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Preliminary studies on formulation of Neem oil based micro emulsion and insecticidal effect on eggplant Mealy bugs

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Abstract

Neem oil based microemulsions were prepared by two surfactants Tween 20 and DMSO (Dimethyl sulphoxide) for preliminary on eggplant mealybugs on Brinjal eggplant (Solanum melogenic) family Solanaceae. The microemulsions has three phases Oil phases, Surfactant phase and aqueous phases. Six separate mixtures of Tween 20 and Neem oil in various weight ratios-1:0, 1:1, 2:2, 3:3, 4:4, and 5:5-were taken and diluted to one litre. The physical factors like pH, Electric conductivity, Transparency, emulsion size determination by light microscope. Pesticide efficiencies were tested in field under greenhouse conditions. Tween 20 based micro emulsions has highest efficiency in first, second and third spray intervals compared to DMSO based micro emulsions on eggplant mealybugs. pH ranges of Tween 20 freshly prepared micro emulsions from 3.76 -5.88; in Electric conductivity 58-170 and one month old emulsions has 3.21 to 6.03; in Electric conductivity 58-170; in Electric conductivity 50-200 whereas DMS based Neem micro emulsions pH ranges from 4.07-6.02 freshly prepared; in Electric conductivity 51-128 and one month old emulsions ranges 5.22-6.02; in Electric conductivity 51-65. Transmission of freshly prepared of Tween 20 microemulsions from 0.27-36.1and one month old emulsions has 0.1 - 60.57 whereas Transmission of freshly prepared of DMSO based Neem microemulsions ranges from 0.1 to 60.1 and one month old emulsions ranges 0.1-30.63. Preliminary studies indicated that Tween 20 based micro emulsion of Neem oil has been better pesticide efficiency compared to DMSO based micro emulsion of Neem oil.

Keywords: Neem oil, Tween 20, DMSO, Mealybugs, Microemulsions.

Introduction

Micro emulsions, which have a range of size 5-200 nm and with very short interfacial tension, are thermodynamically constant isotropic ally transparent dispersions of two immiscible liquids, such as oil and water. They are stabilized by an interfacial layer of surfactant molecules[1]. When compared to conventional creams and lotions, which are made of surfactant, water, and oil, they have a better therapeutic effect[2]. Azadirachta indicia, sometimes known as neem, neem tree, or Indian lilac, belongs to the family Meliaceae. Azadirachta indica, sometimes known as neem, neem tree, or Indian lilac, belongs to the family Meliaceae. One of only two species in the genus Azadirachta, it is indigenous to the Indian subcontinent and the majority of the African nations. Some sources also place its native range as covering Pakistan, Sri Lanka, Thailand, Malasiya, and Indonesia. The tree is most frequently used in India. Tropical and semi-tropical climates are where it is commonly grown. However, India is home to more than 60% of the world's neem species. A particularly valuable tree with a lifespan of 1500–2000 years is the neem. One of the most effective ways to keep high agronomic output however minimizing the harmful effects of pesticides on the environment is currently emerging as the use of botanical pesticides. Compared to synthetic pesticides with longer environmental retention half-lives, botanicals are thought to be more environmentally friendly and less likely to harm beneficial pest, since they breakdown more quickly than the majority of commercial chemical pesticides.

The fate of azadirachtin or neem products has yielded a variety of effects. To present, laboratory research have primarily been used to investigate the fate and effects of azadirachtin[3,4]. After being applied to olive trees, azadirachtin has a half-life of roughly 20 hours and is quickly broken down by sunshine[5,6]. discovered that azadirachtin had a fading time of approximately 20 days in soil at 25°C, and that when the soil was autoclaved, it increased to 31–42 days, showing that azadiractin must be disintegrated by soil bacteria in the top soil. Additionally[7] discovered that azadirachtin has a half-life of 1–12 days. Azadiractin A, Azadirachtin B, Salanine, Nimbin, and other compounds are the primary active components of neem oil. Azadirachtin has the ability to kill a variety of insect species due to its biological features, which include anti-feed ant, growth inhibition, ovipositor deterrent, etc.

High solubilization capabilities for both polar and non-polar chemicals, low interfacial tensions, fine microstructures, and spontaneous production are just a few of the unique characteristics of microemulsions[8]. Microemulsions have shown the capability to protect the pesticide, their controlled release and enhances the solubility of pesticides. These unique

characters microemulsions convert an impending system for both hydrophilic and hydrophobic pesticides. Therefore, micro emulsion systems have been using in several applications of pharmaceutical, industrials, agriculture etc. Neem oil microemulsion stability for the period oflongstorage and use is very crucial due to the presence of active ingredients like azadirachtin and other elements of insecticidal property. Therefore a long period stability various combination of surfactants used by trial and error method.

Aqueous extracts of milled neem kernels have also been used as a pesticide in India for about 2,000 years to control insect infestations[9]. The use of neem-based pesticide has expanded over the earlier 30 years as an outcome of the recent discovered the azadirachtin, the primary active ingredient that gives neem its insecticidal properties[10,11]. A popular biopesticide currently in use, azadirachtin is still the most effective botanical pesticide used in agriculture globally[10,12,13]. But more research is needed to understand how it works, particularly in respect to neurophysiological effects and potential long-term effects.

The purpose of this study is to create a neem microemulsion using neem oil and a surfactant comparison of Tween 20 and DMSO in a particular ratio. This systematic study involves the preparation of neem oil micro emulsion with Tween 20 and DMS which act as a synergist and surfactant. The formulated formulations are less time consuming and are in great in demand in the pharmaceutical and agrochemical industries. These improved formulations are good absorption rates and give superior and long lasting results.

Materials and methods

Neem oil was collected from Biofuel Unit, Karnataka State Akkamahadevi Woman's University Vijayapura-Karnataka. Tween 20 (Polysorbate 20 is a nonionic surfactant and emulsifier), DMSO surfactant (Dimethyl sulfoxide), was taken by Himedia Laboratories. Deionized water collected, Botany Department, Karnataka State Akkamahadevi Woman's University Vijayapura-Karnataka.

Test insects: Eggplant mealybugs were collected from green house, where planted *Solanum melogenic* (eggplant) highly infected with Mealybug species *Coccidohystrix insolita* .It is a common pests in eggplant growing regions in Vijayapura district-Karnataka. This pest not only reduced yields but also wane vigour ultimately die. Brief description of targeted mealy bugs, The Mariana Islands' island of Guam is where the initial document of the eggplant mealybug, *Coccidohystrix insolita* (Green), has been made. There are 17 pairs of cerarii on the edges, 9-segmented posterior ostioles present but missing, and well-developed legs. Each of the anal lobes has a sclerotized ventral bar and is well-developed. On the ventral surface of the abdominal segments, there are many of quinquelocular pores. Numerous multilocular disc pores can be found on the venter of the abdominal segments and a few of these holes can also be found on the segment dorsum. On the sub-margins of the abdominal segments, there are ventral oral collar ducts. No oral rim ducts are present at all.

Preparation of Neem Oil Micro Emulsion: The creation of Neem oil formulations using the micro emulsion techniques. Neem oil and surfactant were first mixed in a 1:1 ratio. The aqueous titration method, which includes adding water incrementally to each weight ratio of oil and surfactants, was employed. Using a magnetic stirrer, each liquid was thoroughly combined to achieve homogeneous dispersion. In order to prevent the incorporation of surface-active contaminants, distilled water was employed in these formulations. Water and ambient temperature were used to titrate the mixture while it was continuously stirred. Distilled water was added in the amount needed for dilution at the point when the mixture started to get turbid. Six separate mixtures of varying weight ratios of oil and Tween, DMSO, 1:0, 1:1, 2:2, 3:3, 4:4, and 5:5 were taken and diluted to a volume of one litre Table-1. The prepared microemulsions are tested various physical characterization and their pesticide efficiencies were examined at field.

The use of surfactant and water for formulations of neem oil because, that could explain commercial neem-based pesticides with additional non-AZA-compounds are not desensitized to them[14]. Same idea was followed in this study in Tween 20, DMSO and distilled water in order to avoid hypersensitization of neem oils for microemulsions preparation.

Table-1: Components are used for preparation of neem oil based micro emulsions formulations.

Constituent I	Constituent II	Constituent III
phase (Oil)	Surfactants	Aqueous phase
Oil (Neem)	Tweens 20	Distilled water
Oil (Neem)	Dimethyl sulphoxide (DMSO)	Distilled water

Physicochemical properties: Characterization of Neem Micro-emulsion: Particle size was observed under transmission light microscope. The size and size distribution of neem oil micro emulsion by using Olympus CKX53 Model.

Surfactant and cosurfactant screening: Tween 20 and DMSO were among the hydrophilic surfactants tested for use in micro emulsion formulations. To create stable formulations, the amount of surfactant was changed while the oil % was kept constant. The stability of this emulsion was evaluated using UV-visible spectrophotometers against distilled water as a blank, measuring percentage transmission at 560nm. For further investigation, the surfactant that produced a stable emulsion

with the highest percent transmittance and demonstrated colloidal stability over 24 hours is chosen**.**

pH: pH meter operating at 25°C was used to determine the formulation's pH. The instrument had a pH of 4.0 calibrations. The digital pH meter model 111 took three additional readings for each formulation similarly Electric Conductivity of the microemulson documented by a pH meter at 25˚C.

Turbidity determination: The Micro emulsion turbidity was determined by measuring the /Transmission at 560nm with a UV-Vis spectrophotometer without dilution. Distilled water was used as blank sample.

Validation of Neem oil micro emulsion for pest management in vitro and Brinjal fields: Initially, six different doses of neem oil micro emulsion with two different surfactants like Tween 20 and DMSO are selected. All micro emulsions were prepared just before one day. There are two micro emulsion tested on the Brinjal mealy bugs in field and in vitro conditions. For this purpose, each dose has five replica similarly six dose have 10 replica respectively DMSO and Tween 20. The parameters were considered neem oil micro emulsion efficiency test on the Brinjal mealy bugs. The following characters are considered total number of mealy bugs before and after death each spraying respective doses of selected micro emulsions. Three successive spraying first day, seventh day and fourteenth day intervals. Similarly, in field conditions in green house of Botany Department.

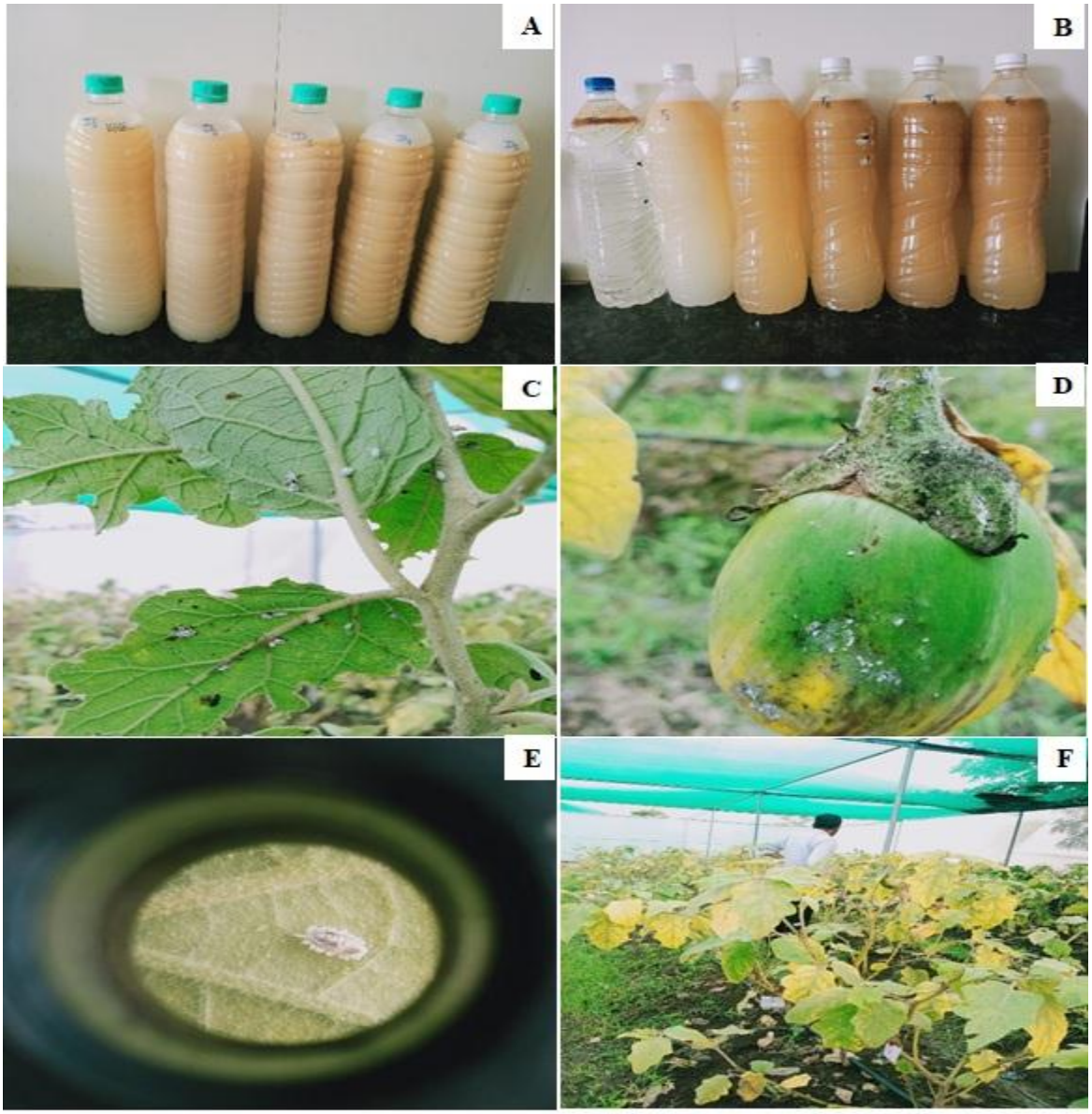

Figure-1: (**A**) Micro emulsion of Neem oil with Tween20; (B) Micro emulsion of Neem oil with DMS; (C-D) *Coccidohystrix insolita* (Brinjal Mealybugs) infected leaves and fruits; (E) *Coccidohystrix insolita* Brinjal Mealybugs; (F) Experimental site at Green house, Dept. of Botany, KSAWU Vijayapura.

Results and discussion

Tween 20 and DMSO were among the hydrophilic surfactants tested for use in micro emulsion formulations. To create stable formulations, the amount of surfactant was changed while the oil % was kept constant. By measuring the droplet size and the percentage of transmission at 560nm using a UV-visible spectrophotometer with distilled water as a control, the stability of this emulsion was determined. For further investigation, the surfactant that produced a stable emulsion with the highest percent transmittance and demonstrated colloidal stability over 24 hours is chosen.

Microemulsion stability: Storage stability at low and high temperatures. Subsequently storage at room temperature for 14 days, the appearance of the prepared Tween 20 and DMSO based Neem oil microemulsions were turbid and uniform, without any precipitate or phase separation. Neem oil microemulsions based on Tween 20 and DMSO had low decomposition rates, indicating that the stability of the microemulsion has not changed significantly. Turbidimetry is the scientific measurement of a substance's transmission or reflection characteristics in relation to wavelength Table-2. It scales a light beam's strength at various wavelengths. The measurement of turbidity is an easy and affordable way to assess the stability of an emulsion. By correlating the particle size distribution, it is an indirect way for assessing the stability of an emulsion. Figure-2 and Figure-3 the turbidity of colloidal systems.

pH: A pH meter operating at 25°C was used to determine the formulation's pH. The instrument had a pH of 4.0 calibration. For each formulation, the reading was done three times.

Electrical conductivity: Using a pH meter at 25°C, the formulation's EC was determined. The instrument had a pH of 4.0 calibrations. For each formulation, the reading was done three times.

The pH value is a measure of the stability of the micro emulsion and is based on the surface properties around the droplet. The compositions' pH values were discovered to be in the acidic range (3.76-6.02) in freshly prepared microemulsion and in one month old microemulsion (3.21-6.03). However, at application concentrations of 0.1% to 1%, the formulations turn virtually neutral Table-3 According to earlier research, high alkalinity or acidity of nano-formulations causes the neem ingredient to degrade, which in turn lowers the formulation's bio-efficacy[15]. Another study found that during storage, pH aggregates and destabilizes the micro emulsion[16]. In the this study, it was found that a botanical adjuvant stabilizes pH to acidic range (the acidic range (3.76-6.02) in freshly prepared microemulsion and in one month old microemulsion (3.21-6.03) to diminish neem's degradation in storage at low, ambient, and high temperatures.

Table-2: Percentage of transmission of different formulations of micro emulsion of neem oils with tween 20 and DMSO.

Freshly Prepared Neem Microemulsons			One month old Neem microemulsons	
Sl.No	Emulsions	% of Transmission@ 560nm	Emulsions	% of Transmission@ 560nm
1	T0	36.10	T0	60.57
2	T1	0.43	T1	1.87
3	T2	0.83	T2	0.10
4	T3	0.27	T3	1.43
5	T4	0.67	T4	0.47
6	T5	1.37	T5	0.10
7	D0	60.1	D0	30.63
8	D1	1.63	D1	0.13
9	D2	1.77	D2	0.10
10	D3	0.10	D3	0.10
11	D4	0.10	D4	0.10

12	D5	2.80	D5	0.83

Table-3: PH and Electrical conductivity of different formulations of micro emulsion of neem oils with Tween 20 and DMSO.

Sl.No	Freshly Prepared Neem Microemulsons				One month old Neem Microemulsons			
	Emulsions	PH	EC (mV)	Temperature	Emulsions	PH	EC(mV)	Temperature
1	T0	5.77	066	25℃	T0	6.03	050	25℃
2	T1	5.88	058	25℃	T1	4.15	150	25℃
3	T2	5.13	098	25℃	T2	3.21	200	25℃
4	T3	4.68	123	25℃	T3	3.48	187	25℃
5	T4	4.00	159	25℃	T4	4.88	111	25℃
6	T5	3.76	170	25℃	T5	4.15	150	25℃
7	D0	5.76	063	25℃	D0	5.93	054	25℃
8	D1	6.02	0051	25℃	D1	5.84	056	25℃
9	D2	5.79	062	25℃	D2	5.94	054	25℃
10	D3	4.92	109	25℃	D3	6.02	051	25℃
11	D4	4.57	128	25℃	D4	5.76	065	25℃
12	D5	4.07	153	25℃	D5	5.22	095	25℃

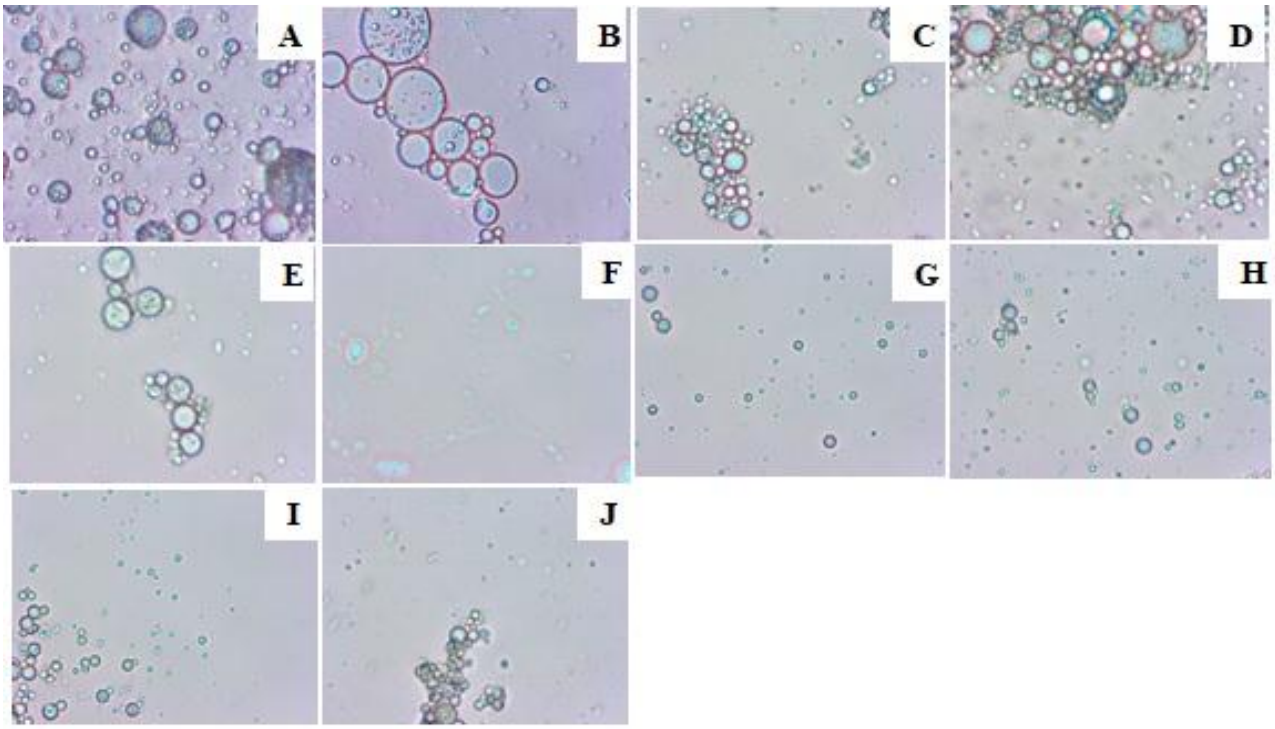

Figure-2: Neemoil with Tween20 microemulsions (freshly prepared [A-E) and one month old [F-J) in different formulation and droplets size under light microscope @10X.

Neem bio insecticide effect on Mealy bugs: Neem oil, water and Tween 20, micro emulsion was prepared and applied on Brinjal Mealy bug and results were presented in Figure-4. These formulations results shown that the highest death rate in T0 and T5 combination in second spray (19.6±0.9 and 19.5±0.7); Death rates decrease in the first spray in six formulations whereas in second and third round sprays increasing death rate all formations .First spray all combinations have shown less effect on mealybugsit may be due, variations in limonoids, known as meliatoxins A1, A2, B1, B2, and azadirachtin, tetranortriterpene and saponins with hemolytic effects. Pesticide efficiencies decreasing in third round spray where first sprays shown highest death rate. This noticed in Tween 20 based neem oils in microemulsions where first spray shown decreasing death rate corresponding increasing death rate in second and third sprays on eggplant mealybugs.

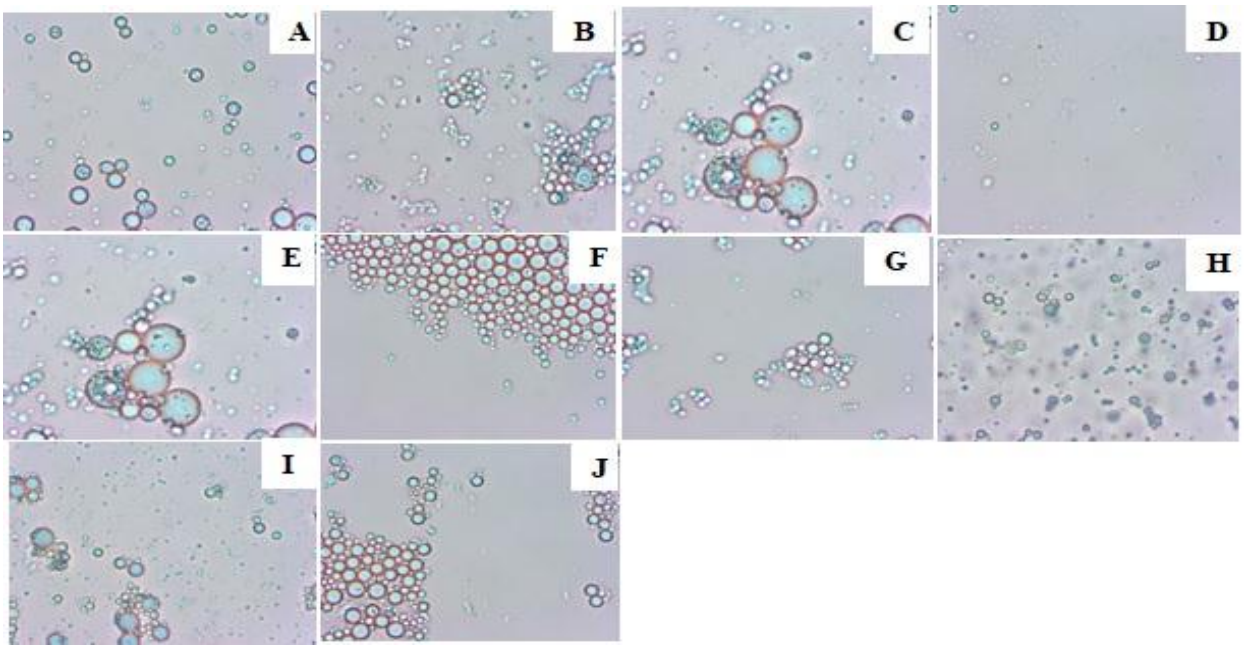

Figure-3: Neem oil with DMSO microemulsions (freshly prepared [**A-E**) and one month old [**F-J**) in different formulation and droplets size under light microscope@10X.

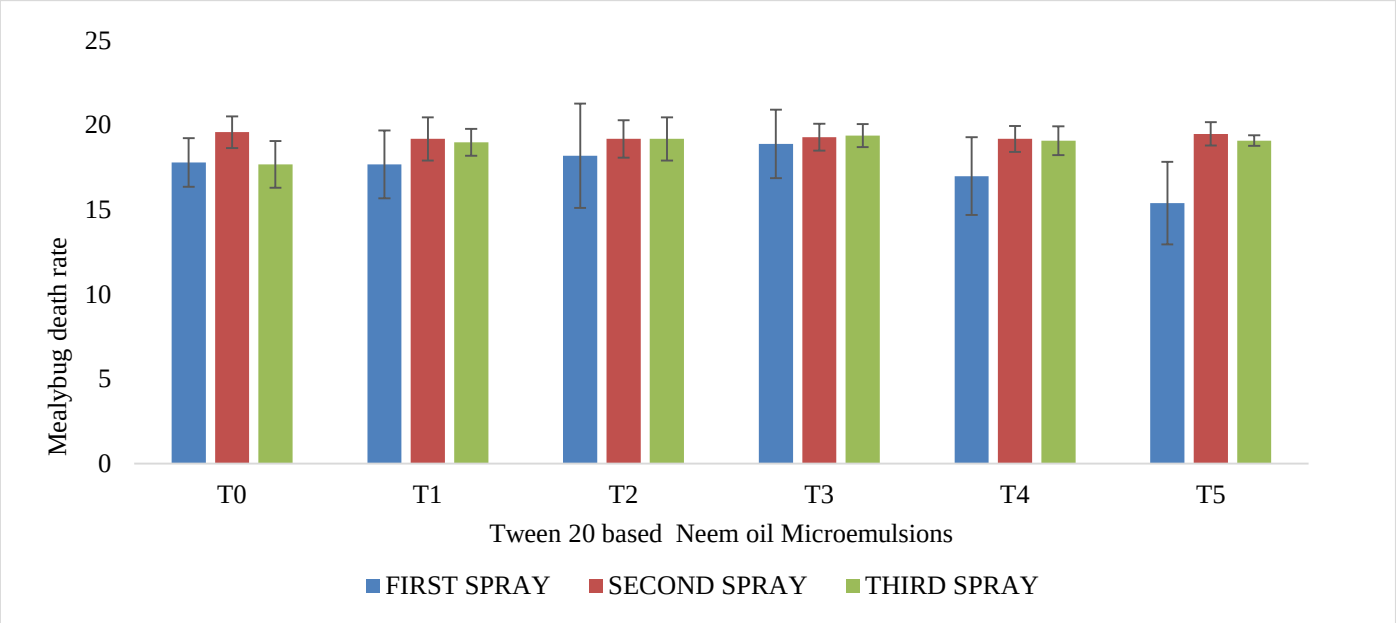

Figure-4: Average death rate of eggplant mealybugs in different concentrations of tween 20 with neem oil based micro emulsions.

Neem oil, water and DMS, micro emulsion was prepared and applied on Brinjal Mealy bug and results were presented in Figure-5. These formulations results shown that all combination in first spray decreasing death rates mean while second and third round sprays death rates increasing compared with first sprays. In case of Neem oil, water with DMSO based microemulsions shown reverse effect compared with Tween 20 with Neem oils micro emulsions Figure-5. Although azadirachtin has been the subject of several studies on neem oil micro emulsions, little is known about how it inhibits eating, although it is likely that it activates the cells that do so, weakening and killing the pest as a result[17]. Azadurachtin has a similar ability to colchicine to prevent mitosis. Additionally, it directly alters the histopathology of the muscles, adipose tissues, and gut epithelial cells of insects, limiting their ability to move and lowering their activity levels during flight[18-20].

Azadirachtin, nimbolide, salannin, nimbin, deacetylnimbin, mahmoodin, epoxy-azadiradione, deacetylgedunin, and gedunin are a few of the most significant neem limonoids[21,22]. The management of pests makes advantage of these substances' antifeedancy, insecticidal, and disruption of insect development capabilities, among others[9]. The majority of the neem triterpenoids, which make up the complete bioactivity of the neem seed extract, were found to be present in the tree in extremely little concentrations[23]. The primary active ingredient, azadirachtin A, accounts for between 72 and 90 percent of the biological action[23,24].

Conclusion

With the help of the nonpolar surfactant Tween 20, the polar surfactant DMSO, and distilled water, a microemulsion of neem seed oil was created. When compared to normal microemulsions, freshly made microemulsions demonstrated superior conduction, stability, less turbidity, and pH. When compared to neem oil microemulsions based on DMSO, those based on tween 20 have reduced stability, and their colour and smell have changed. Tween 20-based microemulsions are more effective than DMS-based microemulsions when it comes to pesticide characteristics. The presence of poisonous compounds in mealy bugs and the target plant by neem oil microemulsions has to be further investigated. How exactly do they kill mealy bugs, and do any beneficial insects also have an adverse impact on these microemulsions to be find out.

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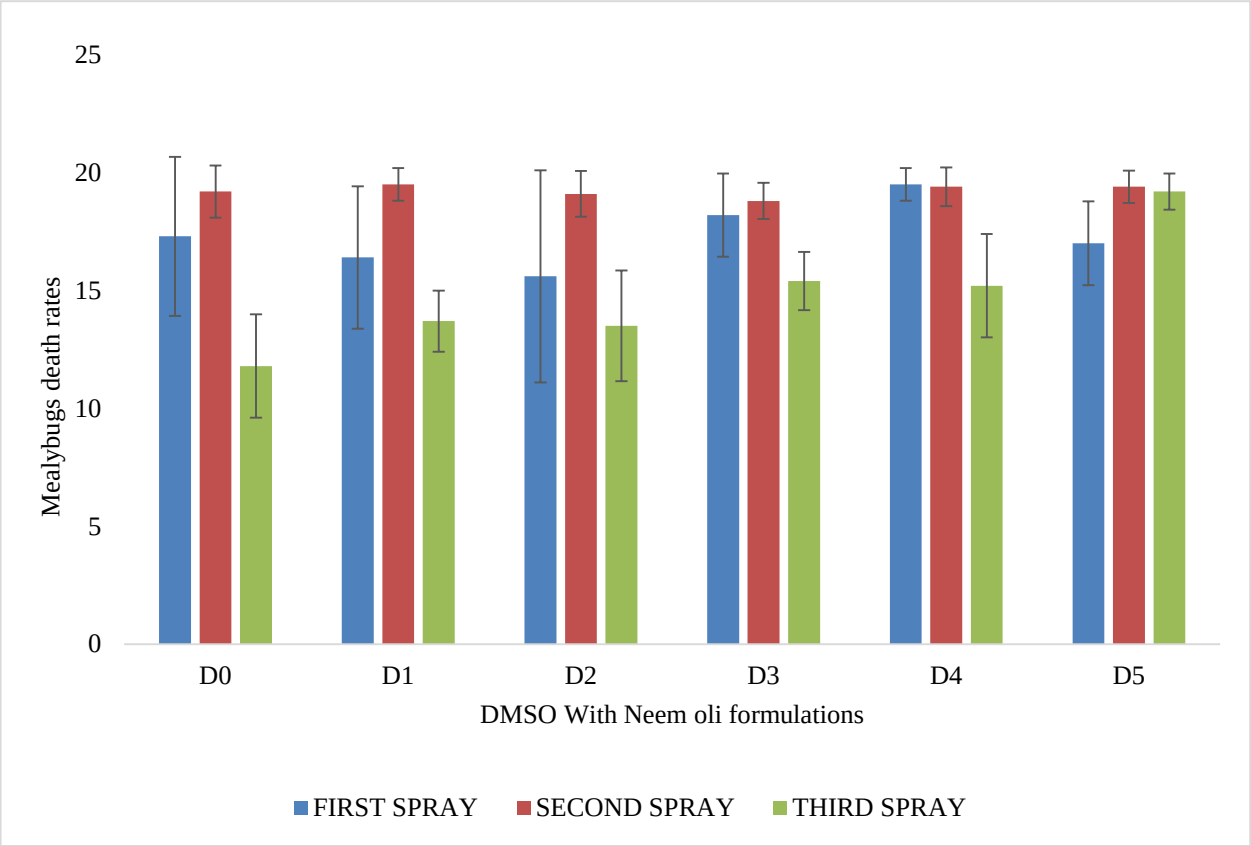

Figure-5: Average death rate of eggplant mealybugs in different concentrations of DMSO with neem oil based micro emulsions.

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