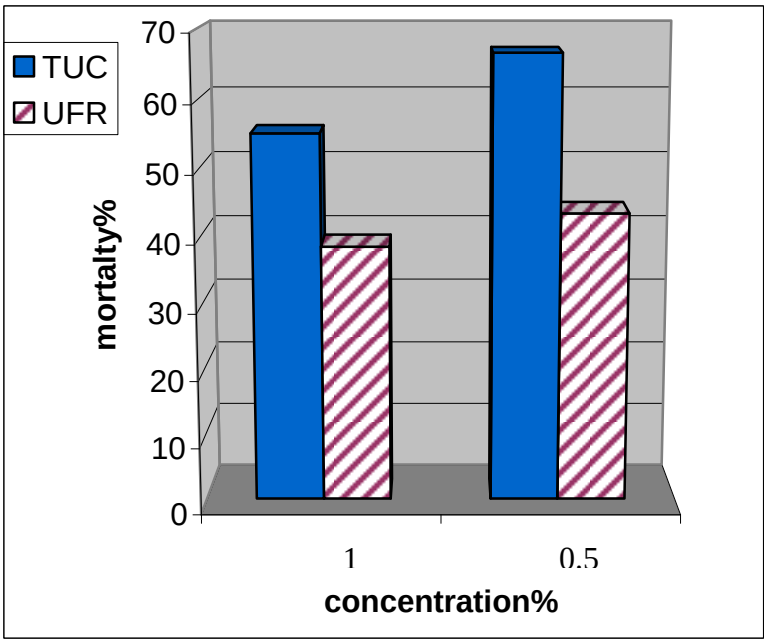
Table (6) Effect of Phenol-Ureaformaldehyde Copolymer (PUC) on nymph mortality of two spotted spider mite *T.urticae*

Treatment	Concentration	Mortality rate %	mortality	mortality

	%	after			mean	mean
		24h	48h	72h		
PUC	0.5	30	61.66	81.66	57.77	65.55
	1.0	50	81.66	88.33	73.33	
UFR	0.5	21.66	36.66	38.33	32.21	31.38
	1.0	18.33	31.66	41.66	30.55	
mortality mean		29.99	52.91	62.49		
R.L.S.d0.01		1.92			1.57	1.92

R.L.S.d (0.01) interaction (Treatment ,Concentration and Time) 3.96
R.L.S.d (0.01) interaction (Treatment and Time) 2.72
R.L.S.d (0.01) interaction (Treatment and Time) 3.34

Egg mortality was increased as shown in Figure (2), reaching 60.83% on PUC treatment after 72h of application, compared with 40.83% on UFR treatment. While no significance for interaction (chemicals materials and concentration) on egg mortality.



S.d (0.01) Treatment 4.23
R.L.S.d (0.01) Concentration 3.76
R.L.S.d (0.01) interaction (Treatment, Concentration) N.S

Figure (2) Effect of Phenol-Ureaformaldehyde Copolymer (PUC) on Egg mortality of two spotted spider mite *Tetranychus urticae* After 72h of application.

The toxicity of phenol was increased, when phenol used as Phenol-Ureaformaldehyde Copolymer at 1.0% concentration as shown in table 5

and 6 , figure 2 compared with Phenol as shown in table 3 and 4 ,figure 1 against two spotted spider mite, the toxic effect found in this study could by attributed to the polymerization of Phenol, this method confirm the possibility of controlled drug release, and can be used to control of the release of active principles in ways suitable for both acute and long-term treatments and it seems enhanced by micro particle adhesion to hairs of the mite body (19,14). Given these encouraging results, further experiments are in progress to assess the suitability of natural active formulations for application as a new tool in integrated control of different pest.

التأثير السام لفينولات الحناء *Lawsonia inermis* و الرمان *Punica granatum* و بوليمرها في *Tetranychus urticae* Koch (Acari : Tetranychidae)

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الملخص

استخلصت الفينولات اولا " من اوراق الحناء *Lawsonia inermis* و قشور ثمار الرمان *Punica granatum* واستعملت مختبرياً في الاختبار الحيوي لتقييم كفاءتها لمكافحة حلم ذات البقعتين *Tetranychus urticae* Koch (Tetranychidae : Acari) ، وكانت فينولات الرمان عند التركيز ١% هي الأكفأ ، إذ سببت نسبة قتل بلغت ٦٠.٥٥ و ٧١.١٠ و ٥٦.٦٦% مقارنة بنسبة القتل ٥٨.٣٣ و ٥٩.٤٤ و ٤٦.٦٦% عند التركيز ١% لمعاملة فينولات الحناء للأطوار البالغ والحوري والبيضة للحلم ذات البقعتين على التوالي.

بعد ذلك حضرت الفينولات الأفضل (الرمان) على شكل بوليمر فينولات اليوريافورمالديهيد ، و ارتفعت سمية الفينول عندما استعمل على شكل بوليمر ، و اثر بشكل فعال في نسبة القتل للطور البالغ للحلم *T.urticae* و بلغت ٦١.٦٦% باستعمال التركيز الأكثر كفاءة ١% بعد ٢٤ ساعة من عملية الرش وازدادت نسبة القتل (٧٥%) بزيادة الوقت حتى ٧٢ ساعة بعد المعاملة ، كما ازدادت نسبة القتل كذلك في الطور الحوري ، إذ تراوحت بين ٥٠ الى ٨٨.٣٣% عند التركيز المذكور أعلاه للفترة بين ٢٤ ساعة وحتى ٧٢ ساعة بعد عملية الرش ، ووصلت نسبة القتل للبيض الى ٦٦.٦٦% نتيجة المعاملة ببوليمر الفينول عند التركيز ١% بعد ٧٢ ساعة من المعاملة . التأثير السام الذي وجد في هذه الدراسة ، قد يعود الى عملية البلمرة للفينول ، إذ إن هذه العملية تعرض إمكانية استعمال الفينول في نظام تحرر مسيطر عليه ، وبذلك يمكن أن تستخدم هذه المواد الطبيعية الفعالة كبدائل لمقاومة الآفات.

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