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IMPACT OF BOTANICAL PESTICIDES DERIVED FROM *AZADIRACHTA INDICA* AND *MELIA AZEDARACHTA* ON THE AGRICULTURAL PEST CLUSTER CATERPILLAR (*SPODOPTERA LITURA*)

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ABSTRACT

Agriculture has had to face the destructive activities of numerous pests like fungi, weeds and insects from time immemorial, leading to radical decrease in yields. With the advent of chemical pesticides, this crisis was resolved to a great extent. Current problems with the use of chemical insecticides and emphasis on low inputs sustainable agriculture have pushed the microbial agents to the fore front for use in IPM systems. In the present study *Azadirachta indica* and *Melia azadirachta* were used to control the *Spodoptera litura*. Study of lethal concentrations, larval duration, mortality, pupal duration, adult duration, synergistic efficacy were carried on using the extracts of *Azadirachta indica* and *Melia azadirachta* at different concentrations was evaluated in the present study. Ethyl acetate extract recorded 80% larvicidal activity at 96h. Ethyl acetate extract of *M. azedarach* showed good larvicidal activity against *S. litura* larvae. Different concentrations showed larval mortality viz. 0.25 (10%), 0.5 (26.66%) 1 (46.66%) and 2 (73.33%). Ethyl acetate and chloroform extracts of both these plants crude equally mixed (1:1 ratio) and tested against pest of *S. litura* larvae. Ethyl acetate mixture showed potential larval mortality 93.33% at 2% concentration. Chloroform extract showed good larval mortality at 83.33% at % concentration. At 2% concentration of mixed ethyl acetate extract of *A. indica* treated showed all insects not emerged from pupal stage. This concentration showed pupal mortality observed dose dependent manner. It could not emerge adult stage from pupal stage. At 1% concentration showed 1. 14 day only survived *S. litura* larvae. It is not possible to produce their next generation. The observed duration of all stages were affected and highly inhibited their treatment of mixed crude extract. Life cycle duration inhibited and growth inhibited gradually. Hence it is inferred that the ethyl acetate extract of *Azadirachta indica* and *Melia azadirachta* can be used further for the solation of active molecules and to develop a new botanical formulation for the management of *Spodoptera litura*.

INTRODUCTION:

India primarily is an agrarian country and provides livelihood to about three fourth of the population and contributes half of the national income. The food gain production has reached to 250 million tons in 2011-12 from 50.8 million tons in 1950-51 during the last sixty years [1]. India's agriculture production has been growing at the rate of 3 percent per annum. India is the fourth largest food grain producer country in the world and offer vast potential for future increase in production[2].Agriculture was the key development in the rise of human civilization, whereby farming of domesticated species created food surpluses that nurtured the development of civilization. Development of agricultural techniques has steadily increased agricultural productivity and the widespread diffusion of these techniques during a time period is often called an agricultural revolution. A remarkable shift in agricultural practices has occurred over the past century in response to new technologies. Modern agronomy, plant breeding, pesticides and fertilizers, and technological improvements have sharply increased yields from cultivation, but at the same time have caused widespread ecological damage and negative human health effects[3]. In the past few decades agriculture has been characterized by enhanced productivity, by use of synthetic fertilizers and pesticides. Pesticides are substances or mixture of substances intended for preventing, destroying, repelling or mitigating any pest. It may be a chemical, biological agent (such as a virus or bacterium), antimicrobial, disinfectant or device used against any pest. They include insects, plant pathogens, weeds, molluscs, birds, mammals, fish, roundworms and microbes that destroy property, spread disease or vectors for disease or cause nuisance[4, 5]. Although there are benefits to the use of pesticides, some also have drawbacks, such as potential toxicity to humans and other animals. The overuse of pesticides and synthetic fertilizers damages the long-term fertility of the soil. The environmental impact of pesticides is often greater than what is intended by those who use them. Over sprayed insecticides and herbicides reach a destination other than their target species, including non target species, air, water, bottom sediments, and food. Though there can be benefits using pesticides, inappropriate use can counterproductively increase pest resistance and kill the natural enemies of pests. Many users are inadequately informed about potential short and long-term risks, and the necessary precautions in the correct application of such toxic chemicals are not always made. Pesticides can contaminate unintended land and water when they are sprayed aerially or allowed to run off fields, or when they escape from production sites and storage tanks or are inappropriately discarded[6].

Natural pesticides are active principles derived from plants for the management of human and animal pest organisms or it can be said to be biologically active ingredients, principally derived from plants, for the management of human and animal pest organisms. With the growing global demand for environmentally sound pest management strategies; there is a need to develop alternative pesticides with minimal or non-ecological hazards. They bio-degradable and their use in crop protection is a practically sustainable alternative. It maintains biological diversity of predators and reduces environmental contamination and human health harzards[7]. Biopesticides is a naturally occurring substances that control pest (biopesticides), microorganisms that control pests (microbial pesticides), and pesticidal substances produced by plants containing added genetic material (plant-incorporated protectants) or PIPs. Biopesticides are biochemical pesticides that are naturally occurring substances that control pests by non toxic mechanisms. Conventional pesticides, by contrast, are generally synthetic materials that directly kill or inactivate the pests. Biopesticides are considered eco-friendly and easy to use. In the USA, the EPA regulates the registration and use of earth friendly bio-pesticides. Biopesticides are key components of integrated pest management (IPM) programmes, and are receiving much practical attention as a means to reduce the load of synthethic chemical products being used to control plant diseases[8]. *Spodoptera litura* is an oriental leafworm moth which is considered as an agricultural pest. It is also known as the cluster caterpillar, cotton leafworm, tocacco cutworm and tropical armyworm. It is found in the Indo-Australian tropics. It is also established on most Polynesian islands where it occurs in a variety of island forms. The larvae feed on a wide range of plants and have been recorded from over 40 mostly dicotyledonous plant families. It is a major pest of many crops. It is vital to control this pest for the sustainable yields[9]. The neem tree (*Azadirachta indica*) from the Meliaceae (mahogany) family, known as margosa or Indian lilac, has long been recognized for its properties both against insects and in improving human health. The seed consist of a shell and 1-3 kernels which contain azadirachtin and its homologous. Both the bark and the leaves also contain biologically active molecules but not high levels of azadirachtin which is found mainly in the seed kernels. The tree is now grown in most tropical and sub-tropical areas of the world for shade, for reforestation programs and in plantations for the production of compound which have toxic and repellent properties against insects[10, 11]. The neem tree (*Melia azadirachta*) originates from the Indian subcontinent and now grows in the fry regions of more than 50 tropical countries around the world. The active compounds, from various parts of the plant with pesticidal, nematicidal, fungicidal, bactericidal, anti-inflammatory, anti-tumor and other medicinal properties. These have found applications in the pesticide, medical, healthcare and cosmetic industries all over the world[12, 13]. In the present investigation, *Azadirachta indica* and *Melia azadirachta* were used to control the *Spodoptera litura*. Study of lethal concentrations, larval duration, mortality, pupal duration, adult duration, synergistic efficacy were carried on using the extracts of the neem at different concentrations was evaluated.

2. MATERIALS AND METHODS

Azadirachta indica and *Melia azedarach* (Meliaceae) leaves were collected on December 2011 from Pachaiyappa's College campus, Chennai, Tamil Nadu.

EXTRACTION OF PLANT MATERIALS

Plant materials were shade-dried, ground into powder by an electronic blender and 300g of plant powder was soaked sequentially in 1000ml with increasing polarity of solvents (Hexane, Chloroform and Ethyl acetate) for 48h with constant shaking. The soaked powder material was filtered through filter paper. The solvent in the filtrate was evaporated under reduced pressure by vacuum rotary evaporator. These concentrated three solvent crude extracts were analyzed for bio-efficacy and active crude extract was further tested for growth inhibition bioassay. Two plants of crude solvent extracts were weighed and stored 4^{o}C until treatments.

HISTORY AND DISTRIBUTION

The genus *Spodoptera* includes 25 species and contains some of the most economically important insects' pests of cultivated crops.

Spodoptera litura: Female moths of *S. litura* lay eggs at night, leaving batches of up to 300 eggs on the under surface of host leaves. Sometimes, females lay eggs on other flat surfaces such as the walls of houses[14]. Newly hatched larvae are very susceptible to dry heat, usually staying on lower leaf surfaces during the day and feeding at night. In their last two instars, they feed only at night and seek shelter during the day under the lowest leaves or in the soil at the base of the host. Males can fly up to 3.1 miles per night when temperatures exceed 68^{o}F. Males mate once each night and avoid previously-mated females[15].

DEVELOPMENT: The development of a pest will guide program actions and influence the selection and success of eradication treatments, the duration of trapping activities, and regulatory functions. Many factors influence the development of insects, including host availability, pest population densities, photoperiod, rainfall, and temperature. Temperature is one of the most important factors influencing the development of all insect life stages. Scientists use site-specific temperature data, along with knowledge of insect development, to predict when pests will be most abundant and likely to damage plants at a particular location. Scientists use temperature data in a tool known as the degree day value.

Degree day values are useful for the following

- Predicting emergence of adults
- Determining the time to begin trapping
- Monitoring cycles of generation during a season
- Monitoring the effect of eradication or suppression measures

DAMAGE

S. litura larvae prefer to feed on young, tender leaves. They may also feed on growing points, young shoots, stalks, bolls, buds, and fruits, often gnawing holes which allow disease or not to enter the host. On newly infested hosts, young larvae feed at numerous small feeding points that eventually spread over the entire leaf. Older instars chew large holes or wholly consume leaves, or mine their way into young shoots or bare sections on young stalks, bolls and buds. They may destroy fruit such as tomatoes and peppers. If larvae feed on a young plant heavily, the plant's development is retarded and it may only produce small or late fruit.

INSECT CULTURE

S. litura larvae were collected from Arani (Periyapalayam), Thiruvallur District near Chennai. They were maintained on castor leaves in the laboratory
at 28 ± 1^{o}C : 11± 1hr photoperiod and 65-70% R.H. adults were released into oviposition chambers for egg laying. Eggs were collected, kept separately and newly hatched larvae were maintained on castor leaves. Freshly emerged 3rd instar larvae were used for the experiment.

TREATMENT

Each crude extracts were treated separately with leaf disc bioassay. Effective crude extracts were mixed with respective solvents and finally growth inhibitory studies were studied. Concentrations 0.25, 0.50, 1 and 2% concentrations were used for all treatments. Synergistic study were conducted and mixed each effective crude extracts 50:50 (%) ratio. The following treatment groups were followed.

- ✓ *Azadirachta indica* (Hexane, Cholorform and Ethylacetate extract)
- ✓ *Melia azedarach* (Hexane, Chloroform and Ethyl acetate extract)
- ✓ *A. indica* (Ethyl acetate extract) + *M. azedarach* (Ethyl acetate extract).

GROWTH INHIBITION

Third instar larvae (average weight: 12.4 mg) were used for growth inhibition bioassay. Leaf discs (4 cm diameter) were dipped in two extracts mixed respective plants at different doses with acetone: 0.25, 0.50, 1 and 2% and acetone alone were used as solvent control. Thirty replicates were maintained for each treatment and control. Each individual the analyzed and the stage were recorded until it died. The following parameters were considered: larval toxicity, larval period duration, pupal duration, pupal period duration and adult duration.

STATISTICAL ANALYSIS: Insecticidal activity lethal concentrations- Probit analysis were estimated EPA 1.5 software. Further larval, pupal and adult durations were analyzed using one way ANOVA. Significant differences between treatments were determined using Tukey's multiple range tests (P≤0.05).

RESULTS

In the present investigation, the impact of botanical pesticide on the agricultural pest cluster caterpillar (*Spodoptera litura*) was assessed. The leaf extract were extracted from *Azadirachta indica* and *Melia azedarach* by using the solvents hexane, chloroform and ethyl acetate. Six solvent extracts of *Azadirachta indica* and *Melia azedarach* were tested against *Spodoptera litura. A. indica* ethyl acetate extract was identified as the most toxic treatment against third instar larvae of *S. litura* at 2% concentration. Ethyl acetate extract recorded 80% larvicidal activity at 96h (Table 1). Ethyl acetate extract of *M. azedarach* showed good larvicidal activity against *S. litura* larvae. Different concentrations showed larval mortality viz. 0.25 (10%), 0.5 (26.66%) 1 (46.66%) and 2 (73.33%). Hexane extract of *M. azederach* showed least level of larval mortality. The larval mortality was directly related to the concentration of the treatments. Chloroform extract of both the plants also recorded high larval mortality. The *A. indica* ethyl acetate extract showed LC50 value of 0.861% and LC90 value of 3.393% (Table 2) and 1.03% and 4.29% for *M. azedarach.* The Chi-square values were significant at P≤0.05 level. The high Chi-square values in the bioassays probably indicated the heterogeneity of the test population. Different solvent crude extracts influenced larval mortality differently. Both of solvent and water control did not showed larval mortality.

SYNERGISTIC ACTIVITY OF *S. LITURA* LARVAL MORTALITY

Ethyl acetate and chloroform extracts of both these plants crude equally mixed (1:1 ratio) and tested against pest of *S. litura* larvae (Table 3). Ethyl acetate mixture showed potential larval mortality 93.33% at 2% concentration. Chloroform extract showed good larval mortality at 83.33% at % concentration. This treatment showed dose dependent manner activity, No larval mortality were recorded both solvent and water control

MIXED CRUDE EXTRACTS OF *S. LITURA* LARVAL DURATION

Normal (water control) larval period was observed in 16.8 days (Table 4). Solvent control showed as 16.4 days. 9.6 days was observed at 2% concentration of mixed ethyl acetate extract. This is the high larval duration inhibition which treated mixed extract.

MIXED CRUDE EXTRACTS ON *S. LITURA* PUPAL DURATION

Pupal duration also inhibited when mixed ethyl acetate extracts from *A. indica* and *M. azederach* (Table 5). Sixty percent (±6 days) inhibited when compared with water control. Significant larval duration inhibition was showed with treated synergistic extracts. Dose dependent activity was observed.

MIXED CRUDE EXTRACTS ON *S. LITURA* ADULT DURATION

Normal (water control) larval period was 6.04 days (Table 6). Solvent control showed as 5.43 days. At 2% concentration of mixed ethyl acetate extract of *A. indica* treated showed all insects not emerged from pupal stage. This concentration showed pupal mortality observed dose dependent manner. It could not emerge adult stage from pupal stage. At 1% concentration showed 1. 14 day only survived *S. litura* larvae. It is not possible to produce their next generation. The observed duration of all stages were affected and highly inhibited their treatment of mixed crude extract. Life cycle duration inhibited and growth inhibited gradually.

4. DISCUSSION

Results on the mortality effects of different concentrations of the *A. indica* and *M. azedarach* leaves extracts on the *S. litura* reported in the present investigation, confirm their potential against economic important cosmopolitan pest Asian army worm. This report is the first for studying the *A. indica* and *M. azedarach* species effects for control of the armyworm insect pest. Generally the botanical insecticides were reported to have less environmental impact than the most commercial synthethic insecticides for example neem insecticides[16]. Consequently, neem leaves crude extracts (i.e hexane, chloroform and ethyl acetate) were screened for the identification of insecticidal potentiality of the plant.

Leatemia and Isman reported that in leaf disc biossays, insect death was occurred due to a combination of starvation and contact toxicity of these extracts and larval mortality observed in all crude extract treated insects[18]. However, ethyl acetate crude extract have the higher toxicity with the lowest LC50 and cause more larval mortalities. Similarly in the present work may indicate the presence of active anti-insect phytocompounds in the ethyl acetate crude extract have the higher toxicity with the lowest LC50 of the both of *M. azederach* and *A. indica* and their synergistic effect. The *A. indica* ethyl acetate extract showed LC50 value of 0.861% and LC 90 value of 3.393% and 1.03% and 4.29% for *M. azederach*. The Chi-square values were significant at $P \leq 0.05$ level.

Effects of the compounds extracted from *M. azedarach* on insects reported by several researchers [18 – 21]. Control of mosquito is essential as many species of mosquitoes are vectors of malaria, filariasis, and many arboviral diseases; and they constitute an intolerable biting nuisance[22 – 23]. Biotechnologists and entomologists agree that mosquito control efficiency should be with selectivity for a specific target organism. New control methodologies aim at reducing mosquito breeding sites and biting activity by using a combination of chemical-biological control methods soothing several advocated biocontrol methods to reduce the population of mosquito and to reduce the man-vector contact. There has been a major concern for the promotion of botanicals as environment friendly pesticides, microbial sprays, and insect growth regulators amidst other control measures such as beneficial insects and all necessitate an intergration of supervised control[24]

The development of insects growth regulators (IGR) has received considerable attention for selective control of insect for medical and veterinary importance and has produced mortality due to their high neurotoxic effects[25, 26]. Although, biological control has an important role to play in modern vector control programs, it lacks the provision of a complete solution by itself. Irrespective of the less harmful and eco-friendly methods, suggested and used in the control programmers, there is still a need to depend upon the chemical control methods in situations of epidemic outbreak and sudden increase of adult mosquitoes. Hence, insecticides are known for their speedy action and effective control during epidemics. Nonetheless, they are preferred as effective control agent to reduce the mosquito population irrespective of their side effects. Recent studies stimulated the investigation of insecticidal properties of plant-derived extracts, and concluded that they are environmentally safe, degradable, and target specific[27]. Muthukrishnan and Pushpalatha evaluated the larvicidal activity of extracts from *Calophyllum inophyllum* (Clusiaceae), *Rhinacanthus nasutus* (Acanthaceae), *Solanum suratense*

(Solanaceae) and *Samadera indica* (Simaroubaceae), *Myriophyllum spicatum* (Haloragaceae) against *Anopheles stephensi*[28, 29]. Several indigenous plants in India and subtropical parts of Asia, such as *Ocimum basilicum, Ocimum santum, Azadirachta indica, Lantana camera, Vitex negundo* and *Cleome viscose* . Senthil Nathan *et al.* were studied for their larvicidal action of the field which collected fourth instar larva of *Culex quinquefaciatus*[30, 31]. Chavan, Zebitz reported that *Leucas aspera, O. santum, Azadirachta indica, Allium sativum* and *Curcuma longa* had a strong larvicidal, antiemergence, adult repellency and antireproductive activity against *A. stephensi*[32, 33]. In addition, *Pelargonium citrosa*, *Dalbergia sissoo* and *Mentha Piperita* were shown to contain larvicidal and growth inhibitory activity against *A. stephensi*[34,35]. The present investigation was conducted to study the effect of Azadirachtin, a neem tree *A. indica* extract, against larvae and pupae of *Culex pipiens* mosquitoes in east of the Algeria. The growth regulatory effect is the most important physiological effect of *M. azedarach* on insects. It is because of this property that family Meliaceae has emerged as a potent source of insecticides. Exposure of *A. stephensi* larvae to sub-lethal doses of neem leaves extract in the laboratory prolonged larval development, reduced pupal weight and oviposition[36]. In the field, delayed phenology of surviving larvae and reduced pupal weight are common occurrence after treatment with neem[37]. The direct and indirect contribution of such effects to treatment efficacy through reduced larval feeding and fitness need to be properly understood in order to improve the use of *M. azedarach* for management of *A. stephensi*. The results of this study indicate the plant-based compounds such as Azadirachtin (compounds present in the Meliaceae plant family seed) may be an effective alternative to conventional systhetic insecticides for the control of *Culex pipiens*, Undoubtedly, plant derived toxicants are valuable sources of potential insecticides. These and other naturally occurring insecticides may play a more prominent role in mosquito control programs in the future[38]. Synergistic activity on *S. litura* larval mortality Ethyl acetate and chloroform extracts of both these plants crude equally mixed (1:1 ratio) and tested against insect pest of *S. litura* larvae. Ethyl acetate mixture showed potential larval mortality 93.33% at 2% concentration. Chloroform extract showed good larval mortality at 83.33% at 2% concentration.

The results of this study will contribute to a great reduction in the application of synthetic insecticides, which in turn will increase the opportunity for natural control of various medicinally important pests by botanical pesticides. Since these are often active against a limited number of species including specific target insects, less expensive, easily biodegradable to non-toxic products, and potentially suitable for use in mosquito control programme[39], they could lead to development of new classes of possible safer insect control agents. Plant allelochemicals may be quite useful in increasing the efficacy of biological control agents because plants produce a large variety of compounds that increase their resistance to insect attack[40,41]. The intensive use of pesticides produces side effects on many beneficial insects and also possess both acute and chronic effects to the milieu[42]. Recently, bio-pesticides with plant origins are given for use against several insect species especially disease-transmitted vectors, based on the fact that compounds of plant origin are safer in usage, without phytotoxic properties, also leave no scum in the environment[43]. Large alterations in the fecundity and sterility of insects exposed to neem have been extensively reported, such as those in the fly, *Ceratatis capitata*[44]; banana root borer, *Cosmopolites sordidus* (Germar)[45] and mosquitoes, *A. stephensi* and *A. culicifacies*[46.] The work published by Khan *et al*., microscopically demonstrated that the decrease in fecundity of *Bactovera cucurbitae* and *Bactocera dorsalis* exposed to neem compound was due to the block of ovarian development[47]. Likewise, mixing of a *C. capitata* by interfering with oogenesis. The block in the ovarian activity of *C. capitata*, resulting from neem compound, was verified by histological observation[48]. Results from the study of Lucantoni *et al*. clearly indicated that the neem treated female mosquito, *A. stephensi*, displayed a delay in oocyte development in the vitellogenesis[49]. As discussed by weathersbee and Tang, the disruption of reproductive capability could lead to substantial population decline over time[50]. Furthermore, Dhar *et al*. revealed that the exposure to neem extract suppressed rather than inhibited oviposition in mosquitoes[46]. The efficacy of Azadirachtin on larvae, pupae, and adult of *Culex pipiens*. Correspondingly in the present study the mode of action and synergism with the biocides under laboratory condition on *S. litura* significantly reduced at maximum concentration 2% ethyl acetate extracts.

Previously and traditionally in many countries simple crude extracts have also been used as insecticides[51]. While plant crude extracts often consist of complex mixtures of active and inactive phytochemicals. Hummelbrunner and Isman reported that the exposure of several plant extracts to the insects causes delayed larval development through decreased growth rates[52]. Similar result was obtained in the present study; fractions treated larval weight was reduced and duration of the larva and pupa were increased. Reduced feeding activity (or increased anti-feedant activity) i.e., decreased consumption of castor leaf area was led to the larval weight reduction of larval and pupal duration extension. Hence, fraction eight has the higher inhibitory potential with increasing concentration on growth, larval and pupal developmental stages of *S. litura*. However, this effective extract shows higher anti feedant and larvicidal activities at higher concentrations. Similarly, Audrey Leatemia and Isman reported that the high concentrations of extracts caused high mortality of larvae even though small portions of the leaf discs were consumed[53]. Likewise in present study 2% concentration of mixed ethyl acetate extract of *A. indica* treated showed all insects not emerged from pupal stage. This concentration showed pupal mortality observed dose dependent manner. It could not emerge adult stage from pupal stage. At 1% concentration showed 1.14 day only survived *S. litura* larvae. Hummelbrunner and Isman reported that botanical extracts protect crops by reducing the fitness of insect herbivores via disruption of larval development, inhibition of larval growth and failure in pupal eclosion[52]. Similarly in the present study most effective eighth fraction deterrent effect evident by the poor feeding, growth and development, led to the development of abnormal pupa and adults. Telang *et al*. stated that malformed adult insects that were produced as a result of plant toxin treatments were short-lived and infertile and these effects could be considered important in the pest population reduction[54.] Biopesticides are an important group of naturally occurring, often show-acting crop protectants that are usually safer to humans and the environment than conventional pesticides, and with minimal residual effects[55]. From the present study results it can be understood that these (*A. indica* and *M. azedarach*) extract treatment is promising in reducing the feeding rate of *S. litura* and might be toxic to the larvae. Among the studied fractions isolated from ethyl acetate crude extract of (*A. indica* and *M. azedarach*) leaves, extracts shows promising antifeedant activity, larvicidal activity and insect development inhibitory activities that the other extracts.

Table 1: Effect of different solvent extracts from *Azadirachta indica* and *Melia azedarach* on *Spodoptera litura* larval motility

Name of Plant	Solvent	Concentration (%)			
		0.25	0.50	1.0	2.0
Azadirachta indica	Hexane	10	20	30	50
	Chloroform	6.66	26.66	40	56.66
	Ethyl acetate	13.33	30	53.33	80
Melia azedarach	Hexane	3.33	10	16.66	30
	Chloroform	3.33	3.33	20	43.33
	Ethyl acetate	10	26.66	46.66	73.33
Solvent control		0			
Water control		0			

Values are represented as percentage of 30 replicates

Table 2. Toxicity (LC_{50} and LC_{90}) on different solvent extracts from *Azadirachta indica* and *Melia azedarach* on *Spodoptera litura*

Name of Plant	Solvent	LC50	LL-UL	LC90	LL-UL	Chi square value
Azadirachta indica	Hexane	22.11	1.30 – 7.96	17.65	5.63 – 877.27	0.124*
	Chloroform	1.46	1.027 – 2.827	8.44	3.850 – 65.41	0.867*
	Ethyl acetate	0.861	0.658 – 1.172	3.393	2.138 – 8.538	0.132*
Melia azedarach	Hexane	4.817	2.25 – 156.15	41.13	8.60 – 108.99	0.087*
	Chloroform	2.47	1.68 – 6.07	9.70	4.51 – 81.53	1.157*
	Ethyl acetate	1.03	0.78 – 1.48	4.29	2.55 – 12.80	0.085*
Solvent control		0				
Water control		0				

LL: Lower limit, UL: Upper limit, LC_{50} and LC_{90} values are expressed as percentage (n=24). *$x2$ values are significant at $p \leq 0.05$ levels

Table 3. Synergistic activity *Azadirachta indica* and *Melia azedarach* on *Spodoptera litura* larval motility

	Concentration (%)			
Treatment	0.25	0.50	1	2
Ethyl acetate extracts of *Azadirachta indica* and *Melia azedarach*	20	53.33	70	93.33
Chloroform extracts of *Azadirachta indica* and *Melia azedarach*	13.33	46.66	60	83.33
Solvent control				
Water control				

Values are represented as percentage of 30 replicates

Table 4. Synergistic activity *Azadirachta indica* and *Melia azedarach* on *Spodoptera litura* larval duration

Treatment	Concentration (%)			
	0.25	**0.50**	**1**	**2**
Ethyl acetate extracts of *Azadirachta indica* and *Melia azedarach*	13.5 ±2.5ab	12.2±1.60c	10.4±1.4cd	9.6±0.67d
Chloroform extracts of *Azadirachta indica* and *Melia azedarach*	14.9±1.70a	13.6±1.94b	11.41±0.64c	10.5±0.38cd
Solvent control	16.4±1.54a			
Water control	16.8±2.21a			

Values in each column followed by the same alphabets are not significantly different by Tukey's test at $p \leq 0.05$

Table 5. Synergistic activity *Azadirachta indica* and *Melia azedarach* on *Spodoptera litura* pupal duration

Treatment	Concentration (%)			
	0.25	**0.50**	**1**	**2**
Ethyl acetate extracts of *Azadirachta indica* and *Melia azedarach*	8.46 ±0.72b	7.14±0.61bc	5.04±0.36d	4.51±0.19e
Chloroform extracts of *Azadirachta indica* and *Melia azedarach*	10.08±0.54a	8.43±0.19b	6.40±0.07c	5.21±0.02cd
Solvent control	11.1±1.42a			
Water control	11.6±1.15a			

Values in each column followed by the same alphabets are not significantly different by Tukey's test at $p \leq 0.05$

Table 6. Synergistic activity *Azadirachta indica* and *Melia azedarach* on *Spodoptera litura* adult duration

Treatment	Concentration (%)			
	0.25	**0.50**	**1**	**2**
Ethyl acetate extracts of *Azadirachta indica* and *Melia azedarach*	3.19 ±1.08ab	2.48±0.46c	1.14±0.02d	-
Chloroform extracts of *Azadirachta indica* and *Melia azedarach*	4.72±1.15b	4.31±0.18bc	3.16±0.51ab	2.54±0.04c
Solvent control	5.43±0.25a			
Water control	6.04±1.12a			

Values in each column followed by the same alphabets are not significantly different by Tukey's test at $p \leq 0.05$

The main advantages of using botanical pesticides like neem are reduced human toxicity[56]. Accordingly *A. indica* and *M. azedarach* extracts is also has the important toxic effect to the *S. litura* and may less effective to the humans. These present study data suggests that the ethyl acetate extract of the leaves of *A. indica* and *M. azedarach* should be further investigated in order to establish their chemical composition and may use in insect post control programmes. It is very likely that in future their role will be more significant in agriculture and forestry. Biopesticides clearly have a potential role to play in development of future integrated pest management strategies hopefully, more rational approach will be gradually adopted towards biopesticides in the near future and short-term profits from chemical pesticides will not determine the fate of biopesticides.

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