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Synergistic effects of monocrotophos with botanical oils and commercial neem formulation on *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae)

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ABSTRACT

The present study was carried out to determine the synergistic effects of pungam oil, neem oil, vijayneem and biosilver nanoparticles (10, 20, 30, 40 and 50%) blended with monocrotophos and screend against third instar larvae of *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) by dermal toxicity bioassay. The dermal toxicity bioassay caused 100% mortality in monocrotophos + neem oil and monocrotophos + biosilver nanoparticles in 10% on eighth day, monocrotophos + pungam oil in 20% on second day and monocrotophos + vijayneem in 50% at ninth day. The highest growth was observed in neem oil and biosilver nanoparticles when blended with monocrotophos. We conclude that either pungam oil or neem oil can be blended with monocrotophos for the management of *S. litura* larvae.

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Key words: Pesticides, biosilver nanoparticles, dermal toxicity, *Spodoptera litura,* synergistic effects, neem formulations.

INTRODUCTION

Spodoptera litura (Fab.) (Lepidoptera: Noctuidae) is a polyphagous pest, widely distributed throughout the Asia (Baskar *et al.*, 2014). It has a wide range of hosts of which 40 species are known in India (Krishnaveni *et al.*, 2013; Singh *et al.*, 1998 and Paulraj, 2001). Traditionally, farmers have been practicing synthetic pesticides to control *S. litura* and hence it has developed resistance against almost all the commonly using pesticides (Anbalagan *et al.*, 2014).

The world-wide use of chemicals accounts for very high risk of toxicity and environmental pollution (Rao *et al.*, 2005). The growing awareness of the hazards of excessive use of pesticides globally has led researchers to search for safer and more environment- friendly alternative methods for insect pest control. Therefore, extensive studies are being carried out to screen plants for pesticidal property. Over the last three to four decades greater attention has been focused on the bioactivity of phytochemicals for their potential as pesticides against phytophagous insect parts (Padmaja and Rao, 2000; Sahayaraj, 2011; Hong Tong *et al.*, 2013; Anbalagan *et al.*, 2014). Neem tree, *Azadiracta indica*, A. Juss, (Meliaceae) is an important source of 99 biologically active compounds and most of these compounds showed antifeedant, ovicidal, larvicidal, oviposition deterrent activity against a number of insects (Schumutterer, 2002; Isman, 2006; Locantoni *et al.*, 2006; Dua *et al.*, 2009; Johnson Stanley, 2014). Pungam oil possesses toxic components such as glabrin, karajn, karajae and pongoglabrone, which are effective against insect pests of stored grains, field and plantation crops (Maria Packiam and Ignacimuthu, 2012). Neem oil peers is less systemic because it is insoluble in water. It should be formulated to make it systemic to enhance its efficacy on sucking pests. To overcome these hurdles, better formulations are being developed (Soundararajan and Lakshmanan, 2009).

Vijayneeem a neem – product has been reported effective against okra leaf hopper, *Amrasca devastans* (Dist.) (Senguttuvan and Rajendran, 2001). Monocrotophos one of the organophosphate of synthetic/ chemical pesticides is becoming a serious threat to public health and environment (Theurkar *et al.*, 2014). Anoharan *et al.*, (2011) report that edible oils did not show any synergistic activity with monocrotophos; however the non-edible oils suppressed the monocrotophos resistance significantly. Studies on larvicidal property of neem oil and monocrotophos at different concentrations against fourth instar larvae of *Pericallia ricini*

(Fab.) revealed that neem oil was highly effective (War *et al*., 2011).

Andrographis paniculata (Burm.) (Acanthaceae) commonly known as "King of Bitters" or "Rice Bitters" (Chopra *et al.,* 1956) (Acanthaceae) and grows abundantly in South-East Asia. It is considered a traditional medicine plant useful to treat malaria (Misra *et al.,* 1992), filarial (Dutta and Bukul, 1982) as well as for pest control (Kupusamy and Murugan, 2006) and as an insect repellent (Prakash and Rao, 1997). Biological synthesis of nanoparticles has received increased attention due to a growing need to develop environmentally benign technologies. Several plant species have been utilized in this regard (Rajesh *et al.,* 2012; Ramar *et al.,* 2014).

So far no report is available on the additive or synergistic effect of monocrotophos with neem oil or commercial neem-based pesticide, pungam oil and biosilver nanoparticles on feeding, survival, growth and development of *S. litura*. However, synergistic activity of cypermethrin (Bingham *et al.*, 2008), pyrithroids (Mohan *et al.,* 2007) and carbamates (Mohan *et al.,* 2007) with neonicotinoids and *Solanum xanthocarpum* extract respectively was available in the litrature. Hence, the present work was undertaken to evaluate the effects of synergistic combinations of pesticides, biopesticides and biosilver nano particles on mortality and growth regulating activities against *S. litura*.

MATERIALS AND METHODS

Collection and Rearing of Pest

Egg masses and larval stages of *S. litura* was collected from the castor and cotton fields at Savalaperi (8^o 49' 55.6" N, 77^o 51' 34.8" E) and Killikulam (8^o 42' 48.66" N, 77^o 48' 44.7" E) TNAU, Tirunelveli, Tamil Nadu, India, were maintained on castor leaves under laboratory conditions (29 ± 1^{o}C temperature; 65-75% RH) in plastic troughs (16 X 7 cm). Laboratory emerged adults were transferred to oviposition chamber (44 X 34.5 cm) and fed with 5 % honey fortified with vitamin mineral mixture (Multivitamin tablet) to enhance oviposition (Anbalagan *et al.,* 2014). Egg masses were collected, sterilized and maintained in moisture with cotton swab Petri dishes for hatching. Laboratory emerged larvae was also maintained again with caster leaves for a generation. Third instar larvae obtained from the second generation were utilized for this experiment.

Source of Pesticidal formulations

Commercial formulations of Monocrotophos (Jeyakrishna Pesticide Limited, Salem) and Vijayneem (Madras Fertilizers Limited, Chennai) and plant oils like neem oil and pungam oil were purchased from the local market and utilized for the experiment.

Synthesis of Silver Nano Material

Five ml of 50% *A. paniculata* leaf extract was added to 100 ml of 10^{-3} M aqueous silver nitrate solution for reduction of Ag^+ ions at room temperature. The colour of the solution changes from light yellow to dark brown indicating the formation of silver nanoparticles which was confirmed by UV- Visual spectrophotometer at 415 nm.

Toxicity bioassay

Monocrotophos (0.03%) was blended with neem oil, pungam oil, vijayneem and biosilver liquid nanomaterial at each in 9:1 (10%), 8:2 (20%), 7:3 (30%), 6:4 (40%) and 5:5 (50%) were prepared for the present experiment. Dermal toxicity bioassays were performed with third instar larvae of *S. litura* tested. 1 ml of the experimental solutions were applied topically on the surface of the thorax and abdominal region of the pest using a fine Camlin brush (1mm). Five replications each with 3 third instar larvae per treatment were used for the experiment. Control category was treated with distilled water. The experimental animals were maintained with normal caster leaves. Mortality was recorded every 24 hrs until 10th day of exposure. After the treatment, animal growth was recorded.

Statistical Analysis

Corrected mortality was calculated as per Abbott correction formulae (Abbott, 1925) and then LC_{30}, LC_{50} and LC_{90} values were derived using Probit analysis method (Finney, 1971). ANOVA for mortality and growth data were performed by SPSS version 20.0.

RESULTS AND DISCUSSION

Monocrotophos readily dissolved in neem oil, pungam oil, vijayneem and nanosilver liquid materials. No precipitate was recorded for any of the

Table 1. Mortality of *Spodoptera litura* third instar larvae on monocrotophos with pungam oil, neem oil, vijayneem and biosilver nano material in dermal toxicity bioassay.

Concentrations (%)	Days										
	1	2	3	4	5	6	7	8	9	10	Mean
	Monocrotophos with Pungam oil										
10	-	-	-	-	13.3±8.2	20.0±13.3	20.0±13.3	33.3±10.54	40.0±12.5	86.7±8.2	21.3±1.0
20	73.3±12.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	97.3±1.2
30	80.0±13.3	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	98.0±7.3
40	-	13.3±8.2	19.9±13.3	33.3±21.1	53.3±13.3	73.3±6.7	79.9±8.2	86.6±8.2	93.3±6.7	100.0±0.0	55.2±8.2
50	-	13.3±8.2	20.0±13.3	20.0±13.3	20.0±13.3	20.0±13.3	26.6±12.5	53.3±16.9	86.7±8.2	100.0±0.0	35.9±3.2
100	-	6.7±6.7	46.7±8.2	73.3±12.5	86.7±8.2	86.7±8.2	86.7±8.2	86.7±8.2	86.7±8.2	86.7±8.2	73.3±8.2
	Monocrotophos with Neem oil										
10	-	-	13.3±8.2	66.7±14.9	86.7±8.2	86.7±8.2	93.3±6.7	100.0±0.0	100.0±0.0	100.0±0.0	64.6±3.4
20	13.3±8.2	13.3±8.2	13.3±8.2	66.7±10.5	80.0±13.3	93.3±6.7	93.3±6.7	93.3±6.7	100.0±0.0	100.0±0.0	66.6±4.7
30	-	-	6.7±6.7	80.0±13.3	86.7±8.2	93.3±6.7	93.3±6.7	93.3±6.7	93.3±6.7	93.3±6.7	63.9±8.9
40	-	-	-	-	13.3±8.2	40.0±12.5	53.3±8.2	73.3±12.5	86.7±8.2	86.7±8.2	35.3±3.6
50	-	-	-	33.3±21.1	40.0±24.5	60.0±24.5	80.0±13.3	86.7±8.2	86.7±8.2	93.3±6.7	48.0±0.5
100	-	6.7±6.7	33.3±10.5	46.7±8.2	60.0±12.5	66.7±14.9	66.7±14.9	66.7±14.9	66.7±14.9	66.7±14.9	54.7±13.4
	Monocrotophos with Vijayneeem										
10	13.3±8.2	33.3±10.5	66.7±14.9	80.0±8.2	93.3±6.7	93.3±6.3	93.3±6.7	93.3±6.7	93.3±6.7	93.3±6.7	75.3±12.0
20	26.7±12.5	26.7±12.5	26.7±12.5	40.0±12.5	53.3±8.2	73.3±12.5	80.0±8.2	80.0±8.2	80.0±8.2	80.0±8.2	56.6±3.5
30	40.0±19.4	66.7±18.3	80.0±20.0	93.3±6.7	93.3±6.7	93.3±6.7	93.3±6.7	93.3±6.7	93.3±6.7	93.3±6.7	83.9±8.9
40	13.3±8.2	66.7±10.5	73.3±12.5	73.3±12.5	73.3±12.5	73.3±12.5	73.3±12.5	73.3±12.5	73.3±12.5	73.3±12.5	66.6±6.4
50	40.0±12.5	53.3±13.3	53.3±13.3	66.7±10.5	80.0±8.2	86.7±8.2	93.3±6.7	100.0±0.0	100.0±0.0	100.0±0.0	77.3±3.2
100	-	-	20.0±13.3	26.7±12.5	33.3±10.5	40.0±12.5	53.3±17.0	60.0±12.5	60.0±12.5	60.0±12.5	35.3±12.5
	Monocrotophos with *A. Paniculata* silver nano materials										*A.*
10	-	-	-	-	20.0±20.0	73.3±19.4	86.7±8.2	93.3±6.7	100.0±0.0	100.0±0.0	47.3±2.8
20	-	6.7±6.7	6.7±6.7	6.7±6.7	20.0±13.3	20.0±13.3	60.0±19.4	80.0±13.3	93.3±6.7	100.0±0.0	40.0±2.0
30	-	-	-	-	-	13.3±13.3	26.7±12.5	39.9±6.7	39.9±6.7	73.3±12.5	55.2±3.8
40	6.7±6.7	6.7±6.7	6.7±6.7	6.7±6.7	6.7±6.7	6.7±6.7	20.0±13.3	26.7±12.5	46.7±8.2	46.7±8.2	18.0±0.2
50	13.3±8.2	13.3±8.2	13.3±8.2	13.3±8.2	13.3±8.2	13.3±8.2	26.7±12.5	26.7±12.5	33.3±14.9	40.0±16.3	20.6±3.8
Control (0.03%)	6.7±6.7	26.7±16.3	73.4±6.7	86.7±8.2	93.3±6.7	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	78.7±21.0

-Indicates no mortality.

combinations and this could be used by the farmers as a combination for pest management programme. Monocrotophos at 0.03% caused only 6.7% mortality with in 24 hrs. However, blending of pungam oil (73.3 and 80.6 at 20 and 30% concentration respectively) whereas monocrotophos with neem oil recorded 13.3% at 10% concentration and vijayneem recorded 40% mortality at 30% and 50%. 20%-30% combination of monocrotophos with pungam oil recorded 100% mortality at 48 hrs. Higher concentration resulted in increase in mortality. Synergistic combination of biological and chemical insecticides yield a promising alternative for insect pest management (Koppenhofer and Fuzy, 2003; Morals – Rodriguez and Peck, 2009). The synergistic formulations of the chemicals generally exhibit faster response than the technical formulations. This response increases the lethal and sublethal impacts of the insecticides (Srivastava *et al.*, 2011). Botanicals play an important role as synergists in insect pest management both economically and ecologically (Baki *et al.,* 2005).

The LC_{50} values (Table 2) of pungam oil is 5.1356 at sixth day and slope (-0.4325), chi-square (277.266) and regression equation (Y= 13.204 + 0.709X) were recorded. Mortality may be due to increased breakdown of proteins by the active principles present in pungam oil, *Pungamia glabra.*

Monocrotophos in combination with Neem oil and vijayneem separately caused more mortality in 10% (8^{th} day) (LC_{50}= 35.7703) and 50% (9^{th} day) (LC_{50}= 25.0143) concentrations respectively (Table 2). The mortality was higher and expressed by slope 8.8177, 6.7732), chi-square (81.165, 8.939) and regression equation (Y= 2.273 + 0.950X, Y= -66.668 + 1.639X) respectively (Table 2). War *et al*. (2011) reported that antifeedant activity of neem oil formulation + endosulfan was significantly higher against *S. litura* larvae than the individual treatments. Recently Krishnaveni *et al.* (2013) reported higher insecticidal activity of pungam oil followed by neem gold than the control category. Rajappan *et al*. (1999) reported neem and pongam oils were tested for their effect on the survival of *Nephotettix virsescens* (Dist.) and reported two neem based formulations are neem oil + pungam oil were tested and compared with monocrotophos. Ayyasamy (2008) reported that continuous feeding of *S. litura* third instar larva with neem oil formulation took nine days to cause 100% mortality whereas the present study reveals that *S. litura* third instar larva took eight days to cause 100% mortality. Besides azadirachtin, neem oil also contains different fatty acids, namely oleic acid, lenoleic acid, linolenic acid, palmitic acid and stearic acid (Valenzuela *et al.,* 2007). The compounds presents in neem oil are reported as strong antifeedants and growth inhibitors against Lepidopteran larvae (Koul *et al.,* 2004). Deota and Upadhyay (2005) reported that azadirachtin, the active ingredient of *A. indica* against *S. litura* showed toxicity and antifeedancy. Sighamony *et al.* (1984) reported that karanj oil (pungam oil) obtained from the seeds of *Pogamia pinnata*, exhibited repellent property at 2.5 mg / cm^3 against insect pests and retained the efficacy upto eight weeks.

The use of plant product chemistry coupled with nanotechnology that reducing pest populations at the larval stage can provide many associated benefits to pest control. Silver nanoparticles are considered to be potential agents for various biological applications and also as pest larvicidal agents. The larvicidal activity of biosynthesis of silver nanoparticles from *Andrographis paniculata* against larvae of *S. litura* is presented in Table 1. Monocrotophos incorporated *A. Panicluata* silver nano particle recorded 100% mortality at 10% concentration on day 9 of experiment. It was expressed by the LC_{50} values (10.2674), slope (2.4903), chi-square (47.520) and regression equation (Y= 109.810 + (-) 0.770X) (Table 2). Kupusamy and Murugan (2010) demonstrated that increased larval and pupal duration, increased mortality, growth regulatory effect, reduced fecundity and decreased adult longevity of *A. paniculata* extracts treated individuals reduced fecundity the overall survival and reproductive capacity of the malarial vector *An. stephensi* (Kuppusamy and Murugan, 2010).

The silver nano particles in the intracellular space can bind to sulphur containing proteins or to phosphorus containing proteins compounds like DNA, leading to the denaturation of some organelles and enzymes (say Rai *et al.*, 2009), which leads to the death of the insect (Ramar *et al.*, 2014).

Table 2. Probit analysis of dermal toxicity bioassay in pungam oil, neem oil, vijayneem and biosilver nano material treated castor leaves fed *Spodoptera litura* third instar larvae.

Biopesticides	LC_{30}	LC_{50}	LC_{90}	slope	Chi-squre	Regression	Df	P (Sig.)
Pungam oil + Monocrotophos	0.3688	5.1356	9.1874	0.4325	277.266	Y=13.204+0.709X	2	0.000
Neem oil + Monocrotophos	12.0762	35.7703	55.7819	8.8177	81.165	Y=2.273+0.950X	2	0.000
Vijay neem + Monocrotophos	12.2487	25.0143	33.5024	6.7732	8.939	Y=-66.668+1.639X	2	0.011
A. paniculata silver nano + Monocrotophos	7.3455	10.2674	11.7753	2.4903	47.520	Y=109.810+(-)0.770X	2	0.000

Significant of pungam oil: the mortality was higher in 20% (df= 5; f= 15.139; p= 0.010) and 30% (df= 5; f= 26.628; p= 0.004) concentration at second day of the exposure. In neem oil and vijayneem the highest mortality was observed in 10% (df= 5; f= 93.477; p= 0.000) and 50% (df= 5; f= 25.149; p= 0.004) of the concentration at eighth and ninth days after the exposure. In biosilver nanomaterial the highest mortality was recorded in 10% concentration (df= 6; f= 326.857; p= 0.000) at ninth day of the exposure.

Weight gain (mg) of the animal was clearly noticed in pungam oil, when compared with control (1086.0 ± 122.6) the 10 % concentration got higher growth (1332.0 ± 72.7) and in all the others, no significant change was observed (Fig. 1).

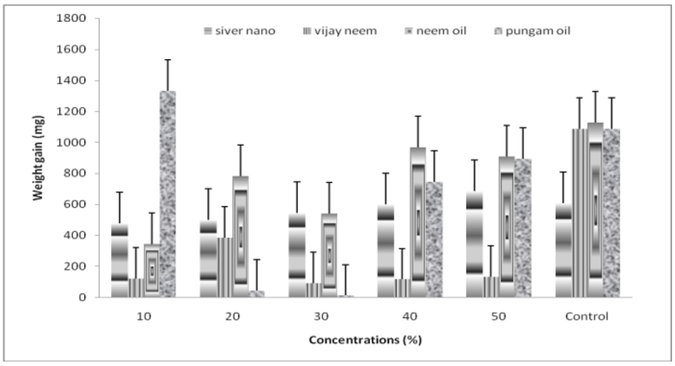

Fig 1. Weight gain (mg) of *Spodoptera litura* third instar larvae fed with pungam oil, neem oil, vijayneem and biosilver nano material treated castor leaves.

But neem oil and vijayneem give lesser growth (1426.0 ± 135.1 and 796.0 ± 139.7) than the control respectively. Azadirachtin acts as growth inhibitor and regulates the animal (Kupusamy and Murugan, 2010). Biosilver nano particles of *A. paniculata* significantly reduced the weight of the animal, 50% concentration showed (687.3 ± 18.1) higher growth than control (606.7 ± 17.5) of the *S. litura* (Fig. 1). Ground plant parts (leaves, stem and roots) of *A. paniculta* significantly inhibited the growth parameters like height, number of production of leaves and seeds per plant of *Parthenium hysterophorous* (Nagaraja and Deshmukh, 2009). *A. paniculata* has naturally bitter taste and with its characteristic features it repels the animals from the castor leaves.

Significant weight gain of the animal was the highest in pungam oil treated category 10% concentration (df= 1;13; f= 45.089; p= 0.000) when compared with control (df= 1;13; f= 4.087; p= 0.05) category. In neem oil and vijayneem give lesser growth (df= 3;13; f= 0.922; p= 0.462 and df= 3;11; f= 6776.425; p= 0.000) than the control category respectively. Biosilver nanoparticles reduced the weight of the animal at 50% (df= 3;11; f= 264.070; p= 0.0005) rather than control (df= 3;11; f= 79.440; p= 0.000) of *S. litura*.

Synergism between botanicals and chemical insecticides against insect pests has been well documented. There is a considerable concern over the last few years about the potential of synergism between synthetic and biopesticides applied to different insect pests (Srivastava *et al.*, 2011). Synergists are therefore, considered straightforward tools for overcoming metallic resistance and delay the manifestation of resistance. Previously, Feng *et al.* (1995), had studied synergistic activity of *Melia tooseudan* extract with malathion against *S. litura*.

The synergistic combinations showed higher mortality (100%) when compared with their individual treatments. All botanicals (pungam oil, neem oil) biopesticides vijayneem and bio silver nano particles, when mixed with monocrotophos gave more mortality and weight loss of animal. Therefore, all the synergistic combinations of biopesticides could be used as a synergistic with monocrotophos to reduce growth and increase mortality. They reduce the quantity of synthetic insecticides and increase their effectiveness in insect pest management. Field studies with monocrotophos and neem oil at 2 % showed the reduction of *S. litura* population (Sahayaraj and Amalraj, 2006) which indicates that this kind of combination could be recommended for farmers. Other laboratory studies with endosulfan and neem oil formulation (War *et al.,* 2011), botanical insecticides with dillapiol (Bhaskar *et al.,* 2009) monoterpanoid and essential oil compounds (Laurin *et al.*, 2001) also confirmed this result.

However, further study is needed to develop more and more synergists that could be utilized in insect pest management programmes to reduce the indiscriminate use of harmful chemical insecticides.

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