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Effect of three neem seed kernel extracts on nutritional indices of *Spodoptera litura* (Fabricius,1775) under laboratory conditions

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Abstract: *Neem tree, Azadirachta indica, possess a repository of biologically active compounds effective against various agricultural crop pests. Spodoptera litura, the tobacco cutworm is one of the most damaging polyphagous crop pest. Three neem seed kernel powder extracts (aqueous, methanolic and hexane) were evaluated for effects on nutritional indices of S. litura larvae. Consumption of neem extracts treated castor leaves for a short duration by sixth instar larvae resulted in reduced consumption, growth rate, efficiency of conversion of ingested food and efficiency of conversion digested food. The effects were more pronounced at higher concentration of extracts. Methanolic and aqueous extracts were more effective as compared to hexane neem seed kernel extract. Interestingly approximate digestibility was higher on neem treated food. The results obtained from the study indicate towards the multiple ways neem- chemicals exert the adverse effects ranging from suppression of feeding to impairment of conversion of food for biomass production.*

Keywords: *Neem seed kernel powder extracts, tobacco caterpillar, nutritional indices.*

1. INTRODUCTION:

Spodoptera litura (Lepidoptera: Noctuidae), the tobaaco caterpillar is one of the most damaging cosmoplitan agaricultral crop pest infesting number of importnat crop plants [1]. With a wide distribution range [2], host plants across different plant families [1] and rapidly growing resistance to various chemical insecticides, this pest species becomes highly concerning [3-8]. Loss of crop yield may range from 26-100% depending on infestation level and damage inflicted to crop plants [9,10], leading to huge economic losses. As the management of this pest relies heavily on application of chemical insecticides it has devloped resisitance to various insecticides such as organophosphate, carbamate, pyrethroids and some selected newer chemistry insecticides [3-8]. Resistance towards chemical insecticides in insect pests species has turned a serious issue. The extensive application of chemical insecticides exerts strong selection pressure on exposed pest population and favours selection of gene(s) responsible for resistance in survivng population of insect pest [11]. Also, use of chemical insecticides have been associated with health hazards, adverse ecological outcomes, environmental toxicity and potential risk of entering the food chain via various routes. According to World Health Organization (WHO), 3 million people get exposed to poisoning and approximately 200,000 dies every year, globally, due to agricultural pesticides [12].Moreover, there has been a surge of interest in organic crop production [13,14] as organically grown food crops tend to be more nutritionally rich and contain higher percentage of antioxidants as comapred to conventionally grown [15]. As a result it becomes imperative to look for environmentally and biologically safer alternates with high efficacy agaiainst insect pests. Neem, referred to as Indian lilac [18] and scientifically known as *Azadirachta indica* is an excellent source of various biologically active metablotites, exhibiting range of activities impacting feeding, growth, development , reproduction and survival of the insect pests [16-19]. Adverse behavioural and physiological effects of neem on lepidopteran larvae have been reported earlier [16,17,18]. However, there is not much information avaialble regarding effect of neem on nutirtional indices of *S.litura* [20,21]. The objective of the present study is to examine and compare the efficacy of various crude extracts from neem tree seeds on nutritional indices of *S. litura* larvae.

2. MATERIAL AND METHODS:

Insect rearing and extract reapration was done as described by Das & Singh (2012)[19].

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.

Extracts: Neem fruits were collected, processed and various extracts were prepared according to Das & Singh, (2012). The 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 @ 0.2%. The solution was always prepared fresh. Control solution was prepared with distilled water and Triton-X-100 @ 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%.

Food consumption and nutritional indices

Effect of different concentrations of NKME, NKHE and NKAS on various parameters of nutritional indices was studied by feeding 6th instar larvae (L6) on castor leaves treated with neem extracts, for 24 h, using a standard gravimetric technique [22]. For the experiment, late penultimate stage larvae were selected and kept individually in plastic Petri dishes overnight, provided with wet cotton swab to prevent desiccation. Next morning newly molted 6th instar larvae were selected, with an almost empty gut due to overnight starvation. Each larva was weighed individually, which constituted the initial fresh weight of the larva (IFWL). 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. For control, leaves were treated with solvent only. These treated leaves were weighed separately, which provided the initial fresh weight of the food (IFWF). Newly molted L6 larvae were kept individually in plastic jars (10.5 cm x 10 cm) bottom lined with filter paper and allowed to feed on treated or control castor leaves for 24 h. Five replicates of 10 larvae each were used for different concentrations of each extract. Fresh leaves (weighed) were kept without larvae under similar conditions to estimate the natural loss of moisture for calculating corrected weight of the ingested food. Larvae were removed after 24 h and weighed individually to get the final fresh weight of the larva (FFWL). Faces discharged by larva was separated from leftover food and weighed. Uneaten food and leaves without larvae were also weighed to get final fresh weight of food (FFWF). Weight gain by individual larvae after 24 h of feeding was calculated by subtracting the initial fresh weight of the larva (IFWL) from final fresh weight of the larva (FFWL). Amount of ingested food was determined by subtracting the corrected weight of uneaten food (FFWF) from the weight of food initially provided (IFWF).

The following nutritional indices were calculated:

Consumption Index (CI) = weight of ingested food /feeding period x average weight of the insect

Approximate Digestibility (AD) = [weight of ingested food – weight of faeces/ weight of ingested food] x 100

Growth Rate (GR) = weight gain during the feeding period/ feeding period x mean body weight of larvae during the feeding period

Efficiency of Conversion of Ingested food to body substance (ECI) = [weight gain by larva/ weight of ingested food] x 100

Efficiency of Conversion of Digested food to body substance (ECD) = [weight gain by larva/ weight of ingested food – weight of faces] x 100

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

Consumption Index (CI)

Consumption Index (CI) of L6 larvae fed for 24h on castor leaves treated with neem extracts NKME, was significantly lower ($p < 0.05$) in treatments (o.5%, 1%, 2% and 4%) as compared to control. C.I., on the whole were significantly lower at 2% and 4% concentrations than at 0.5% and 1% concentrations. A similar trend was observed in case of larvae fed on castor leaves treated with NKAS, where consumption index was significantly higher in control as compared to treatments. However, consumption indices recorded at concentrations 0.5% and 1% were statistically identical and no significant difference ($p > 0.05$) was observed between 2% and 4% concentrations. In case on larvae fed on castor leaves treated with NKHE, consumption index was significantly higher in control at all tested concentrations,

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except at the lowest treatment level, i.e. 0.5%. A dose dependent effect was reflected at the higher concentrations level i.e. 1%, 2% and 4%. However, no significant difference was found between the consumption index of larvae fed on leaves treated with 0.5% and 1% (p> 0.05). A significant level of interaction was absent between concentration levels and extract types. The effect of NKME and NKAS was statistically same at all concentration levels, however, NKHE was less effective at all concentrations except the highest (4%) as compared to NKME and NKAS as the consumption indices recorded for larvae were higher. (Fig1.1).

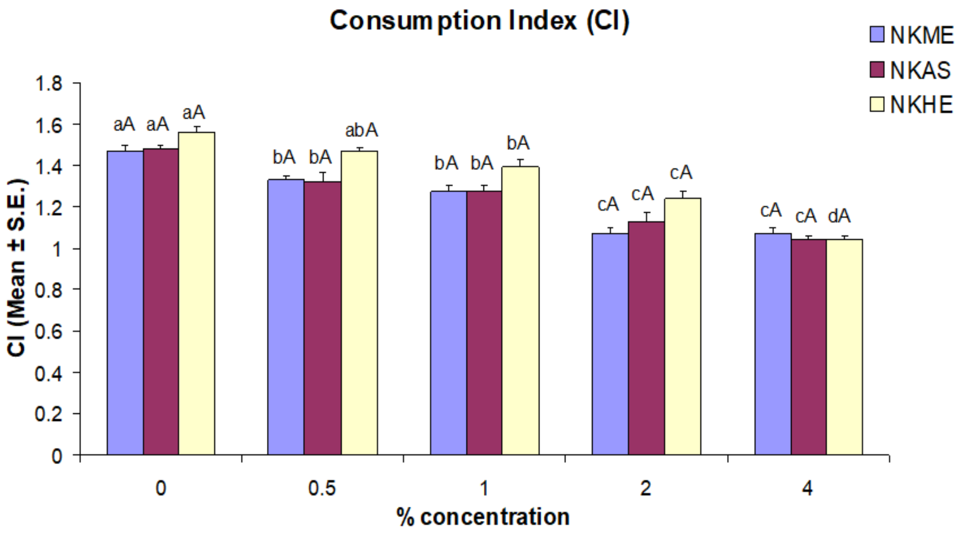

Fig.1.1: Consumption Index (CI) for the last instar *S.litura* larvae fed on castor leaves treated with methanolic, aqueous and hexane neem kernel extracts. Bars superscripted with different lower case letter are significantly different ($p<0.05$) in an extract type and bars superscripted by different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

Approximate Digestibility (AD)

Approximate digestibility (AD) of 6th instar larvae for the castor leaves treated with methanolic extract of neem kernel (NKME) was significantly higher (p, 0.05) than that of control treated leaf surfaces. AD values were statistically similar of the last instar larvae fed on castor leaves treated with different concentrations of NKME. A similar trend was observed in case of NSKS treatments, where larvae fed on NKAS treated leaves had significantly higher AD values as compared to those fed on control leaves. However, no significant difference in AD was recorded for castor leaves treated with different concentrations of NKAS. Larvae exhibited the same phenomenon in case of NKHE too. No significant difference was observed between AD of larvae fed on castor leaves treated with different concentrations of non-polar extract of neem (NKHE). When compared for efficacy, a significant interaction between concentration level and nature of extract was absent i.e. all three extract had same level of activity at all concentrations tested (Fig. 1.2).

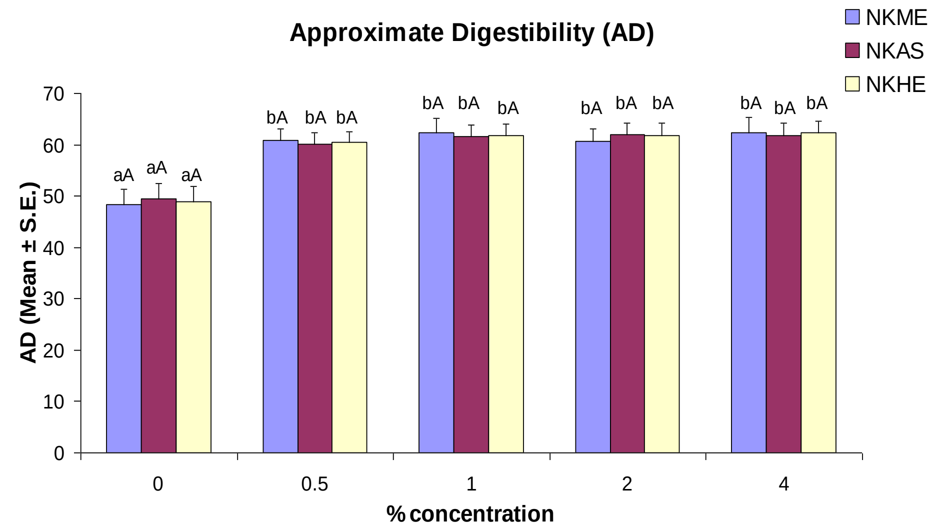

Fig. 1.2: Approximate Digestibility (AD) of *S.litura* larvae fed on castor leaves treated with methanolic, aqueous and hexane neem kernel extracts. Bars superscripted with different lower case letter are significantly different ($p<0.05$) in an extract type and bars superscripted by different upper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

Growth Rate (GR)

Growth rate of *S. litura* larvae was significantly higher for those fed on control leaves in comparison with GR observed on leaves treated with neem kernel powder methanolic extract (NKME). Growth rates of larvae on castor leaves treated with 1%, 2% and 4% concentration were statistically similar. Growth rates of larvae fed on leaf surface treated with NKAS were also lower than those of fed on respective control and the difference was always significant ($p< 0.05$). Amongst treatments, highest GR was achieved in case of 0.5% concentration. However, growth rates of 6th instar larvae fed on leaves treated with 2% and 4% NKAS were statistically equal but significantly lower than GR observed for larvae fed on leaves treated with lower concentrations (0.5% and 1%) of NKAS. In case of NKHE also the larvae in control group experienced significantly higher growth rate as compared to those in treatment groups. Statistically similar GR was observed for larvae fed on 0.5%, 1% and 2% NKHE treated leaves. The lowest growth rate of larvae was recorded at 4% concentration and that was the only concentration significantly different ($p< 0.05$) from rest of the treatments. Significant interaction was found between different concentrations and type of extracts. The GR of last instar larvae at lowest concentration 0.5% was statistically different for three neem kernel powder extracts used. This was highest for hexane, followed by aqueous and minimum for methanolic extract. The same trend was also observed at 1% concentration for different type of extracts. GR of larvae fed on leaves treated with 2% and 4% of NKME was statistically similar to GR of larvae fed on similar concentrations of NKAS. However, GR of larvae fed on 2% and 4% concentrations of NKHE was significantly higher than the larvae fed on same concentrations of the other two tested extracts (Fig. 1.3).

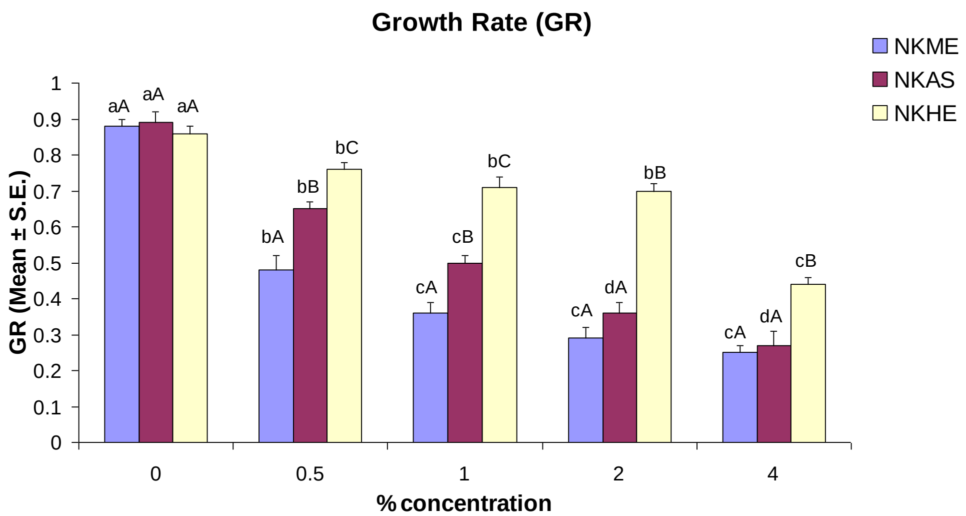

Fig.1.3: Growth Rate (GR) of *S.litura* pupae obtained from larvae fed on castor leaves treated with methanolic, aqueous and hexane neem kernel extracts. Bars superscripted with different lower case letter are significantly different ($p<0.05$) in an extract type and bars superscripted by different upper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

Efficiency of Conversion of Ingested food (ECI)

S. litura larvae had significantly higher ECI value on control leaves as compared with leaves treated with various concentrations of NKME, NKAS and NKHE. Efficiency of conversion of ingested food of larvae was statistically same for leaves treated with all, except 0.5% concentrations of NKME extract, where it was significantly higher than other treatments. ECI values of larvae fed on castor leaves treated with NKAS were significantly different at 0.5% and 1% and significantly higher than ECI of larvae fed on 2% and 4% NKAS treated leaves. However, the difference between 2% and 4% concentrations was not significant. In case of NKHE treatment groups, the difference obtained in ECI values were not significant at 0.5 and 1% concentrations. Significant level of interaction was found between concentration level used and the nature of extract. At 0.5% concentration level ECI of larvae fed on NKME treated leaves were significantly lower than those fed on NKAS or NKHE at the same concentration. At 1% concentration all three extracts exhibited significantly different activity. The ECI values obtained at 2% and 4% of NKHE were significantly higher than ECI values obtained for NKME and NKAS that were statistically equal at these concentrations (Fig. 1.4).

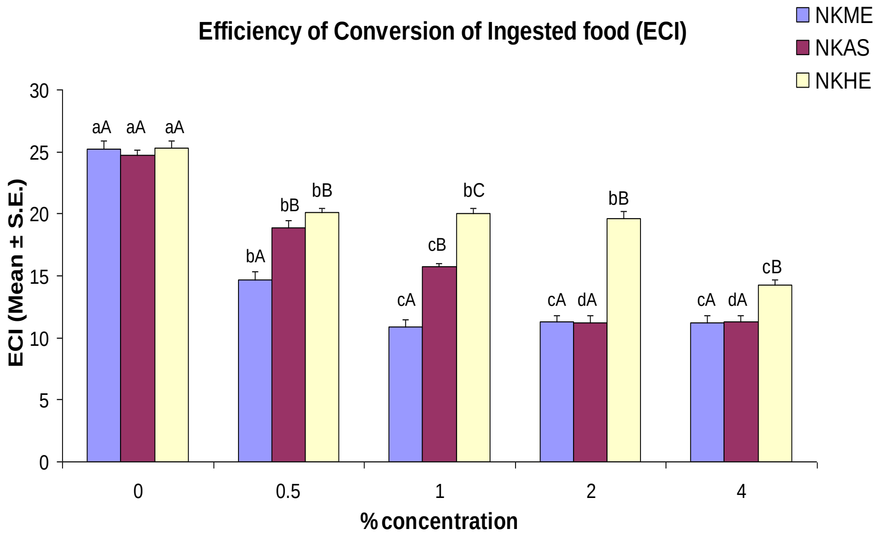

Fig.1.4: Efficiency of Conversion of Ingested food (ECI) for *S. litura* larvae fed on castor leaves treated with methanolic, aqueous and hexane neem kernel extracts. Bars superscripted with different lower case letter are significantly different ($p<0.05$) in an extract type and bars superscripted by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

Efficiency of Conversion of Digested food (ECD)

ECD values of larvae fed on leaves treated with NKME, NKAS and NKHE were significantly lower ($p< 0.05$) as compared their respective controls. Significant level of interaction was found between concentration level and nature of extracts ($p\leq 0.001$). NKME and NKAS had statistically same activity at 05%. The activity of NKME, NKAS and NKHE was significantly different ($p<0.05$) at 1%. The activity of NKME was the maximum as the ECD values recorded were lowest as compared to those recorded for larvae fed on NKAS and NKHE treated leaves at this concentration (1%). The activity of extracts at 2% concentration was statistically similar for NKME and NKAS. Moreover, this activity was statistically higher than NKHE at the same level of concentration (Fig. 1.5).

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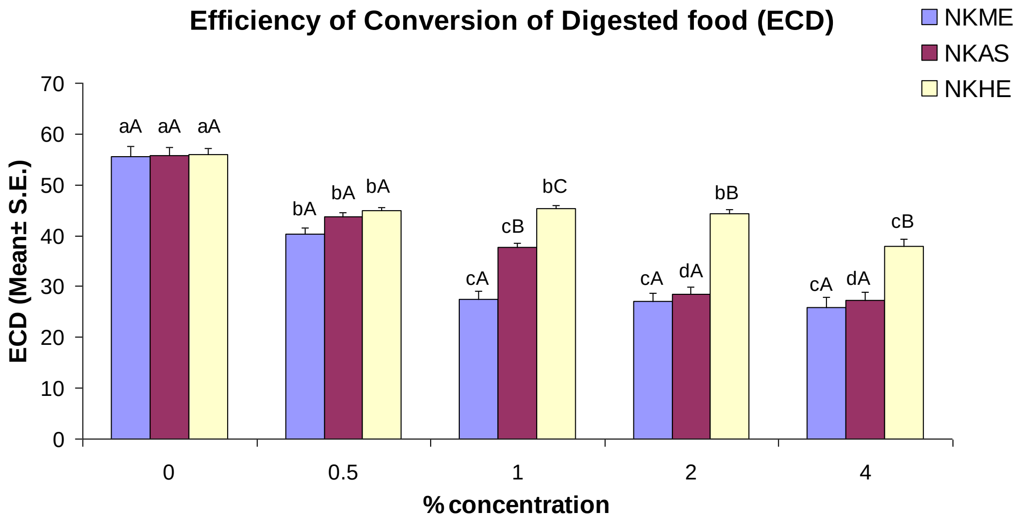

Fig. 1.5: Efficiency of Conversion of Digested food (ECD) of *S. litura* larvae fed on castor leaves treated with methanolic, aqueous and hexane neem kernel extracts. Bars superscripted with different lower case letter are significantly different ($p<0.05$) in an extract type and bars superscripted by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

4. DISCUSSION :

The results of this study indicated that neem seed kernel power extracts exerted both short term and long term adverse effects on *S. litura* larvae, as reflected through reduced consumption of food as well as by reduced efficiency of conversion of ingested food and efficiency of conversion of digested food. Insect growth results from adequate energy and biomass accumulated through ingestion of sufficient food, fulfilling its nutrient requirements. The ingested food after assimilation and conversion into energy and biomass leads to insect growth [23]. In the present study, neem seed kernel powder extracts were found to have detrimental effect on the food consumption by sixth instar larvae of *S. litura* larvae as reflected by reduced consumption index values. However, larvae exhibited increased AD values, probably indicating towards a strategy to compensate for reduced consumption and utilization of food in order to maintain growth rates or could be a result of greater retention of food in the midgut attributed to azadirachtin induced gut motility inhibition [24,25], though digestibility and food retention duration in the gut may not be closely correlated [26]. Reduction in ECI and ECD values, two highly important measures of food utilization and its conversion into biomass also suggests a negative effect of neem seed kernel powder extracts on *S. litura* larvae. ECI is an overall measure of an insect's ability to utilize food that it ingests, for growth. A drop in ECI indicates that more food was metabolized for energy and less being converted to body substance. Similarly, decreased ECD also resulted from increased proportion of digested food metabolized for energy. This could be attributed to compounds present in the extracts. Together the reduced ECI and ECD values indicate towards a post –ingestive toxicity effect rather than a simple antifeedant effect causing a mere reduction in CI. Similar results were found in case of fourth instar larvae of *Cnaphalocrocis medinalis* fed on rice leaves treated with aqueous extract of neem seed kernels [27]. These results are not surprising as azadirachtin can act both as an antifeedant as well as toxin and the two activities can be independent of each other [20]. Azadirachtin incorporated diet resulted into reduced food ingestion in case of *Heliothis virescens* larvae [28]. However, these larvae were able to digest treated diet as good or better than control diet. AD value was significantly higher on azadirachtin incorporated diet as compared to control, though ECI and ECD values were reduced. Similar suppressing effects of azadirachtin on food utilization efficiency were also reported in case of other insects [29,30,31]. There could be several underlying mechanisms responsible for the impaired conversion efficiency of ingested food observed in case of neem extract or neem- based insecticides ranging from reduced food digestion and nutrition uptake ability to impairment of conversion of ingested food to growth or a combination of both the factors [32].

Azadirachtin, the main active component of neem tree, can exert its effect via midgut epithelial cells leading to impaired secretion of various digestive enzymes and a consequent disruption of nutrient absorption [33,34]. Another probability could be allocation of more energy into induction of azadirachtin detoxification enzymes as compared to biomass investment [35,36], resulting into decreased ECI and ECD and hence reduced growth rate. Reduced growth rate observed in case of larvae fed on the neem extract treated leaves rate thus, can be attributed to a combination of decreased food intake as well as the reduced ability to convert ingested food into biomass.

5. CONCLUSION:

This study has immense practical utility as *S. litura* is a highly polyphagous agricultural pest and causes enormous damage to various crop plants. The larvae are voracious feeders and heavy infestation leads to complete defoliation of crop plants. Conventionally, the pest is managed by synthetic pesticides that has led to various agro- ecological issues. Moreover, there is a growing interest in organic production of various food crops and application of neem based natural or processed products as insecticides is highly acceptable in organic farming. This study depicted the potential of using crude neem seed extracts for management of this insect pest. As there are significant differences in the efficacy of the different neem seed kernel extracts with respect to the adverse effects on growth and survival of the insect as demonstrated in the study using nutritional indices, selection of optimum extract keeping in mind the efficacy, ease of preparation and economic cost becomes crucial. Based on the results of this study author recommends neem aqueous seed extract as an effective, readily available and economic solution for management of *S. litura*.

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