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Improvement the efficacy and physical properties of some recommended pesticides on cotton leaf worm, *Spodoptera littoralis* (BOISD.) by using different nano surfactants

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Abstract
The toxicity effect of two different chemical synthesized nano-surfactants : anionic surfactant (N-(2-hydroxyethoxy)-O-(2-hydroxyethyl)-N-(2-(2-mono mercapto acetate)) hydroxyl ammonium-4-dodecyl benzene sulfonate) and nonionic surfactant (Di Phosphate monopoly ethylene glycol mercapto acetate, tri ethanol amine monophosphate polyethylene glycol oleiate) has been studied. Also the effect of these nano synthetic Surfactants on physic-chemical properties and efficiency of Nasrthrin 25% EC (Cypermethrin) and Tak 48% EC (Chlorpyrifos) pesticide formulations has been measured against 2nd instar larvae of cotton leafworm, *Spodoptera littoralis* (BOISD.). LC_{50}s and LC_{90}s values were measured and tabulated. Such formulations are being proven to be as effective as traditional formulations due to their inherent ability to achieve controlled delivery of their respective active ingredients. The changing in the surface tension and pH values were affected on the insecticidal activity of the tested pesticides and also affected by difference of surfactant with type of pesticide.

Keywords: Nano-surfactants, Surface tension (ɣ) and *Spodoptera littoralis*

Introduction
Nano emulsions have a droplet sizes of 20-200nm in oil / water or water / oil types. There formulations make beneficial in numerous industries like cosmetic, pharma and food and as agrochemical formulations [1, 2] due to their low viscosity, good stability, dispersity, and transparent properties. The use of chemical pesticides for agricultural pest control and the consequent damage to the ecosystem at air, water and soil levels has become a factor of common knowledge. Nanoemulsion formulations allow the delivery of pesticides and prevent the premature degradation of the active ingredient by addition of low surfactant concentrations [3, 4]. The toxicity towards non-target organisms is decreased by using a controlled release strategy [5, 6]. The effects of adjuvants(surfactants) used in pesticide formulation, have been widely studied. Many adjuvants, including surfactants, are biodegradable in the soil environment, and thus their effects on the biodegradation of a pesticide in soil and sediment may be limited, as shown in field trials. In (2006), Guang-Guo Ying studied the Fate behavior and effects of surfactants and their degradation products in the environment [7]. Xiarchos and Doulia (2006) measured the solubility of alachlor pesticide by using of nonionic surfactants [8]. N. Raman *et al.* (2008) studied the insecticidal activity of the Schiff – base derived from anthranilic acid acetoacetanilide and its copper complex on *Spodoptera litura* [9]. Castro *et al.* (2014) improved the pesticide efficacy and the absorption of foliar to be useful for biocides, defoliants and growth regulators by adding surfactants. Also, the additives were used to decrease cost and pollution [10]. Nuruzzaman *et al.* (2016) developed the releasing properties of nanoencapsulated pesticide by storing the encapsulated active ingredients from dissolution and increasing the activity of pest control for a longer time [11]. The surfactants simplified the transport of chlorpyrifos pesticide under equilibrium and dynamic hydrologic conditions [12]. Two common surfactant application practices in agrosystems: pesticide spraying and irrigation with waste water had been studied by Dollinger *et al*. (2018) [12].
In our new search we will study the efficacy of two different chemical nano-surfactants: anionic surfactant (N-(2-hydroxyethoxy)-O-(2-hydroxyethyl)-N-(2-(2-mono mercapto acetate) hydroxyl ammonium-4-dodecyl benzene sulfonate) and nonionic surfactant (Di Phosphate monopoly ethylene glycol mercapto acetate, tri ethanol amine monophosphate polyethylene

glycol oleiate) complexes. the effect of these complexes on physic-chemical properties and insecticidal activity of Nasrthrin 25% EC (Cypermethrin) and Tak 48% EC (Chlorpyrifos) pesticide formulations against 2nd instar larvae of cotton leaf worm, *S. littoralis* (BOISD).

Materials and Methods

Growing of cotton leaf worm

Cotton leaf worm, S.littoralis (BOISD.) was rearing in the Laboratory of Plant Protection Research Institute, Egypt. It were cultured under controlled conditions (30±2 ºC and 65±5% R.H.) on castor oil leaves for several generations. The cotton seeds were sowed in Plastic bags (25X 40X15 cm) included soil with peatmus. The seeds were planted then irrigated and fertilized as required for two month [13].

Tested Insecticide

Nasrthrin 25% EC (Cypermethrin) and Tak 48% EC (Chlorpyrifos) pesticide formulations.

Synthesis and Characterization of the Nano surfactant complexes:

Synthesis of (N-(2-hydroxyethoxy)-O-(2-hydroxyethyl)-N-(2-(2-mono mercapto acetate) hydroxyl ammonium- 4-dodecyl benzene sulfonate) by esterification reaction of 0.3mole of triethanol amine (T.E.A) with 0.3 mole of mercapto acetic acid in presence of toluene as a solvent Then was neutralized with dodocyl benzene sulfonate to give the final product nano- anionic surfactant with Chemical formula ($C_{26}H_{47}NO_{10}S_2$).

Synthesis of (Di Phosphate monopoly ethylene glycol mercapto acetate, tri ethanol amine monophosphate polyethylene glycol oleiate) by esterification reaction of 3mole of polyethanol glycol with 3mole of phosphoric acid to give polyethanol glycol phosphate compound. 4mole of product was reacted with 1mole of triethanol amine then reacted with 2 mole of oleic acid and 2mole of mercapto acetic acid. The product was purified in p-toluene sulfonic acid and reflux. A final product, nano-nonionic surfactant with Chemical formula ($C_{83}H_{165}NO_{41}S_2P_3$).

Description of the synthetic surfactant complexes by Micro Elemental Analysis: These analyses were achieved by a Vario Elementar (National Research Center, Cairo, Egypt) [14].

Transmission electron microscopy (TEM): Particle size and TEM image were measured by a (JEOL-JEM-2100) electron microscope operating at high resolution (200KV). TEM samples were prepared by dipping of fine sample powders onto a copper handful coated with holey carbon foil and dried at room temperature [15].

Studying the effect of adding of synthetic nanosurfactants on physico-chemical properties of tested pesticides formulations:

Determination of Surface tension (ˠ), critical micelle concentration (CMC), Effectiveness (πcmc) and Minimum surface area (Amin): Surface tension measurements were obtained using De-Nöuy Tensiometer krüss K-6 with a platinum ring. Freshly aqueous solutions of the synthesized Schiff base ammonium compound and their metal complexes were prepared in double distilled water in a wide concentration rang of (10 to 0.01ml.mole/l) at 25ºC. The effectiveness of the synthetic surfactants is calculated from the following equation: $\pi_{CMC} = \gamma_{\circ} - \gamma_{CMC}$ Where, $\gamma_{\circ}$ is the surface tension of the bidistilled water. ,γ_{CMC} is the surface tension of the critical micelle concentrations. The area occupied by each surfactant molecule at the air-water interface at the complete saturation or at complete coverage (Amin) can be calculated as follows: Amin = 10^{16} / [μmax . Nav] Where, Amin = minimum surface area in nanometer square (nm^2) Where,μmax = maximum surface excess and N_{av} = Avogadro's number (6.023 X 10^{23}).

Measurement of acidity of the prepared surfactants , the applied insecticide and mixture sprays solution (surfactant + insecticide): by using automatic pH Meter-2601.

Study the insecticidal activity:

The chemical insecticides may provide a great insecticidal activity and decreasing their application rates, pollution and costs by adding surfactants [16]. The insecticidal activity are affected by Surfactant type, surface tension of synthesized surfactants**,** Pesticide type and pH of mixed pesticide solution with surfactant.

Study the insecticidal activity of the applied insecticide formulations, prepared surfactant complexes and their mixtures: The experiments were carried out in the laboratory-field of Plant Protection Research Institute, ARC, Dakahlia, Egypt. The method of indirect exposure was used throughout the present investigation. Five concentrations of each tested pesticides formulations, Nasrthrin 25% EC (Cypermethrin) formulation (20, 40, 80, 160 and 320 ppm) and Tak 48% EC pesticide formulation (0.1, 1.0,1.5, 2.0 and 2.5 ppm) against 2nd instar larvae of cotton leafworm were used. Leaves of cotton plants were divided into five replicates. Five concentrations of each new nano surfactants were tested against the two of cotton aphids, anionic surfactant (N-(2-hydroxyethoxy)-O-(2-hydroxyethyl)-N-(2-(2-mono mercapto acetate) hydroxyl ammonium- 4- dodecyl benzene sulfonate), (500, 1000, 3000, 5000 and 7000 ppm) and nonionic surfactant (Di Phosphate monopoly ethylene glycol mercapto acetate, tri ethanol amine monophosphate polyethylene glycol oleiate), (500, 1000, 2000, 4000 and 6000 ppm). Then we mixed the calculated LC_{50} values of the tested new surfactants with the tested pesticides formulations. Every five Plastic bags contained leaves of cotton plants were sprayed in each concentration of each tested pesticides, synthetic surfactants and their mixtures in clean water as untreated check (control). Each 10 of 2nd instar larvae of ***S. littoralis*** (BOISD**.**) were transferred to the treated Plastic bags contained leaves of cotton plants, which then covered with muslin cloth held in position by rubber bands. After 48 hours the alive larvae was counted.

Statistical analysis: The mortality percentages were calculated and plotted by LDP line program. The tested compounds were compared for their efficiency on 2nd instar larvae of cotton leaf worm according to their LC_{50} and LC_{90} of the toxicity lines. Toxicity index (Ti) was calculated using equation as follow: Ti=(LC_{50} of the most toxic insecticide/ LC_{50} of less toxic insecticide)X100.

Results and Discussion

Characterization of the synthetic surfactant complexes: Elemental Analysis is a process used to determine the elemental composition of organic compounds as showed in table (1). The calculation of the elemental composition by mass is defined as the empirical formula for a compound. This small footprint analyzer needs less than 1 mg of sample for simultaneous C, N, H, S, O, Cl, Br, F and P analysis. Transmission electron microscopy (TEM) is used to determine morphology – size, shape, arrangement of particles on scale of atomic diameters as showed in table (2). The stabilization of these surfactants due to the formation of nano shells. TEM images of anionic and nonionic nanoparticles as prepared by liquid reflux process showed in figure (1), revealing high aggregation due to they have a highly molecular weight due to agglomerate on synthesis. So, nano-nonionic surfactant has a higher particle size than nano-anionic surfactant.

Table 1: *Micro elemental analysis of the synthetic surfactants.*

Surfactants	Chemical Formula (Molecular Weight)	Elements Percentage (%)
N-(2-hydroxyethoxy)-O-(2-hydroxyethyl)-N-(2-(2-mono mercapto acetate) hydroxyl ammonium- 4-dodecyl benzene sulfonate	$C_{26}H_{47}N O_{10}S_2$ (597.26)	C: Calculated 52.24, Found 52.92 H: Calculated 7.93, Found 8.23 N: Calculated 2.34, Found 2.31 O: Calculated 26.76, Found 26.14 S: Calculated 10.73, Found 10.49
Di Phosphate monopoly ethylene glycol mercapto acetate, tri ethanol amine monophosphate polyethylene glycol oleiate	$C_{83}H_{160}NO_{43}S_2P_3$ (2015.90)	C: Calculated 50.04, Found 50.09 H: Calculated 8.40, Found 8.36 N: Calculated 0.74, Found 0.70 O: Calculated 33.01, Found 32.96 S:Calculated 3.21, Found 3.22 P: Calculated 4.60, Found 4.67

Table 2: Particle size of the synthetic surfactants.

Surfactant symbol	size(nm)
N-(2-hydroxyethoxy)-O-(2-hydroxyethyl)-N-(2-(2- mono mercapto acetate) hydroxyl ammonium- 4- dodecyl benzene sulfonate	20– 30
Di Phosphate monopoly ethylene glycol mercapto acetate, tri ethanol amine monophosphate polyethylene glycol oleiate	30– 45

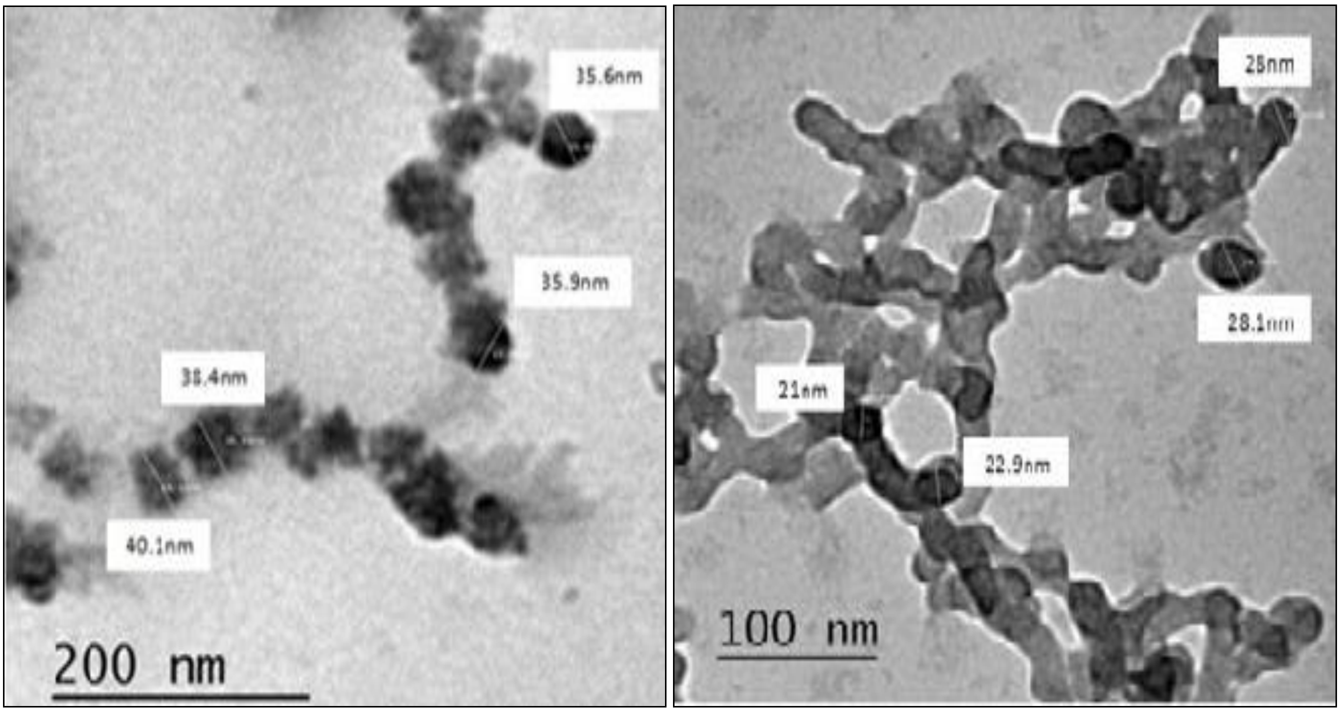

Fig 1: TEM image of : A) Nano anionic surfactant, N-(2-hydroxyethoxy)-O-(2-hydroxyethyl)-N-(2-(2-mono mercapto acetate) hydroxyl ammonium- 4- dodecyl benzene sulfonate. and B) Nano nonionic surfactant, Di Phosphate monopoly ethylene glycol mercapto acetate, tri ethanol amine monophosphate polyethylene glycol oleiate.

Studying of the physico-chemical properties:

Surface tension were measured by De-Nöuy Tensiometer krüss K-6 with a platinum ring. Then we were calculated effectiveness from the equation: $\pi_{CMC} = \gamma_{\circ} - \gamma_{CMC}$. The minimum surface area were calculated as follows: $A_{min} = 10^{16} / [\mu_{max} . N_{av}]$,as shown in table (3). The concentration of the surfactant at which no additional decrease in the surface tension is defined as the critical micelle concentration. (CMC) [17, 18]. The effectiveness of the synthesized anionic surfactant is more than nonionic surfactant. From the data in the table (3), it is clear that the maximum surface excess decrease by increasing the hydrophobic chain length. the maximum surface excess of the synthesized surfactants is arranged as follow: nonionic > anionic. Data in table (4) indicates that anionic surfactant (triethanol amine mono mercapto acetate dodocyl benzene sulfonate) has pH value less than (more acidic) that of nonionic surfactant (triethanol amine mono phosphate poly ethylene glycol oleiate, diphosphate monopoly ethylene glycol mercapto acetate). In case of Nasrthrin 25% EC (Cypermethrin) and Tak 48% EC formulations mixed with the tested Nano prepared surfactants, the surface tension values are arranged as anionic Surfactant mixed with each tested pesticide formulations more decreased than nonionic Surfactant mixed with tested pesticides. while Tak 48% EC formulation has higher surface tension without any tested surfactants. But the pH value are arranged as anionic surfactant mixed with tested pesticide formulations more acidic than with nonionic Surfactant. The applied nanosurfactants decreased pH and surface tension values of insecticide spray solution.

Table 3: Surface properties of the two prepared.

Compound	CMC X 10^7 Mole/L	γ_{CMC} mN/m	π_{CMC} mN/m	μ_{max} x10^{11} mol.k^{-1}.cm^{-1}	A_{min} nm^2
N-(2-hydroxyethoxy)-O-(2-hydroxyethyl)-N-(2-(2-mono mercapto acetate) hydroxyl ammonium- 4- dodecyl benzene sulfonate.	1.01	28.82	38.10	17.40	9.98
Di Phosphate monopoly ethylene glycol mercapto acetate, tri ethanol amine monophosphate polyethylene glycol oleiate.	1.56	38.80	34.91	9.62	17.20

Table 4: The pH and surface tension values

Compound	pH	Surface Tension
N-(2-hydroxyethoxy)-O-(2-hydroxyethyl)-N-(2-(2-mono mercapto acetate) hydroxyl ammonium- 4- dodecyl benzene sulfonate.	2.35	36.82
Di Phosphate monopoly ethylene glycol mercapto acetate, tri ethanol amine monophosphate polyethylene glycol oleiate	5.03	31.89
Nasrthrin 25% EC (Cypermethrin).	8.6	45.06
Nasrthrin 25% EC (Cypermethrin)+ N-(2-hydroxyethoxy)-O-(2-hydroxyethyl)-N-(2-(2-mono mercapto acetate) hydroxyl ammonium- 4- dodecyl benzene sulfonate.	6.9	33.89
Nasrthrin 25% EC (Cypermethrin)+ Di Phosphate monopoly ethylene glycol mercapto acetate, tri ethanol amine monophosphate polyethylene glycol oleiate.	7.2	35.07
Tak 48% EC.	3.80	56.00
Tak 48% EC + N-(2-hydroxyethoxy)-O-(2-hydroxyethyl)-N-(2-(2-mono mercapto acetate) hydroxyl ammonium- 4- dodecyl benzene sulfonate.	3.25	34.01
Tak 48% EC + Di Phosphate monopoly ethylene glycol mercapto acetate, tri ethanol amine monophosphate polyethylene glycol oleiate.	4.91	44.70

Study the insecticidal activity properties

Study the efficacy of the synthetic nano surfactants: The obtained results in table (5) show that the synthetic nonionic surfactant (Di Phosphate monopoly ethylene glycol mercapto acetate, tri ethanol amine monophosphate polyethylene glycol oleiate) confirmed to be the most effective compound against laboratory strain of 2nd instar larvae of the cotton leafworm followed by synthetic anionic surfactant (N-(2-hydroxyethoxy)-O-(2-hydroxyethyl)-N-(2-(2-mono mercapto acetate) hydroxyl ammonium- 4- dodecyl benzene sulfonate). The prepared anionic surfactant has surface tension more less than nonionic surfactant. While, pH value of nonionic surfactant more than anionic surfactant, so the insecticidal activity increases with decreasing surface tension value.

Study the efficacy of tested pesticides formulations: The obtained results in table (5) show that Nasrthrin 25% EC (Cypermethrin)showed the $LC_{50's}$ of 24.64 ppm. However, $LC_{90's}$ reached 58.20 ppm. While, Tak 48% EC formulation showed the $LC_{50's}$ of 0.30 ppm. However, $LC_{90's}$ reached 1.98 ppm. Tak 48% EC (Chlorpyrifos) gave highly efficient against 2nd instar larvae of the cotton leaf worm than Nasrthrin 25% EC (Cypermethrin). the insecticidal activity increases with decreasing pH value of Tak 48% EC (Chlorpyrifos) than pH value of Nasrthrin 25% EC (Cypermethrin).

Study the efficacy of nanosurfactant complexes adding to the tested insecticides formulations: The obtained results in table (5) show that Nasrthrin 25% EC (Cypermethrin)mixed with synthetic anionic (An) surfactant with($LC_{50's}$ =1.93 ppm, $LC_{90's}$ = 10.29 ppm) proved to be the most effective compound followed by Nasrthrin 25% EC (Cypermethrin)with synthetic nonionic surfactant (NI) with ($LC_{50's}$ =5.87 ppm, $LC_{90's}$ = 27.20 ppm) and Nasrthrin 25% EC (Cypermethrin) with($LC_{50's}$ =24.64 ppm, $LC_{90's}$ = 58.20 ppm). The insecticidal activity of tested Nasrthrin 25% EC (Cypermethrin) insecticide with the tested surfactants as mixtures found to be depend on decreasing of surface tension and pH values than Nasrthrin 25% EC (Cypermethrin) insecticide alone solution. Nonionic surfactants make part of most pesticide formulations [19]. They enhance pesticide performance and pesticides promote or inhibit the photolytic degradation of nonionics [20]. Also, *Tak* 48% *EC* (Chlorpyrifos) with nano-anionic surfactant proved to be the most effective compound with ($LC_{50's}$ =0.009ppm, $LC_{90's}$ = 0.75 ppm) followed by *Tak* 48% *EC* with nano-nonionic surfactant (NI) with ($LC_{50's}$ =0.05 ppm, $LC_{90's}$ = 1.01 ppm) and *Tak* 48% *EC* only formulation with ($LC_{50's}$ =0.3 ppm, $LC_{90's}$ = 1.98 ppm) as shown in table (5). The insecticidal activity of tested *Tak* 48% *EC* insecticide with the tested surfactants as mixtures found also to be depend on decreasing of surface tension and pH values. The behavior of amphoteric surfactants is dependent on the value of pH. The ability of changing charge from net cationic to anionic is depending on changing value of pH from low to high, while zwitterionic behavior at intermediate pH [21]. They have a clear biological activity. As, anionic surfactants can bind to bioactive macromolecules such as peptides, enzymes, and DNA. Surfactants were obligated with proteins and peptides and may change the folding of the polypeptide chain and the surface charge of a molecule. This can improved the biological function[22]. The adsorption behavior of nonionic surfactant adding to pesticides is matrix dependent due to the ability of the compound to ionise at environmentally relevant pH [23].

Table 5: The insecticidal activity of the two prepared surfactant complexes, Nasrthrin 25% EC (Cypermethrin), Tak 48% EC and their mixture solutions against 2nd instars. larvae of the cotton leafworm

Treatment	LC_{50}(ppm)	LC_{90}(ppm)	Toxicity index (Ti)	Slope
N-(2-hydroxyethoxy)-O-(2-hydroxyethyl)-N-(2-(2-mono mercapto acetate) hydroxyl ammonium- 4- dodecyl benzene sulfonate.	1168.70	6397.72	52.71	1.736
Di Phosphate monopoly ethylene glycol mercapto acetate, tri ethanol amine monophosphate polyethylene glycol oleiate.	616.01	4555.70	100	1.475
Nasrthrin 25% EC (Cypermethrin).	24.64	58.20	7.83	2.400
Nasrthrin 25% EC (Cypermethrin)+ N-(2-hydroxyethoxy)-O-(2-hydroxyethyl)-N-(2-(2-mono mercapto acetate) hydroxyl ammonium- 4- dodecyl benzene sulfonate.	1.93	10.29	100	1.324
Nasrthrin 25% EC (Cypermethrin)+ Di Phosphate monopoly ethylene glycol mercapto acetate, tri ethanol amine monophosphate polyethylene glycol oleiate.	5.87	27.20	32.88	2.034
Tak 48% EC.	0.30	1.98	3.00	1.181
Tak 48% EC + N-(2-hydroxyethoxy)-O-(2-hydroxyethyl)-N-(2-(2-mono mercapto acetate) hydroxyl ammonium- 4- dodecyl benzene sulfonate.	0.009	0.75	100	0.511
Tak 48% EC + Di Phosphate monopoly ethylene glycol mercapto acetate, tri ethanol amine monophosphate polyethylene glycol oleiate.	0.05	1.01	18.00	0.541

Conclusion

It was concluded that, the main role of using Nano anionic and nonionic surfactants as adjuvants with Nasrthrin 25% EC (Cypermethrin)and Tak 48% EC (Chlorpyrifos) insecticide formulations to increase the effectiveness of insecticide by changing the properties of the water (physico-chemical) that will be used for dilution of pesticide by decreasing pH and surface tension values. The wet ability and spreading of insecticide on the treated surface increase by reducing of the surface tension of the insecticidal spray solution, and thus increases insecticidal activity [24]. Also, the decrease in pH value indicates an increase in positive charge of spray solution leading to increase of the attraction between spray solution and treated plants leaves surface, which have a negative charge, thus increase the insecticidal efficiency [25]. The end outcome is a successful pest control with less environmental damage.

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