Combined toxicity effects of neem oil and mild soap on *Myllocerus viridanus* in *Morus alba*

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ABSTRACT

The insect *Myllocerus viridanus* in mulberry cultivation (*Morus alba*) causes significant harm to sericulture industry throughout the year which hampers leaf quality, silkworm rearing, and cocoon production. In this study, the treatment with neem oil (*Azadirachta indica*) in five different concentrations (1 to 5ml/L) along with mild soap solution (Khadi) to assess the mortality of *M. viridanus* at laboratory conditions. The lethal concentrations (LC_{30} – 3.096; LC_{50} – 3.471; LC_{90} - 4.589) were determined by spraying of the neem oil solution to adults of *M. viridanus*. The results showed that neem oil effectively suppressed *M. viridanus* and that the mortality of *M. viridanus* increased gradually (34 %, 44 %, 60 %, 78 %, and 100%) over the control as the concentration of neem oil increased.

KEYWORDS: Neem oil, *M. viridanus*, LC_{30}, LC_{50}, LC_{90}, probit analysis, *Morus alba*

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INTRODUCTION

Mulberry (*Morus spp*.) is the only food plant of *Bombyx mori* L. and cultivated nearly in 34,600 acres of Tamil Nadu. Of late, indiscriminate use of nitrogenous fertilizer and pesticides led to several pest infestations and diseases which resulted in low yield of mulberry leaves and its quality (Ravikumar et al., 2010). Due to poor leaf quality, silkworm rearing and cocoon production was adversely affect. Therefore, routine application of pesticides is inevitable to safeguard the crop from the pests within a short period after pruning so as to take up *B. mori* rearing in time. The silkworms are highly sensitive to harmful chemical pesticides, and the remaining extended effects in the mulberry garden are restricted (Sakthivel *et al.*, 2012). Sakthivel and Qadri (2010) stated that chemical pesticides eliminate the pest and eliminate other beneficial insects from the field. Heavy usage of a chemical pesticide affects the insect population besides numerous consumers such as birds, fishes, other animals, and contaminates groundwater sources, agricultural field soil, and finally dangerous to human health (Isman, 2006).

Neem (*Azadirachta indica*) belongs to the Meliaceae family, is the native tree of the Indian sub-continent, and grows in many subtropical countries such as Africa, Australia, Central, and South America (Schmutterer, 1990). Copping and Mean, (2000), Senthil Nathan et al., (2005) reported that chemical pesticides are being replaced by bio-insecticides, which cause very little and are low toxic to humans and the environment. Raizada *et al.* (2001) state that neem is one of the greatest reliable plant sources of bio-insecticides. SenthilNathan *et al.* (2005) reported that neem plant's leaves and seed extract have deleterious effects on insects. The principal insecticidal component in azardirachtin extracts is a limonoid; this organic compound was first reported by Ruscoe (1972). Azadirachtin is vigorous on nearly 550 species of insects (Schmutterer, 1990). Mordue *et al.* (2005) reported that neem extracts adversely affect insect's feeding behaviour, regulation of growth, development and oviposition. The primary source of azadirachtin was extraction from the Indian neem plant (Rharrabe et al., 2008). Mordue et al., (2005) noted that relatively little detail on the biochemical activity of Azardirachtin has been identified to date,

which affects the feeding patterns of insects, development explicitly, and reproduction, thus decreasing their population density. As bio-insecticides, the impact of azadirachtin is low on non-target or beneficial insects (Mordue, 1993; Naumann and Isman, 1996; Mordue et al., 2005). The present work is design to observe the mortality rate of *M. viridanus* by application of neem oil at five different concentrations.

MATERIALS AND METHODS

Experimental animals

Myllocerus viridanus of same stage were collected from the experimental mulberry field (Latitude 11°21'08.72" N / Great circle 78°06'30.43 E) Elur, Namakkal District of Tamil Nadu. Thus collected insects were grown in clear plastic boxes (14 x 14 x 5.5 cm) covered with aerated muslin fabric (10 insects/box) and were fed with existing mulberry leaves at a temperature of 24 ± 1°C, and relative moisture at 75 ± 5 percent in the laboratory. *M.viridanus* was fed with fresh mulberry leaves throughout the experimental period.

Preparation of neem oil bio-insecticides

The five concentrations of neem oil insecticides were prepared (1 to 5 ml of neem oil and 1 L of 0.5% *khadi* soap solution) by mixing required quantity of neem oil slowly by stirring in 1 L of 0.5% *khadi* soap solution, removed fat bodies floated and used for study at room temperature.

Data recording

In this experiment, five concentrations of neem oil (1, 2, 3, 4, 5 ml /1 L) were used to evaluate LC_{50} values, with distilled water as the control. The experiment was carried out using foliage of mulberry soaked in a solution of neem oil insecticides (Treatment) and distilled water (Control) for 10 seconds and shade dried at room temperature for 20 minutes. The control and treated mulberry leaves was fed to the experimental *M. viridanus* to evaluate its effect of different concentrations of neem oil. Insect mortality was recorded once at six hours interval. The LC_{30}, LC_{50}, and LC_{90}values and at 95% confidential limit were calculated by the Probit analysis (Finney, 1971).

Corrected mortality

In quantifying the efficiency of bio insecticides, when an authentic count of the live and dead insects in either treated or untreated is available, to consider the mortality due to natural causes. The insect population was calculate for corrected efficacy percentage (Abbott, 1925).

Characterization of the formulations

On a Bruker Tensor-27, FT-IR Spectrophotometer using ATR (Attenuated Total Reflectance) technique, FT-IR analysis of 1-5ml / L neem oil was recorded (Sampath Kumar, 2013).

Analysis of statistics

The mortality data was to be used to calculate the corrected mortality using the Abbott correction technique. The slope and regression equations of LC30, LC50, and LC90 were measure by using the probit analysis approach utilizing the corrected mortality values (Finney, 1971). A one-way ANOVA and post-hoc means split by Tukey's HSD test was use to evaluate mortality ($p < 0.05$). The mistreatment of the SPSS and Origin Pro-applied mathematics program scheme was carried out in both tests.

RESULT

Laboratory experiment study

The exposed pesticide impact of neem oil over every 6, 12, 18, and 24 hrs on mortality rate of *M. viridanus* was record. The mortality rate of *M. viridanus* against neem oil was represented in Table 1 as no mortality in control, and mortality started from 6^{th} hr in 2 – 5 neem oil concentrations and at 12^{th} hr mortality began from 1- 5 (ml/L). Increased concentration of neem oil elevated the pest mortality with statistical significance ($p< 0.000$). In 5 ml/L neem oil, 100% mortality was record in 24hrs, while the most negligible mortality registered in 1 ml/L concentration (34%) at the end of the experimental period.

Probits Analysis

The mortality rate of *M.viridanus* against contact bioassay of *A.indica* oil and soap solution shows high statistical significance at 6 hours LC_{50} values 5.999 (Y = 8.5143x + -

2.2857; R^2 = 0.9233; $p < 0.000$) and 24hr LC_{50} values of 3.471, (Y = 18.514x + 6.381; R^2 = 0.978; $p < 0.001$) was represented in Table 2. Statistical significance between Pest and Neem oil concentration hasbeen established during 12 hours and 18hours of experimental period.

Table 1. The corrected mortality (%) on *M. viridanus* insects treated with the different combinations of neem seed oil (ml/L) and mild soap.

Neem Seed Oil Concentration (ml/L)	Mortality Rate (%)			
	6 Hours	12 Hours	18 Hours	24 Hours
Control	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}
1	0.00 ± 0.00^{c}	12.00 ± 3.74^{cd}	30.00 ± 4.47^{c}	34.00 ± 5.10^{d}
2	16.00 ± 5.10^{b}	24.00 ± 5.10^{c}	42.00 ± 5.83^{bc}	44.00 ± 6.78^{cd}
3	30.00 ± 4.47^{ab}	40.00 ± 3.16^{ab}	54.00 ± 2.45^{b}	60.00 ± 3.16^{c}
4	28.00 ± 3.74^{ab}	36.00 ±4.00^{ab}	50.00 ± 5.48^{b}	78.00 ± 3.74^{b}
5	40.00 ± 4.47^{a}	52.00 ± 5.83^{a}	76.00 ± 5.10^{a}	100.00 ± 0.00^{a}

Total means followed by different lower case letters in a column among various concentrations for neem seed oil significantly different (Tukey's HSD test, $p< 0.05$).

FT-IR analyze

The active functional group compounds in neem oil were analyzed by FT-IR spectrum based on the peak values. Figure -1 reveals the functional groups present in neem oil, which confirms the occurrence of different chemical constituents such as alkyl halides, alcohol, alkanes, alkyne, amines, hydroxyl group, phenols, carboxylic acids, and derivatives of neem oil insecticides. The peaks between 666.62 cm^{-1} and 675.16 cm^{-1} was assigned to C-Br stretching in Alkyl halides. The vibrations present in 1103.47 cm^{-1}, 1104.25 cm^{-1}, and 1163.75 cm^{-1} indicate Amines, Alcohol, and Phenolic compounds. A weak stretch of C-H at 1465.02 cm^{-1}indicates the presence of Alkanes. Similarly, C=C stretching of alkanes was attributed in the peaks at 1637.20, 1637.43, 1637.44, 1637.55,and 1638.06 cm^{-1}. The peak at 1742.13 cm^{-1} indicates the occurrence of carboxylic acids and their derivatives. The weak bands occur at 2124.59, 2118.72, 2107.56, 2114.62, and 2114.75 cm^{-1}corresponding to the C≡C bonding structures were liable for the presence of the alkynes group. The broad strong stretching peaks at 2858.00 cm^{-1}, 2926.77 cm^{-1}, 3329.88 cm^{-1}, 3333.69 cm^{-1}, 3328.38 cm^{-1}, 3345.61 cm^{-1} and 3348.35 cm^{-1} which are assigned to the alkane and alcohol.

Table 2. Probit analysis of neem seed oil treated *M. viridanus.*

Hours	LC_{30}	LC_{50}	LC_{90}	Slope	Chi-square	Regression	R^2	Sig.
6	3.663	5.999	20.036	4.134	14.284	Y = 8.5143x + -2.2857	0.9233	0.000
12	2.472	5.151	30.977	1.232	12.016	Y = 9.9429x + 2.4762	0.9416	0.218
18	3.034	4.421	11.096	1.741	14.109	Y = 12.914x + 9.7143	0.892	0.082
24	3.096	3.471	4.589	3.298	13.577	Y = 18.514x + 6.381	0.978	0.001

LC: lethal concentration; significant at $p < 0.05$ level

The assimilation of O-H stretching at 3881.63 cm^{-1} corresponds to vibration modes in the hydroxyl group.

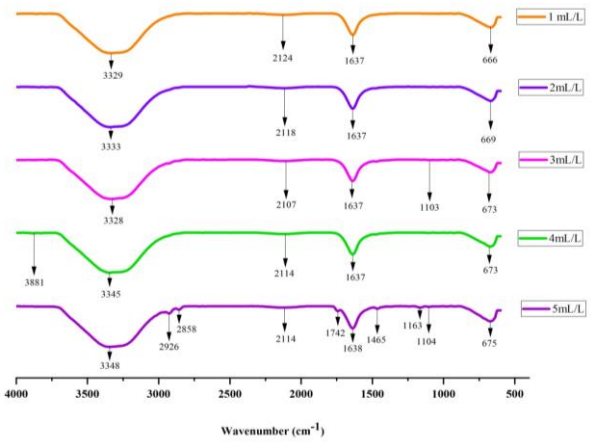

Fig 1. FT-IR spectra of Neem seed oil and Mild soap different combination.

DISCUSSION

The continuous application of chemical pesticides in the agricultural field causes mortality of pests, beneficial insects, and domestic animals and finally makes frailty to human health. Research has been attentive to biodegradable, natural pesticides to reduce pollution risk and effectively control pest infestation. The oral application of neem oil/ soap solution at 5ml/L showed 100% mortality of *M. viridanus*, while other concentrations showed a decline and least toxic effect. The Probit analysis of neem oil recorded on the 24^{th} hours; LC_{50} values of 3.471, ($Y = 12.914x + 9.7143$; $R^2 = 0.978$; $p < 0.001$), while cold pressing of neem seed oil control phytophagous pests such as mites and soft bodied insects.

The exclusion of the neem oil contains triterpene azadirachtin, which has 0.2 to 0.6% of azadirachtin, the active ingredients (technical grade material) used for natural pesticide (Isman *et al.*, 1996). The azadirachtin blocked the prothoracic gland, which released the ecdysteroids hormones in premature insects, contributing to incomplete ecdysis. For many species, Azadirachtin is a potent anti-feedant (NRC, 1992), fish (Wan et al., 1996), pollinators (Naumann and Isman, 1996) and is considered non-toxic to mammals (Rat oral acute LD_{50} > 5000 mg kg^{-1}). The effect of azadirachtin on various natural enemies is exceedingly varied (Lowry and Isman, 1995; Spollen and Isman, 1996). Neem products for natural enemies, pollinators, and other non-target living things are harmless (Singh and Singh, 1996; Ranga Rao et al., 2007). Azadirachtin, easily degraded in sunlight on olives that emerge in Italy; azadirachtin has a half-life of approximately 20 hours (Caboni *et al.*, 2002).

Neem oil, which appears to cause 90 percent of the effects on most pests, contains at least 100 biologically active compounds. Additional ingredients include meliantriol, nimbin, nimbidin, nimbolides, fatty acids (Oleic, Stearic, and Palmitic), and salannin. The main content of neem by-products is the oil extracted from the seeds; smaller azadirachtin found in the other areas of the neem tree but also used for oil extraction (Nicoletti et al., 2012). It has been indicating that synthetic sepsis with arbuscular mycorrhiza may increase the amount of azadirachtin in the neem seeds (Venkateswarlu et al., 2008). *A. indica* oil treatment for LC_{50} values 4.421 is no significance for the probit analysis ($p < 0.082$). Brahmachari (2004), states that azadirachtin inhibits feeding behaviour, cause fatigue and death of the pest. Azadirachtin, salannin, and other limonoids in neem oil inhibit the final step in the transition of ecdysone to the active hormone, 20-hydroxyecdysone, which governs the progression of insect metamorphosis. However, these effects are presumably secondary to the effort of azadirachtin to prevent the production of successful cell division microtubules (Morgan, 2009). Azadirachtin can almost interrupt the development of the cardiac brain-corpus complex of prothoracicotropic hormone and allatoropin, resulting in a lack of fecundity and fertility (Mulla and Su, 1999).

The functional groups, chemical structure and strength of bioactive elements in the neem oil were clearly established by FTIR examination. In our study, the presence of vibration of 1103.47 cm^{-1}, 104.25 $cm^{-1,}$ and 1163.75 $cm^{-1,}$ respectively, indicates amines, alcohol, and phenols. The existence of primary amines is established by N-H vibration (Silverstein and Webster, 1996), while presence of C=O stretching in carboxylic acids and derivatives

indicate peak 1742.13 cm^{-1}. The prominent oxygenated purposeful groups O-H, C-O, C=O, and aromatic compounds displayed that de-oil seed was extremely hydro-oxygenated and, therefore, acidic (Mulimani and Navindgi, 2016). The results presence peaks at 2858.00, 2926.77, 3329.88, 3333.69, 3328.38, 3345.61, and 3348.35 cm^{-1} which are assigned the alkane and alcohol frequency broad, strong stretching. The absorbance band at 3289 cm^{-1}, 2926 $cm^{-1,}$ and 2927 $cm^{-1}$is possibly due to the OH stretch band of phenols or alcohols that might be responsible for stabilizing the azadirachtin content in neem oil (Bhatia et al., 2013).

It hasbeen concluded that neem oil has potential insecticidal effects on insect pests of mulberry crops. Green revolution in early 20^{th} century introduces various chemical pesticides, which slowly eradicate our ancestral practice of natural plant base pesticides. Today our farmers are forced back to natural farming practices, thereby use organic manure and plant based pesticides to save mother earth from soil pollution.

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