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Studies on the antifungal activity of natural dyes and their application on textile materials

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ABSTRACT

The present study was taken up to assessment of antifungal activity of quaternary chitosan acrylic acid (Q CS-PAA) nano particles mordant dyed fabrics. The results indicate that the samples treated with Q CS-PAA exhibit higher inhibition activity than the untreated samples. The percentage of mycelium growth inhibition at higher concentration of 8oo ppm was varied from 68.06% on Fusarium solani to 64.09% on Penicillium decumbens. Findings of present study indicate that there are variation in tolerance to nano-QCS-PAA between different fungi Treatments with logwood dye in combination with alum and ferric chloride mordants inhibit fungal growth of both fungi. On the other hand, treatment with cochineal dye alone and in combination with mordants were not effective against the above mentioned fungi (0.00 mm zone of inhibition). The results showed an increase in inhibition % when dyeing cotton tissue was dyed with rhubarb dye by 6.95%, while opposite trend was occurred when the polyester fabric treated with rhubarb dye by 8.21%. Results clearly showed that treatment of textile with logwood dye only resulted in a decrease in efficiency of Q CS-PAA on the growth of fungus, as the zone of inhibition were dropped to 12.69, 20.65 and 8.69 % in polyester,, cotton and blend than non-dyed fabrics respectively. Polyester treated with Q CS-PAA + cochineal dye with alum and ferric mordants lead to increase inhibition zone by 11.11 and 35.48% respectively in comparison to treatment with Q CS-PAA + cochineal dye only. The treatment of Polyester/cotton blend by mordants lead to an increase by 6.95% . On the hand, opposite trend was observed with ferric mordant , as the zone of inhibition were dropped to 0.00 in polyester/ cotton blend treated with ferric .

Keywords: Natural dyes, antifungal activity, Textile, mordants, fabrics, fungi

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INTRODUCTION

Textile materials and clothing are known to be susceptible to microbial attack, as these provide large surface area and absorb moisture required for microbial growth [1,2]. Natural fibers have protein and cellulose, etc, which provide basic requirements such as moisture, oxygen, nutrients and temperature for microbial growth and multiplication [3]. This necessitates the development of clothing that could provide a desired antimicrobial effect.

The antimicrobial properties of chitosan and its derivatives has been widely explored [4-7]. QCS-PAA nanoparticles were synthesized and their fungicidal activities against plant fungus were examined. Also, coating chitosan on conventional fibers appears to be more realistic, since they do not provoke and immunological activity for textiles are toxic to humans and do not easily degrade in the environment. Numerous studies on the antimicrobial activity of chitosan and its derivatives against most economic plant pathogens have been investigated [5,7,8,9,10]. Therefore, these compounds are considered as useful pesticides in the control of pathogenic microorganisms.

Natural dyes are very important for textiles as well as to replace synthetic dyes, because of non-polluting, non-carcinogenic, biodegradable and non-toxic and eco-friendly [11-13]. Also, the natural dyes are environmental friendly, they are attracting the awareness of people [14,15].

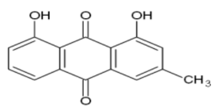
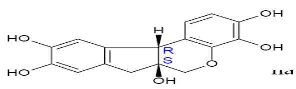
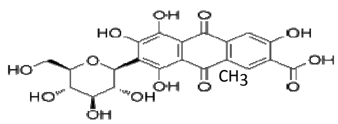
Several studies on the application of natural dyes have been reported [16] to protect the environment for indiscriminate exploitation and pollution by industries. Gupta and Laha [17] found that cotton fabric treated with dye *of Quercus infectoria* plant in combination of alum, copper and ferrous mordants shows good activity at 12% concentration inhibiting microorganisms growth by 45-60%. They added that the microbial growth inhibition increase to 70-90% when alum and cupper are used as mordant. However, the antimicrobial activity completely lost when ferrous sulphat is used.

The present study aims to evaluate the effect of nano-QCS-PAA and some natural dyes and mordants on the antifungal properties of some fungi cellulose filter paper discs and textile fabrics on which nano-QCS-PAA and natural dyes have been applied.

MATERIALS AND METHODS

1. Fabrics:
Cotton and cotton/polyester blend fabrics were received from EL Shorbagy- Egypt, while polyester was received from Golden Tex Company- Egypt.

2. Dyestuffs:
Three natural dyes, in powder form were used throughout this work:-

English Name	Latin name	Structure	The source
1-Extracted rhubarb natural dye	Rheum emodi		Toblamas Co. U.S.A.
2--Extra- Logwood Purple Natural Dye	Haematoxylum		Toblamas Co. U.S.A.
3-Extra- Cochineal Natural Dye	Dactylopius coccus costa		Toblamas Co. U.S.A.

3. Chemicals
Chitosan, with initial 85% degree of deacetylation from Sigma (U.S.A), was used throughout this study.

Laboratory grade reagents were obtained from different sources as follows:

Chemical Name	Chemical Compound	The Source
Acrylic Acid	$CH_2=CHCO_2H$.	ELgomhorya Company, Egypt
Ammonium Persulphate	$(NH_4)_2S_2O_8$	ELgomhorya Company, Egypt
Sodium Bisulphate	$NaHSO_4$	ELgomhorya Company, Egypt
3-chloro-2hydroxy propyl triethyl ammonium chloride (Quat.)	$Cl\,CH_2 - CH(OH) - CH_2N^{+}(C_2H_5)^{-}_3Cl$	Was laboratory prepared by reaction of TEA:HCl with Epichlorohydrine mol/mol
Sodium Carbonate	Na_2CO_3	ELgomhorya Company, Egypt
Ferric chloride	$FeCl_3$	ELgomhorya Company, Egypt
Alum	$KAl\,(SO_4)_2.12H_2O$	ELgomhorya Company, Egypt

4. Preparation of quaternary chitosan acrylic acid nano solution:
QCS-PAA nano particles was synthesized according to [18,19] as in case of obtaining CS-PAA nanoparticles based on an electro static induced interaction between the high positively charged prepared QCS and negatively charged acrylic acid (AA).

5. Mordanting:
Two chemical mordant's namely, alum (10g/l) and ferric chloride (5g/l) were used. The dyed discs or fabrics was treated with mordants at 80C for 60min.

6.Treatment with nano-QCS-PAA and dyeing with natural dyes:
The fabrics (cotton, polyester, and cotton /polyester blended fabric) were treated with 1% nano-QCS-PAA solution with 4% crosslinker (o.w.chitosan) through -Pad once- with pick up 70-80%, air dried and then cured at 130°C for 3min. The fabrics were dyed in bath contain 2 % natural dye, 2 g/l, wetting agent and 2 g/l dispersing agent, at pH 5 and 90°C for 60min. The samples were then mordanted with alum or ferric chloride at 80°C for 60 min then soaped and rinsed with hot and cold water.

7- Assessment of antifungal assay:
7.1: Mycelium growth inhibition: *in-vitro* assay was performed on growth medium treated with different concentrations (0.0, 1000, 2000, 4000 and 8000 ppm) of QCS-PAA nano particles against *Fusarium solani* and *Penicilium decumbens* obtained from Plant Pathology Department, NRC of Egypt . The agar plates were inoculated by a 5mm disc of 7 day- old fungi and then incubated at 27˚C until it fully grow of control plates. Radial inhibition was calculated when growth of mycelia in the control plate reached the edge of the petri-dish. The toxicity of the nanoformulation to growth of fungi in term of percentage inhibition of mycelial growth was calculated by using the formula

% inhibition =dc- dt/dc x100 according to [20].

Where dc= av. increase in mycelial growth in control, dt= av. increase in mycelial growth in treatment.

7.2: Agar well diffusion method: QCS-PAA nano particles of different concentrations were screened for antifungal activity by agar well diffusion method with sterile cork borer of size 6.0 mm according to [21]. 72 hours old cultures grown on PDA medium were used for inoculation of fungal species on PDA plates. An aliquot (0.2ml) of inoculums was introduced to molten PDA and poured in to a petri-dish. After solidification, the appropriate wells were made on agar plate and 500 µl of nanoparticle solution, homogenized using an ultrasonic cleaner, filled in deep blocks. Incubation period of 4-5 days at 27±2˚C was maintained for observation of antifungal activity by measuring zone of inhibition of fungal growth surrounding the well. The zone of inhibition was measured in mm and the experiment was carried out in four replicates.

8. Statistical Analysis:
Data obtained was statistical analyzed using Duncan's multiple range test according to [22].

RESULTS AND DISCUSSION

1. Effect of nano-QCS-PAA on fungal growth:

1.1 -Effect on colony growth:

The data in Table (1) represented the effect of QCS-PAA nano-formulation on the percentage inhibition of mycelial growth of *F. solani* and *P. decumbens* fungi. A positive correlation was reported between the concentrations and colony growth, with the increasing of nano-QCS-PAA concentration a significant increase was found for the two fungi. Also, the sensitivity of the two fungi to nano- formulation was significantly varied, as the % inhibition of *Fusarium solani* for example was significantly high (52.60 mean % inhibition) than *P. decumbens* (50.45).

Table (1): Antimicrobial activity (% inhibition) of nano-QCS-PAA nanoformulation on the linear growth (mm) of *Fusarium solani* and *Penicilium decumbens*

Tested fungi	*Fusarium solani*		*Penicillium decumbens*	
QCS-PAA (ppm)	Growth (mm)	% reduction	Growth (mm)	% reduction
0(control)	90.00 A	0.00	80.75 B	0.00
1000	62.50 C	30.56	63.75 C	21.05
2000	47.50 D	47.22	40.50 E	49.84
4000	34.25 G	61.94	37.25 F	53.87
8000	28.75 H	68.06	29.00 H	64.09
Mean	52.60 A	-	50.45 B	-

-Four replicates were used for each treatment

-Values followed by the same letter are not significantly different at $P \geq 0.05$ according to Duncan's multiple range test.

Data also showed that the percentage of mycelium growth inhibition at higher concentration of 8oo ppm was varied from 68.06% on *F.solani* to 64.09% on *P. decumbens*. Findings of present study indicate that there are variation in tolerance to nano-QCS-PAA between different fungi. According to [23] the degree of fungicidal activity has directed relationship with concentrations of nano-formulation. Almost, similar results were previously obtained by [9,24,25]. Antimicrobial activity expressed as growth reduction of fungi, could be explained, as the amino groups in the degree of fungicidal activity has directed relationship with concentrations of nano-formulation by socking at the cell surface and binding with DNA synthesis [26].

1.2- Effect on zone of inhibition:

The results of the zone of inhibition activity assays are shown in Table (2). Almost, the same results were observed as mentioned before on the effect of nano Q-CS-PAA formulation on fungal linear growth. *F. solani* and *P. decumbens* were highly susceptible to nano-QCS-PAA, as maximum inhibition zones of 25.75 mm and 20.75 mm, respectively were observed at 800ppm. The inhibition zone for the two fungi were gradually increased with increase in concentration of nano-QCS-PAA formulation. As mentioned before the antifungal activity could explained as chitosan derivatives, being polycationic materials, binds to the anionic surface of microorganism cell.

Table 2: Testing the anti fungal activity of nano-QCS-PAA formulation against *Fusarium solani* and *Penicilium decumbens.* (Zone of inhibition values in mm)

Tested fungi	*F.solani*	*P.decumbens*
QCS-PAA	Growth (mm)	Growth (mm)
0(control)	0.00 G	0.00 G
1000	0.00 G	9.75 E
2000	8.50 F	14.50 D
4000	14.25 D	18.50 C
8000	25.75 A	20.75 B
Mean	9.70 B	12.70 A

-Four replicates were used for each treatment

-Values followed by the same letter are not significantly different at $P \geq 0.05$ according to Duncan's multiple range test.

3. Effect of dyeing with natural dyes on zone of inhibition:

The present study in Table (3) demonstrated that untreated filter paper disc samples showed zero percentage fungal reduction, as the untreated filter paper discs (control) show clear fungal growth under them with no zone of inhibition. Filter paper discs treatment with logwood dye without mordant showed maximum zone of inhibition against *F. solani* and *P. decumbens* reached 11.00 and 10.75 mm respectively without significant difference. Moreover , rhubarb dye also showed highest antifungal activity with the inhibition zone of 8.75 and 10.00 mm against *F. solani* and *P. decumbens* fungi respectively. Treatment with logwood dye in combination with alum and ferric chloride mordant inhibit fungal growth of both fungi.

On the other hand, treatment with cochineal dye alone and in combination with mordant were not effective against both fungi (0.00 mm zone of inhibition). The results from these experiment indicated that these natural dyes had antifungal activity both on solutions and substrate. Many investigators reported that common natural dyes are potent antimicrobial agents owing to the presence of a large amount of tannins flavonoids and quinonoids [27]. Similar work on antifungal activity of natural dyes were done by [28,29]

Table (3). Antifungal activity of cellulose paper discs treated with natural dyes and mordant with alum and ferric chloride against *Fusarium solani* and *Penicilium decumbens.* (Zone of inhibition values in mm). (Zone of inhibition, mm)

Tested fungi	***F.solani***	***P.decumbens***
Dyes and mordant	**Growth (mm)**	**Growth (mm)**
Untreated (control)	0.0 h	0.00h
Treated with Rhubarb Dye without Mordant	8.75cd	10.00b
Treated with Rhubarb Dye + alum Mordant	9.50bc	0.00h
Treated with Rhubarb Dye + ferric Mordant	0.00h	0.00h
Treated with Logwood Dye without Mordant	11.00a	10.75a
Treated with Logwood Dye + alum Mordant	7.50f	5.50g
Treated with Logwood Dye + ferric Mordant	8.50de	7.50f
Treated with Cochineal Dye without Mordant	0.00h	0.00h
Treated with Cochineal Dye + alum Mordant	7.50f	0.00h
Treated with Cochineal Dye + ferric Mordant	0.0h	0.00h

-Four replicates were used for each treatment

-Values followed by the same letter are not significantly different at $P \geq 0.05$ according to Duncan's multiple range test.

4. The antifungal activity of nano-QCS-PAA treated fabrics:

4.1- Dyeing with rhubarb:

The effect of nano- QCS-PAA deying rhubarb dye on the antifungal activity against *F. solani* are shown in Table (4). The untreated textile fabrics (control) show clear fungal growth under them with no zone of inhibition, indicating that the textile fabrics by itself does not inhibit fungal activity. In addition, different fabrics treated with nano- QCS-PAA alone showed antifungal activity against *F. solani*. These results were in line with those [30,31] who assessed the effect of quaternary ammonium compounds on textile fabrics . Polyester fabric treated with nano-QCS-PAA+ rhubarb dye with alum mordant lead to significant increase the inhibition zone from 31.00 to 37.00 mm (19.35% increase) in comparison to treatment with nano-QCS-PAA+ rhubarb dye with ferric mordant.

Cotton tissue treated with nano-QCS-PAA dyed with rhubarb and mordant alum also exhibited a highly significant effect on % inhibition *of F. solani* compared with ferric mordant (11.59% increase). Whereas, opposite trend was occurred when the polyester fabric was treated with nano-QCS-PAA dyed with rhubarb without mordant (8.21% decrease).

Concerning the effect of mordant stabilizer of blend fabric on inhibition zone, the treatment of blend by alum and ferric mordants lead to an increase by 5.22 and 1.71% respectively without significant differences .

Table (4):Effect of nano-QCS-PAA treated fabrics dyeing with rhubarb and with alum and ferric chloride mordant on zone of inhibition (mm) of *F. solani*

Treatment	**Fabrics**		
	polyester	**cotton**	**Blend**
Untreated (control)	**0.0 h**	**0.00h**	**0.00h**
Treated with Q CS-PAA only	**33.50de**	**38.75ab**	**28.75f**
Treated with Q CS-PAA + rhubarb dye (without mordant	**30.75ef**	**40.75a**	**28.75f**
Treated with Q CS-PAA + rhubarb dye with Alum mordant	**37.00bc**	**38.50ab**	**30.25f**
Treated with Q CS-PAA + rhubarb dye with Ferric mordant	**31.00ef**	**34.50de**	**29.25f**

-Four replicates were used for each treatment

-Values followed by the same letter are not significantly different at $P \geq 0.05$ according to Duncan's multiple range test.

4.2- Dyeing with logwood:

Results in Table (5) showed that different fabrics treated with nano-QCS-PAA only increased the inhibitory effects against *F. solani* over the untreated fabrics (control)**.** Results clearly showed that nano-QCS-PAA textile fabrics treated with logwood dye only resulted in a decrease in the efficiency of nano-QCS-PAA on fungal growth, as the zone of inhibition were dropped by 12.69, 20.65 and 8.69 % in polyester,, cotton and blend fabrics than non-dyed fabrics respectively.

On the other hand, textile fabrics treated with nano-QCS-PAA dyed with logwood ferric mordant lead to significant increase in the zone inhibition from 29.25 to 38.50mm (31.62% increase) for polyester, from 30.75 to 35.75mm (16.26 increase) for cotton and blend textile from 26.25 to 27.00mm (2.78% increase). These results indicates that ferric mordant provide protection against *F. solani* growth.

Table (5):Effect of nano-QCS-PAA treated fabrics dyeing with logwood dye mordant with alum and ferric chloride on zone of inhibition (mm) of *F. solani*

Treatment	Fabrics		
	polyester	cotton	Blend
Untreated (control)	0.0 h	0.00h	0.00h
Treated with Q CS-PAA only	33.50c	38.75a	28.75de
Treated with Q CS-PAA + logwood dye (without mordant	29.25d	30.75d	26.25f
Treated with Q CS-PAA + logwood dye with Alum mordant	37.50ab	38.75a	28.75de
Treated with Q CS-PAA + logwood dye with Ferric mordant	38.50a	35.75b	27.00ef

-Four replicates were used for each treatment

-Values followed by the same letter are not significantly different at $P \geq 0.05$ according to Duncan's multiple range test.

3- Dyeing with Cochineal:

As mentioned before from Tables (4&5) different fabrics treated only with nano-QCS-PAA showed antifungal activity against *F. solani* over the untreated fabrics (Table, 6). Results also clearly showed that polyester fabric treated with nano-QCS-PAA dyed with cochineal dye stabilize with alum mordant lead to significant increase inhibition zone from 31.50 to 35.0 mm (11.11increase).The same trend was also observed with ferric mordant leading to increased by 33.33% , compared to nano-QCS-PAA + cochineal dye treatment.

Table (6):Effect of nano-QCS-PAA **treated fabrics dyeing with Cochineal dye mordant with alum and ferric chloride on zone of inhibition (mm) of *F. solani***

Treatment	Fabrics		
	polyester	cotton	Blend
Untreated (control)	0.0 h	0.00h	0.00h
Treated with Q CS-PAA only	33.50cd	38.75b	28.75f
Treated with Q CS-PAA + Cochineal dye without mordant	31.50de	38.25b	28.75f
Treated with Q CS-PAA + Cochineal dye with Alum mordant	35.00c	38.00b	30.75ef
Treated with Q CS-PAA + Cochineal dye with Ferric mordant	42.00a	41.50a	0.00g

-Four replicates were used for each treatment

-Values followed by the same letter are not significantly different at $P \geq 0.05$ according to Duncan's multiple range test.

On the other hand, blend fabric treated with nano-QCS-PAA dyed with cochineal stabilize by alum mordant lead to significant increase in the zone inhibition from 28.25 to 30.75mm (6.25% increase), while opposite trend was observed with ferric mordant , as the zone of inhibition were dropped to 0.00mm. Decline of antifungal activity in the case of ferric chloride mordant samples could be the consequence of complex formation between active functional groups of the dye with chloride mordant. The same conclusion was reported by Gupta and Laha (2007) who reported that the use of ferric salt suppresses the activity of dye, most likely due to the iron binding capacity of tannins.

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