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Effect of Three Neem Seed Kernel Extracts on Performance of *Spodoptera litura* (Fabricius, 1775) Under Laboratory Conditions

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Abstract: ***Larvae of Spodoptera litura (Fabricius, 1775) are voracious feeders and cause huge economic losses to various crops. Non-judicious use of chemical pesticides has led to development of insecticide resistance and environmental hazards as well. Neem has been proven as an effective, ecofriendly and economical botanical pesticide to manage the pest but the performance of S. litura larvae exposed to crude neem seed extracts has not been studied in detail. The purpose of present study was to examine the growth inhibitory sub-lethal and lethal effects of three different neem extracts on neonate larvae and identify the most effective extract and the concentration. Three neem seed kernel extracts (aqueous, methanolic and hexane extracts) were assayed against larvae of Spodoptera litura Fabr. using leaf-dip bioassay methodology. Extracts (0.5% to 4%) were tested by allowing neonate larvae to feed individually for 24 h, and then transferring to untreated leaves. Larvae were monitored throughout their life span until adult emergence. All three neem extracts exhibited insect growth inhibitory and toxic effects and adults presented a range of malformations, however, methanol extract was found to be the most potent at all concentration levels.***

Keywords: neem seed kernel extracts, common cutworm, growth inhibitory, sub-lethal, lethal

1. Introduction

Spodoptera litura (Fabricius, 1775) (Lepidoptera: Noctuidae), the common cutworm is a devastating insect pest species which attacks and damages many important crops [1] resulting in great economic losses [2]. *Spodoptera litura* has earned the status of one of the costliest insect pests in terms of control after developing insecticide resistance. Currently, its management is largely dependent on spraying infested fields with synthetic insecticides, which has led to a number of problems such as increased risk to non-target organisms, ecological imbalance, hazard to pesticide appliers and environmental contamination with the potential of affecting the entire food chain [3, 4]. Moreover, the frequent use of insecticides exerts tremendous selective pressure on *Spodoptera* populations, further increasing the potential for development of resistance. Together, these factors make it imperative to look for an environmentally safe and sustainable alternative to chemical insecticides [5, 6, 7]. Plant-derived chemicals can be safely incorporated as part of Integrated Pest Management strategy against insect pests as they are eco-friendly, economic, readily available and have no or insignificant effect on non-target organisms and most importantly as they possess arrays of compounds with diverse modes of actions. Thus, insects find it comparatively difficult to develop resistance against them [8, 9]. Any bio-pesticide that promises efficacy with reduced ecological side effects should be promoted for pest control schemes [10]. All these factors make crude extracts, consisting of a mixture of compounds, a promising alternate to chemical insecticides. Neem, with a repository of active metabolites exhibiting profound biological activity against insects, has gathered much attention, globally. The various modes of action of neem include effects on feeding, metamorphosis, and reproduction [11, 12, 13]. Lepidopteran larvae are highly sensitive to neem [14] and have been studied to examine effects on growth and development [15, 16], digestion and nutrition [17], physiology [18] and feeding inhibition [19]. Feeding inhibition is an interesting phenomenon and can be attributed to presence of an antifeedant. An antifeedant is a chemical that does not kill the insect pest directly but prevents feeding on the treated crop, as a result insect dies out of starvation.

Laboratory and field trials with neem seed kernel extracts and other commercial neem derivatives/ products have amply demonstrated that these materials are effective against *S. litura* [19, 20, 21, 22]. Development of resistance also occurs slowly than to synthetic pesticides [23]. The present study was conducted to investigate the effects of three different neem seed kernel extracts on performance of neonate larvae of *S. litura* by observing various biological attributes of its life histroy.

2. Material and Methods

Insect rearing and extract reapration was done as described by Das & Singh [24].

Insect rearing: A laboratory culture of *S. litura* was maintained on leaves of castor, *Ricinus communis* L., at 27±2°C, photoperiod of 14: 10 (L: D) and 65-75% R. H. Leaves were replenished every 24 h till pupation. Aseptic conditions were maintained in the insectary to prevent microbial infection. The adults were provided with cotton swabs soaked in 10% honey solution as food.

Extracts: Ripened neem fruits were collected from Delhi University Campus, New Delhi, India. The fruits were depulped and shade-dried. Dry seeds were stored at ambient temperature indoors until needed. These seeds were decorticated and ground in an electric grinder to a fine powder. The` neem seed kernel powder (NSKP) was used for making extracts. The moisture content of the seeds was 10% and azadirachtin content was 0.7%.

A 4% (w/v) neem seed kernel aqueous suspension (NKAS) was prepared by placing a muslin bag containing NSKP in distilled water for 12 h. The liquid obtained was then filtered through organza cloth. Lower concentrations (2%, 1% and 0.5%) were prepared by serial dilution, and an emulsifier (Triton-X-100) was added[at]0.2%. The solution was always prepared fresh. Control solution was prepared with distilled water and Triton-X-100[at]0.2%. Methanolic (NKME) and hexane (NKHE) extracts were initially prepared from 10% (w/v) suspension of NSKP that was allowed to soak for 24 h; and then filtered through Whatman No.1 filter paper with addition of more solvent. The filtrate was concentrated in a rotatory evaporator at 40°C under reduced pressure and the concentrate was refrigerated for up to 6 weeks until used. Control solution consisted of 10% solvent (methanol or hexane) and 0.5% Triton-X-100 in distilled water. This solution was mixed with concentrated extract at 4% (v/v) and serially diluted to 2%, 1% and 0.5%.

The effect of methanol, aqueous and hexane neem kernel extracts (NKME, NKAS, and NKHE, respectively) on performance of *S. litura* larvae was studied by using leaf dip bioassay. Castor leaves were washed and air-dried. These leaves were dipped in the appropriate concentration of the extract for 5 seconds, air-dried at room temperature, and offered to neonate larvae (L1, first instar larvae). Larvae in controls were provided with leaves dipped in solvent only. Larvae were allowed to feed individually for 24 h, and then transferred to untreated leaves. Larvae were monitored throughout their life span until adult emergence. Different parameters were used for assessing the effects viz., larval and pupal duration, percent pupation/mortality, pupal weight, percent adult emergence and longevity. Ten larvae were tested for each concentration and five replicates were made.

Data analysis: Statistical analyses were done using Sigma stat 2.0. Significance between mean responses of insects under different conditions was determined by performing Fisher's test (F –test), followed by one-way and two-way AVOVA. Means were separated using Tukey's test.

3. Results

Effect on larval period

Larval period was significantly extended irrespective of the extract type as compared to control when first instar larvae (L1) fed for 24h on castor leaves treated with methanolic, hexane and aqueous neem kernel extract (NKME, NKHE and NKAS respectively). However, larvae failed to survive when fed on NKME 2% and 4% concentrations. The mean larval duration was statistically same in case of larvae fed on leaves treated with 4% and 2% concentrations in NKAS and NKHE. Among three extracts the shortest larval period at 1% and 0.5% concentration was recorded for larvae fed on castor leaves treated with NKAS. However, no significant difference was observed between NKME and NKHE at these concentration levels (Fig1.1).

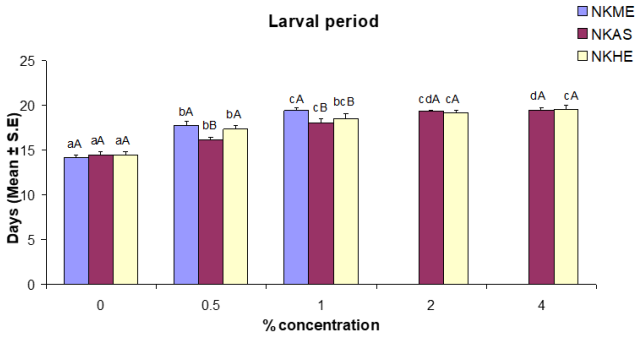

Figure 1.1: Larval period (in days) for *S. litura* larvae fed on castor leaves treated with methanolic, aqueous and hexane neem kernel extracts. Bars superscripted with different lower case letter are significantly different ($p<0.05$) in an extract type and bars superscripted by different upper case letter are significantly different ($p<0.05$) across the extract types.

Effect on Percent Pupation

The percentage of successful pupation was reduced in case of larvae fed on treated leaves irrespective of extract type, as compared to those fed on solvent treated (control). Larvae fed on treated leaves exhibited range of deformities (Fig A) and died during different larval stages. No pupae were obtained in case of NKME at 2% and 4% concentrations as larval mortality was 100%. Amongst three extracts (NKME, NSKAS, and NKHE), NKME was found to be most effective in terms of its effect on pupal formation. At 0.5% concentration the percent pupation in case of NKME was 36%, which was significantly lower than NKAS and NKHE (54% and 68%, respectively). At 1% concentration NKHE was least effective as the pupal formation was highest. The activity of NKAS was significantly better than the NKHE at 2% as well as 4% concentration as evident from the percentage of pupae formed. However, NKME could not be compared for the activity as 100% larval mortality was obtained at these two concentrations (Fig.1.2).

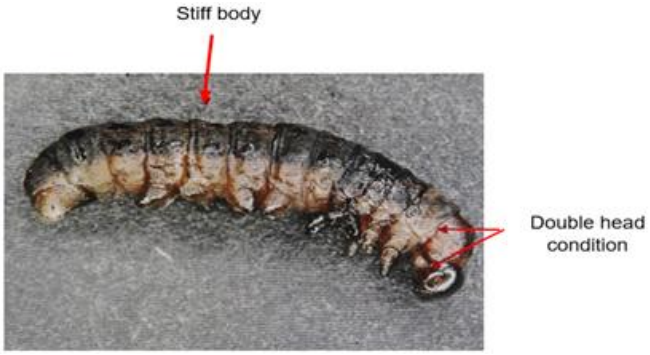

Figure A: Deformed larva of *S. litura*

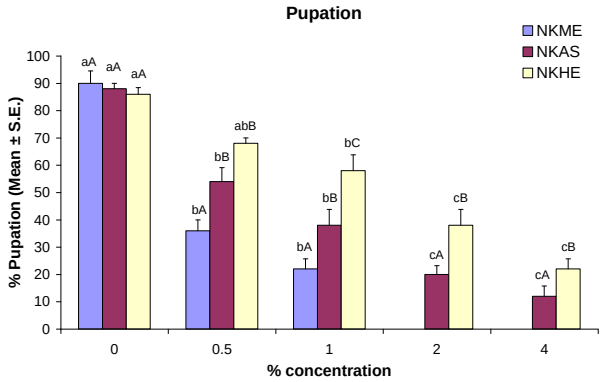

Figure 1.2: Percent pupation of *S. litura* larvae fed on castor leaves treated with methanolic, aqueous and hexane neem kernel extracts. Bars superscripted with different lower case letter are significantly different ($p<0.05$) in an extract type and bars superscripted by different upper case letter are significantly different ($p<0.05$) across the extract types.

Effect on pupal weight

The pupae obtained from larvae who had fed on NKME, NKHE and NKAS treated castor leaves for 24 hrs. as first instar were significantly lighter in weight than those formed in the respective controls. A dose dependent effect was observed for pupal weight at all concentration levels in case of NKAS. In case of NKHE extracts pupae formed in treatments were significantly lighter as compared to control group pupae. NKME was found to be most effective as compared to NKAS and NKHE at 0.5% and 1% concentrations as the pupae weighed significantly lighter. At 2% and 4% concentrations NKME could not be compared for the activity as no larvae survived to pupate. The performance of NKAS at these two concentration was significantly better than NKHE as evident from the pupal weight recorded (Fig.1.3).

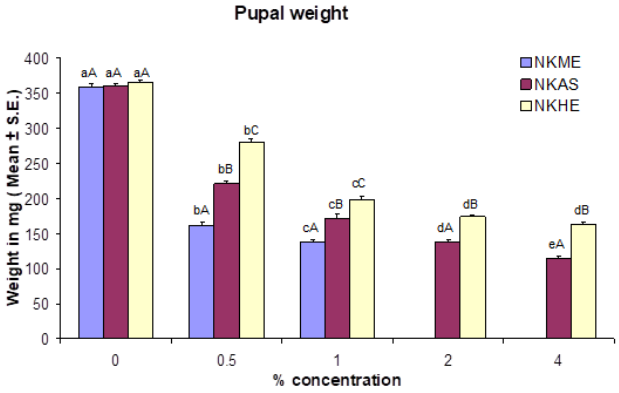

Figure 1.3: Weight of *S. litura* pupae obtained from larvae fed on castor leaves treated with methanolic, aqueous and hexane neem kernel extracts. Bars superscripted with different lower case letter are significantly different ($p<0.05$) in an extract type and bars superscripted by different upper case letter are significantly different ($p<0.05$) across the extract types.

Effect on Pupal Period

Effect of all three extract types was apparent in terms of pupal period as pupae exhibited a prolonged development period as compared to their respective control groups. Pupae were formed in NKAS and NKHE treatments on 2% and 4% concentrations but could not survive. Therefore, the efficacy of three extracts could be compared for only 0.5% and 1% concentrations and it was found that NKHE was more effective than NKAS & NKME as the pupal duration was maximum. However, the activity of NKME and NKAS was not significantly different in terms of their effect on pupal duration (Fig.1.4).

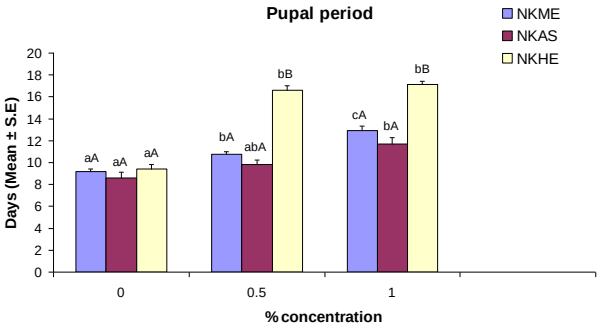

Figure 1.4: Pupal period (in days) for *S. litura* larvae fed on castor leaves treated with methanolic, aqueous and hexane neem kernel extracts. Bars superscripted with different lower case letter are significantly different ($p<0.05$) in an extract type and bars superscripted by different upper case letter are significantly different ($p<0.05$) across the extract types.

Percent adult emergence (out of pupae formed)

Emergence of adults was severely affected in case of pupae derived from larvae who had fed on NKME leaves but was not significantly different for NKAS and NKHE. The maximum effect was exerted by NKME as indicated from significant reduction in adult emergence percentage at 0.5% and 1% concentration. However, the performance of NKAS and NKHE was statistically same these concentration levels (Fig.1.5). Deformed adults were observed irrespective of extract type and concentration (Fig B) and in many cases adults failed to emerge out of pupae and died with pupal sclerites attached to various parts of body.

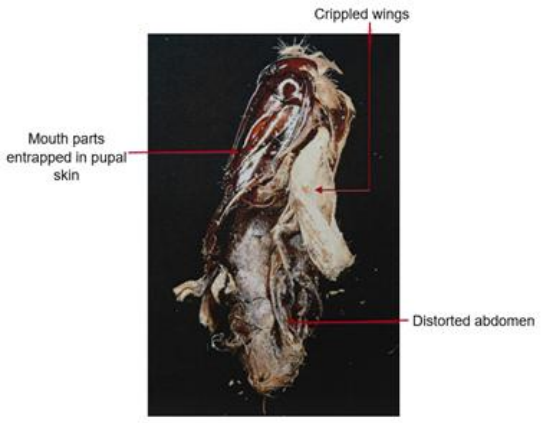

Figure B: Deformed adult of *S. litura*

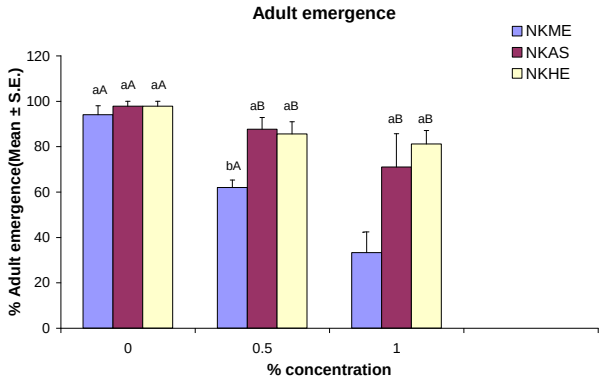

Figure 1.5: Percent adult emergence of *S. litura* larvae fed on castor leaves treated with methanolic, aqueous and hexane neem kernel extracts. Bars superscripted with different lower case letter are significantly different ($p<0.05$) in an extract type and bars superscripted by different upper case letter are significantly different ($p<0.05$) across the extract types.

Effect on percent survival (from larva to adult)

The survival of *S. litura* was effected negatively with the larvae were fed on treated castor leaves, in all extract types. The adverse effects of treatments were manifested in the form of a significantly lower percentage of larvae completing development and reaching adulthood as compared to their counterparts in control. NKME was found to be more effective as the percent survival was significantly reduced in comparison with NKAS and NKHE (fig.1.6) at 0.5 % and 1% concentration. At 0.5 % concentration the efficacy of NKAS and NKHE was statistically same. However, NKHE was found to be least effective at 1% concentration (Fig.1.6).

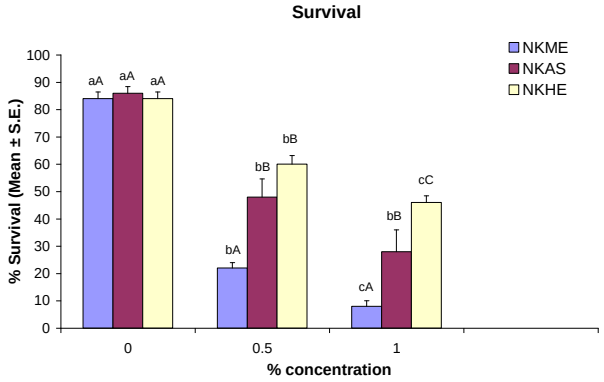

Figure 1.6: Percent survival of *S. litura* larvae fed on castor leaves treated with methanolic, aqueous and hexane neem kernel extracts. Bars superscripted with different lower case letter are significantly different ($p<0.05$) in an extract type and bars superscripted by different upper case letter are significantly different ($p<0.05$) across the extract types.

Longevity

The adults derived from the larvae fed on NKME treated castor leaves for 24 hr., experienced a significantly shorter life span as compared to those in control. Highest longevity (6.70 days) was observed for adults in control which was substantially reduced to 4.25 days in case of adults derived from larvae fed on leaves treated with 0.5% concentration. A further reduction (2.50 days) was observed in the longevity of adults when larvae were fed on the leaves treated with 1% concentration. Adults derived from larvae fed on castor leaves treated with NKAS also suffered reduction in their longevity. The adults obtained from control group larvae enjoyed a life span of 6.66 days which was almost double the duration recorded for adults at 0.5% concentration (Fig.1.7). At 1% concentration the adult life span was shortened to 2.54 days. Longevity was also affected when larvae were fed on castor leaves treated with NKHE. The adult life span was of 6.75 days in the control group. However, there was significant reduction in longevity at 0.5% concentration. At this concentration the life span was approximately half (3.78 days) as compared to the control (6.75 days). Further reduction was recorded at 1% concentration as the life span shortened to 2.86 days. When the activity of extracts was compared it was found that the performance of all the extracts was statistically same (fig 1.7) at 0.5 % and 1% concentration.

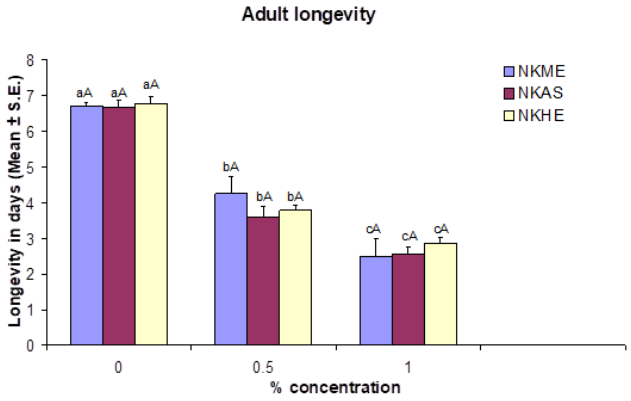

Figure 1.7: Longevity of *S. litura* adults obtained from larvae fed on castor leaves treated with methanolic, aqueous and hexane neem kernel extracts. Bars superscripted with different lower case letter are significantly different ($p<0.05$) in an extract type and bars superscripted by different upper case letter are significantly different ($p<0.05$) across the extract types.

4. Discussion

The deleterious effects of neem extracts were visible in the form of retardation of larval development and toxicity induced larval mortality at higher concentrations. The effect was most pronounced in case of NKME where larvae fail to complete the larval development period at higher concentrations (2% and 4%). However, in case of NKAS and NKHE larval mortality at the same concentrations was maximum at the end of larval period. Larvae died either as stiffened and blackened prepupae or larval-pupal intermolts and no pupation occurred. These results corroborate with the earlier findings of [20] who found that second instar (L2) *S. litura* larvae fed on methanloic extract of neem seed kernel, neem oil or azadirachtin treated castor leaves, failed to moult successfully into next instar. Prolonged development and induced mortality were also reported in *Trichoplusia ni*

and *Spodoptera exigua* [25] when larvae were fed neem seed extract incorporated artificial diet. In both species larval mortality was more pronounced during ecdysis, indicting an insect growth regulator like activity. Similar growth inhibitory effect of neem oil in *S. litura* larvae were also reported when fed on treated castor leaves from third instar (L3) onwards till pupation [26].

The observed extended larval period and mortality could be attributed to combined antifeedant and detrimental physiological effects of neem seed kernel extracts. Feeding behavior results from integration of sensory signals, recieved from the insect 's 'chemoreceptors (taste) present on tarsi, mouthparts and oral cavity, by central nervous system. Stimulation of deterrent cells present in chemoreceptors and blockage of sugar receptor cells stimulation by azadirachtin [15, 27, 28] resutling in feeding deterrency, alone could account for starvation and death of insects. The impaired physiological effects manisfested as prolonged larval period resulting in delayed pupation and abnormal moults in case of larvae that could withstand the deleterious effects of neem extracts can be explained by disruption of neuroendocrine control of moulting mediated by azadirachtin, its derivatives and metabolites [29, 30]. The physiological effects of azadirachtin are exerted both directly via cell and tissues and indirectly via endocrine system. Cells and tissue take up azadirachtin and results could be manifested as paralyzed muscles, necrosis of midgut cells and absence of midgut enzyme production. Indirect effects of azadirachtin can explain the delayed and abnormal moults as observed in the present study. The pupae formed were not phenotypically normal and exhibited extensive malformations. The pupae were small and weighed significantly less than their counterparts in control. The fate of the insect enclosed inside pupae was also not so secured as most of them died during pupal stage while others faced difficulties at the time of eclosion to adult and were partially successful to emerge into malformed adults. The percentage adult emergence was significantly lower in case of NKME as compared to the other two. Kaur et al., [31] also reported a similar effect of neem extracts on *S. litura*. Extended pupal durations, reduced pupal weight and adult emergence were recorded for pupae obtained from larvae fed on extract treated leaves. These observations could be explained knowing the ability of azadirachtin to impair neurosecretory system of the brain, resulting in blockage of the release of morphogenetic peptide hormones e. g. prothoracicotropic hormone and allatostatins that in turn modulate the functioning of prothoracic glands and the corpora allata respectively. Moulting hormone released from the prothoracic glands is responsible for new cuticle formation and ecdyses whereas juvenile hormone (JH) from the corpora allata controls the formation of juvenile stages at each moult. Disruption in any of these events by azadirachtin results in diverse effects seen as moult disruption and moulting abnormalities [12]. In the present study, insect growth regulatory activity of neem impairing metamorphosis at various stages of development and associated morphogenetic defects and mortality during different stages of development (Fig A, B) are of particular interest as together they reduced the overall survival of the insect. Due to deaths at every step of development the overall survival of insect was much reduced as compared to the control. Percentage survival was also lowest in case of NKME.

The adverse effects of neem extracts were manifested in case of adults too as various malformations e. g. deformed wings, antennae, proboscis, and swollen abdomen. Many adults due to highly crippled wings switched to hopping, this may turn them an easy prey under field conditions thus hampering population build up. Adults from neem extract treatments also had a significantly reduced life span as compared with respective controls. Adults from neem extract treated groups lived for a significantly shorter time period than control adults. These findings resemble that of Jeyablan & Murugan who They demonstrated that *Helicoverpa armigera* adults had reduced longevity when third instar larvae fed on deacetylnimbin, 17-hydroxyazadiradione, gedunin, salannin and deacetyl-gedunnin treated cotton leaves [32]. The reason for reduced adult longevity is unclear and additional information on adult endocrine system and the molecular mechanism involved is required to explain this effect.

In the present investigation growth regulatory effect on larvae due to feeding on castor leaves treated with the hexane extract was much less pronounced than the methanolic or aqueous extract. This could be attributed to presence of low content of effective chemicals in non-polar extract. Hexane extract of neem seed kernel powder contain extremely non-polar as well as semi-polar limonoids. Mostly these limonoids, responsible for biological activity include protomeliacins, azadirones, gedunin, vilasinins, salannins, nimbins and traces of azadirachtin [33]. Out of the array of these compounds, few may be responsible for insect growth regulatory activity like salannin, which is three to four times more than the concentration of azadirachtin in oil but the activity is less than that of azadirachtin [21]. In the present study, extracts of neem seed kernel powder were found to severly impact performance of *S. Litura* larvae. The three neem extracts impaired various biological attributes of larvae compared with their respective controls. Extracts exhibited insect growth regulatory activity at lower concentrations while at higher concentrations induced mortality that could be a combined result of starvation due to antifeedant effect as well as toxicity. The study also revealed that NKME and NKAS were more effective as compared to NKHE, which could be due to lower concentration of of main active componenta azadirachtin in non-polar solvent. The results obtained clearly indicate that neem extracts have the potential to influence the population dynamics of *S. litura*. The study indicates that NKAS can be used effectively to control this pest. At present, the pest is predominanatly managed by synthetic chemical persticides that contaminate environment, adversely impact living and non-living components of ecosystem and enter the living systems through food chain. Use of these neem based crude extracts can reduce the burden of chemical insecticides and lead to economic gains for small-scale farmers, resulting from increased agricutural prodution and cost cutting on chemical

insecticides. The abundance of neem tree (*Azadirachta indica*) in most of the developing countries, lacking intensive infrastructure and the ease of preparing the eco-friendly NKAS turns it into an inexpensive and interesting candidate with high potential to manage *S. litura*. Further research studies are required to modify this crude extract to get quick knowckdown effects.

Acknowledgment

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