Effect of three neem extracts on oviposition behaviour and egg- hatchability of *Spodoptera litura* (Fabricius, 1775) under laboratory conditions

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Abstract- *Spodoptera litura* (Fabricius) is a polyphagous pest and the larvae being voracious feeders inflict enormous economic losses to various crops. Indiscriminate use of chemical insecticides has led to insecticide resistance and issues of environmental concern. *Azadirachta indica* A. Juss is known to possess a plethora of chemicals affecting behaviour and physiology of phytophagous insects. Oviposition is a crucial step in the life cycle of insect pests as it has a direct bearing on the population build-up and depends on a range of stimuli perceived by the gravid females. Neem extracts have been shown to deter oviposition by gravid females and adversely affect egg hatching in number of insect pests. In the present study three neem seed kernel extracts were assessed for the oviposition deterrent activity. Three neem seed kernel extracts (aqueous, methanolic and hexane extracts) were assayed against gravid females of *Spodoptera litura* Fabricius, using single-choice bioassay. Effect on egg hatchability was also studied using the three extracts (0.5% to 4%). A dose-response relationship was established for concentration and extract types, with respect to oviposition deterrent index. Methanolic extract was the most effective while hexane extract had no deterrent effect. Ovicidal activity was also maximum in case of methanolic extract, followed by aqueous and least in case of hexane extract. A significant interaction was found between concentration and type of extracts with respect to ovicidal activity.
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Index Terms- neem seed kernel extracts, *Spodoptera litura*, oviposition deterrent, ovicidal activity

I. INTRODUCTION

Oviposition position behaviour of phytophagous insects is the central force directing evolution of insect-plant interactions. As oviposition is directly linked with reproduction, it has tremendous impact on population dynamics of herbivore insect pests. After successful mating and maturation of eggs the next task a gravid female needs to accomplish is, finding a suitable host plant and accepting it before laying the eggs. This is a crucial step and egg laying female needs to carefully choose the food plants for the optimal performance and survival of the offspring [1,2]. Lepidopteran mothers rely on chemical cues (volatile or contact), visual cues, or a combination of the two, while selecting plant surfaces as oviposition sites. The stimuli perceived from plant surfaces thus are of paramount importance in decision making by the gravid females for selecting oviposition sites. Female assesses compounds from the plants to ascertain the suitability of selected plant for oviposition. The sensory cues that elicit or inhibit oviposition by female, clearly play an important role in the survival of phytophagous insects because the hatching larvae, especially in lepidopterans, are often relatively immobile and thus depend on the judicious choice of food plant by the mother [3, 4]. Oviposition deterrents are chemicals that deter an insect from egg laying and can be used as an important weapon in integrated pest management. Compounds from neem tree, *Azadirachta indica* A. Juss, are known to interfere with the oviposition behavior of the gravid females, though the number of studies regarding this aspect is not as much as on the other aspects of behavior. Oviposition deterrence by various neem products has been shown for various insect orders including Lepidoptera. Within Lepidoptera, neem-based oviposition deterrence has been reported in *Corcyra cephalonica* [5,6], *Heliothis armigera* [7] and *Spodoptera litura* [8,9]. Chemicals from neem are also believed to affect egg-hatching, adversely [10].

Unfortunately, past studies have utilized variously prepared extracts and suspensions of different parts of the neem tree, and/or neem seeds from diverse sources. The likely result is that varying amounts of the different active ingredients of neem make comparisons between studies difficult. In the present study an attempt was made to compare the effect of three neem extracts on the egg-laying behavior of the moth *Spodoptera litura* (Fabricius). Effort was also made to evaluate the effect of these extracts on the hatchability of eggs laid by *S. litura* females.

II. MATERIALS AND METHODS

Insect rearing "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 the Delhi University campus area. 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. This 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%. This 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%" [11].

Oviposition response

The experimental cage was made up of plexiglass (40 x 20 x 20 cm) having side walls of plexiglass and a small window (10 x10 cm) on the front wall fixed with a long muslin sleeve (30 cm length). The cage was divided into 3 sectors, (i) treatment/stimulus sector (10 cm), (ii) middle sector (20 cm) and (iii) control/blank sector. Treatment sector contained the castor leaf treated with desired concentration of neem extract while at the opposite end castor leaf treated with solution served as control. The bottom of cage was lined with filter paper that also had this sector demarcation. Four diet cups with cotton swab containing 10-15% honey solution were kept in the middle sector of the cage as adult food. The newly emerged moths were initially kept individually in separate plastic jars (10.5 x 10 cm). these moths were paired on second night in cage (40 x 20 x 20 cm). these mated females were used for oviposition bioassay on third night as females in culture were observed to lay highest number of eggs on third night.

Oviposition response choice bioassay

The oviposition study was made using single choice bioassay. Tender freshly excised castor leaves with intact petioles were selected for application of extracts. One castor leaf was dipped in appropriate concentration of extract for 10 seconds and air dried at room temperature. The petiole of leaf was dipped in water, contained in a narrow neck glass bottle. The mouth of the bottle was plugged with cotton. Control leaf was dipped in respective control solution, air dried and plugged in another bottle. Treated and control leaves were kept in the middle of the two end sectors of the cage i.e., in the treatment and control sector, respectively. Five pairs of males and mated females were released in oviposition cage at 6 p.m. Next morning the eggs laid by females, were counted. Each bioassay was performed with separate group of males and females and was replicated five times.

Oviposition Deterrence Index (ODI)

Oviposition deterrence index was calculated on the basis of number of eggs deposited by females on castor leaves treated with different extracts and their respective control solutions.
ODI was calculated using the formula: ODI = (C –T)/ C+T) x 100
where C= No. of eggs on control leaf and T= No. of eggs on treated leaf

Effect of extracts on hatchability of eggs

To evaluate the efficacy of the neem extracts in reducing the hatchability of eggs, the eggs were treated topically with 50 µl each of the extract concentration separately, air dried and then kept for observation. The number of neonates emerged was recorded. The effect was quantified by calculating the percent hatchability of eggs in treatments and control.

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.

III. RESULTS

Effect on oviposition behavior

Castor leaves treated with methanolic (NKME) extracts of neem kernel were observed to have deterrent effect on oviposition of *S. litura* females. Gravid females deposited 324.60 eggs on castor leaves treated with the lowest concentration (0.5%) of NKME. This was significantly higher as compared to the eggs laid by females on the leaf surface treated with higher concentrations of NKME but almost half the number of eggs that were deposited by females on leaves treated with control solution. However, eggs laid by females on castor leaves treated with 1% and 2% concentrations (262.40 and 206.40 eggs, respectively) were statistically same (Fig.1). The number of eggs deposited by females on the surface of leaves treated with 4% concentration of NKME was the lowest (165.40 eggs) but statistically same as that of eggs recorded on 2% NKME treated leaf surface.

Deterrent effect of neem kernel aqueous treatments (NKAS) was also observed on the egg-laying of *S. litura* as significantly lower number of eggs was deposited by females on NKAS treated leaf surface as compared to control surface (Fig. 1). Mean number of eggs recorded from the leaf surface treated with 0.5% concentration was 463.20, which was statistically lower than control but higher than the surfaces treated with higher concentrations (1%, 2% and 4%) of extract (Fig. 1). However, the mean number of eggs laid by females on leaf surface treated with 1%, 2% and 4% was not statistically different.

No significant effect of NKHE on egg laying of *Spodoptera litura* females was observed as the number of eggs laid by females on extract treated leaves of castor was statistically similar to that of eggs deposited on the surface of leaf treated with control solution. The mean number of eggs deposited by gravid females on leaf surface treated with 4% concentration was 627.60, which was lower than the number of eggs laid by females on control leaf surface but statistically there was no significant difference between these two.

A significant level of interaction was observed between the various concentrations and the nature of the extracts. When the extracts were compared it was established that NKME was most effective as the number of eggs laid on leaves treated with NKME was significantly lower than that of eggs laid on NKAS and NKHE treated leaves. The oviposition deterrence effect was significantly higher for NKME as compared to NKAS at highest and lowest concentrations, i.e. 4% and 0.5%. However, at concentrations 1% and 2% the number of eggs present on NKME and NKAS treated leaves was statistically same. No oviposition deterrent effect was observed for NKHE as the number of eggs laid on the surfaces treated with this was same as control surface (Fig. 1).

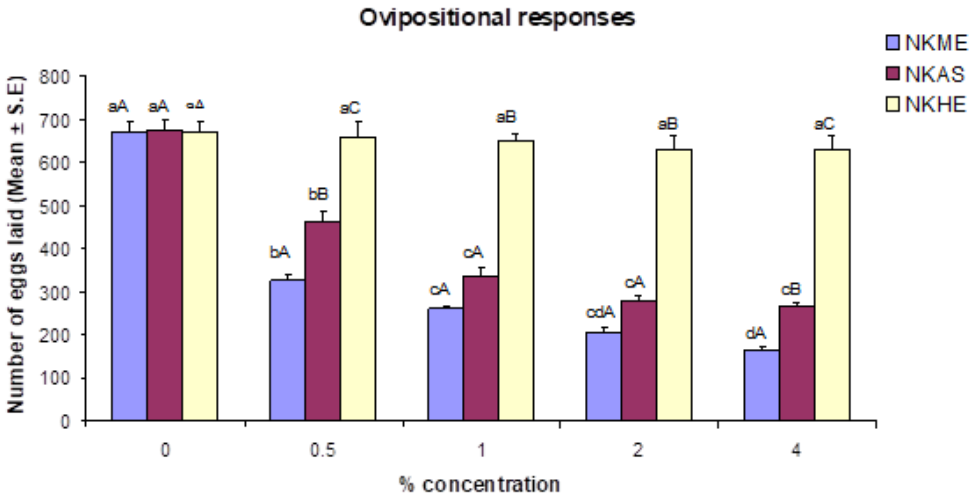

Fig: 1 Ovipositional responses of *S. litura* females towards castor leaves treated with methanolic, aqueous and hexane neem kernel extracts in single-choice bioassays. Bars represent the mean values and vertical line above each bar indicates S.E. Bars superscripted with different lower case letter are 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.

Oviposition deterrence index (ODI)

The oviposition by females was deterred to various extents by different NKME treatments (Fig. 2). The deterrency imparted by various concentrations was significantly different ($p < 0.05$) at all levels. The oviposition deterrence (ODI) obtained at 0.5% concentration (34.89 %) was almost half as compared to the ODI recorded at 4% concentration (60.42 %). A dose dependent relationship was clearly visible at all concentrations, i.e. with the increase in concentration of extract corresponding increase in ODI was recorded. Oviposition deterrence index recorded for castor leaves treated with 0.5% concentration of NKAS had a significantly lower value (18.76%) as compared to the ODI at higher concentrations of NKAS (Fig. 2). The ovipositional deterrence index calculated for NKHE treatments at different concentrations was statistically similar. The ODI values varied from 3.52% to 1.05% at 4% to 0.5% treatment concentrations (Fig. 2).

The effect of different levels of concentrations was dependent on the type of extracts. A significant interaction was present between the two factors, i.e. concentration and extract. When the activity of three extracts was compared using ODI as the basis, all three extracts differed significantly. NKME emerged out as the most potent. At the lowest concentration level (0.5%)the deterrency exerted by NKME was 34.89% which was almost double the effect of NKAS at 0.5% (18.76%). NKHE was the least effective among the three extracts at all concentrations (Fig. 2). NKAS was having a level of activity significantly lower than NKME but always higher than NKHE ($p < 0.05$) at all concentrations.

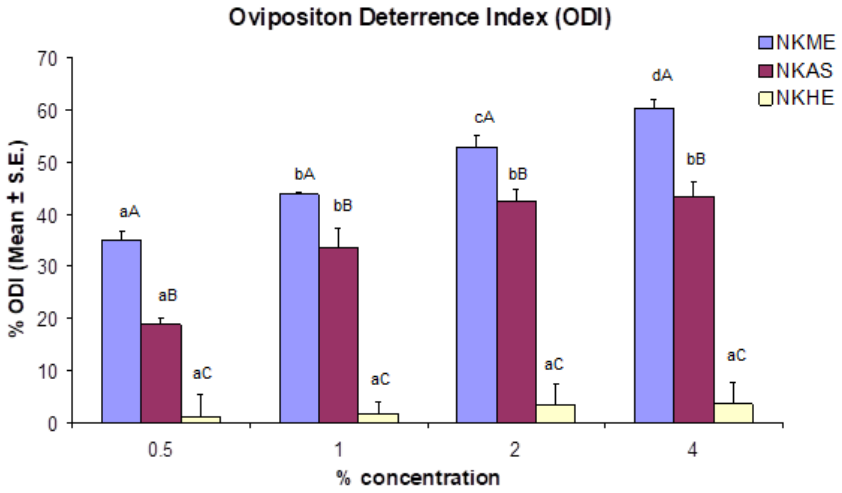

Fig. 2 Oviposition deterrence index (ODI) *of S. litura* females to methanolic, aqueous and hexane neem kernel extracts in single-choice bioassays. Bars represent the mean values and vertical line above each bar indicates S.E. Bars superscripted with different lower case letter are significantly different ($p<0.05$) in an extract type and bars superscripted by different upper case letter are significantly different ($p<0.05$) across the extract types.

The oviposition deterrence index clearly depicted the positive dose-response relationship of NKME concentrations which was not evident when the response was assessed on the basis of number of eggs present on the leaves. The significance therefore of calculating this index was that it provided a much better view of the response at concentrations that had apparently an overlapping effect.

Effect on egg-hatchability

The hatching percentage of eggs treated with NKME extract was significantly reduced in treatments as compared to those in control groups. Egg hatchability was affected in a dose dependent fashion. Among different treatments hatchability of eggs was maximum at 0.5% concentration where 48.08% of eggs gave way to larval emergence as compared to 21.92% at 4% concentration which was minimum (Fig. 3). The hatching percentage of eggs was also affected when treated with NKAS, in a dose dependent manner. The mean percentage of eggs hatching successfully as first instar larvae at 0.5% concentration was 65.84% which was 74.68% of that taken place in control group. At 4% concentration 27.20% of eggs allowed larval hatching and that were only 30.85% of that of control (Fig. 3). Eggs treated with NKHE also showed a significant reduction in hatchability as compared to those treated with

solvent solution ($p<0.05$). Hatchability of eggs reduced as the concentration of extract increased. Eggs treated with 0.5% concentration exhibited 74.40% hatchability which was statistically the highest hatching percentage observed among different treatment groups. The hatching percentage was significantly low (32.48%) at 4% concentration and it almost half of that observed at 0.5% concentration.

When the extracts were compared to assess their activity in terms of egg-hatching percentage it was found that a significant interaction was present between concentration and type of extracts. NKME was significantly better ($p < 0.05$) at all concentration levels tested as compared to the other two. The percent hatching was 48.08%, 65.84% and 74.40% at 0.5% concentration in NKME, NKAS and NKHE, respectively. The efficacy of NKAS was always much higher than NKHE and it was established that NKHE had the least effect on egg- hatching of *S. litura* (Fig. 3).

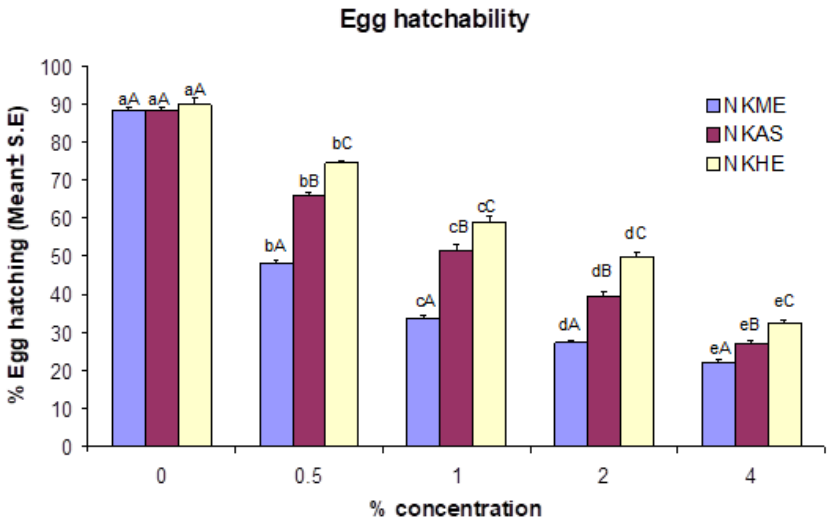

Fig 3 Hatchability of *S. litura* eggs treated with methanolic, aqueous and hexane neem kernel extracts. Bars represent the mean values and vertical line above each bar indicates S.E. Bars superscripted with different lower case letter are significantly different $p<0.05$) in an extract type and bars superscripted by different upper case letter are significantly different ($p<0.05$) across extract types

IV. DISCUSSION

Effect on oviposition behaviour: The results in present study were in contrast with the findings of Nauman and Isman [12] who reported crude neem oil induced oviposition deterrence in *S. litura*. A possible explanation for this discrepancy in results could be the difference in the amount of bioactive components in the material used and the processing. These results are in confirmation of the study conducted by Joshi and Sitaramaiah [8]. They observed a massive reduction (96%) in the number of eggs laid by *S. litura* females on tobacco seedlings treated with neem seed suspension. Ayyangar and Rao [9] also reported a similar deterrent effect of methanolic neem seed kernels extract. The result in present study differ from that of Klemm and Schmutterer [13] who reported an increased egg laying by *Plutella xylostella* on cabbage leaves when sprayed with aqueous neem seed extracts. Oviposition deterrence effect of neem was also demonstrated in *Spodoptera exigua* [14] where three neem based insecticides Agroneem, Ecozin and Neemix as well as neem leaf powder extract (aqueous) used at various concentrations in no-choice and choice bioassays. Neem leaf poweder delivered the highest activity followed by Agroneem, Ecozin and Neemix, respectively. It was suggested that the effect was associated with azadirachtin concentration. However, Saxena and Rembold [7] working with *Heliothis armigera* females reported that azadirachtin alone had no oviposition deterrent effect. It has been suggested that compounds in neem other than azadirachtin may be responsible for oviposition deterrent effects [15,16,17].

Effect on egg hatchability: The ovicidal effect of extracts was reflected in the form of significant decrease in the percentage of larvae hatching out from treated eggs. A positive dose-dependent response was visible in case of all three neem seed kernel extracts (NKME, NKAS and NKHE). However, significantly higher hatching in case of NKHE at all concentrations as compared to the other two extracts suggested a comparatively low activity. These results suggested that either there was a quantitative or qualitative difference in compounds causing hatching inhibition in case of NKHE. The compounds responsible for this activity might be present at a relatively low concentration in hexane extracts or chemically different and less active compounds were responsible for the effect. The maximum effect was obtained in case of NKME followed by NKAS suggesting involvement of some polar compounds. An aqueous extract of neem seeds applied as a spray was reported to significantly reduce the egg viability of *Maruca vitrata* [18]. A similar effect of

methanolic extracts of neem seeds on hatchability of *Earias vitella* eggs was made by Gajmer et. al. [19]. Egg viability was also adversely affected when eggs were laid on a treated surface, were allowed to hatch there only. In *Spodoptera exigua*, Greenberg et. al. [14] observed that neem based insecticides and neem leaf powder extract cause egg mortality at various concentrations. It was suggested that that effect was associated with azadirachtin concentration.

V. CONCLUSION

Oviposition by an insect constitutes a critical step in determining the magnitude of its population growth. The array of chemicals presents in or on the surface of the plants may attract or repel the gravid females and thus influence deposition of eggs. Plant-chemicals adversely affecting the cascade of events leading to oviposition by the females are of profound interest as they can play important role in integrated pest management. The results of the bioassays on the ovipositional response of the *S. litura* females indicate oviposition deterrent as well as ovicidal effects of neem seed extracts and suggest the presence of oviposition deterrent and ovicidal compounds in polar extracts of neem seed kernel. These chemicals are either absent or present below critical concentrations in hexane extract, that fail to trigger the oviposition deterrent response. The oviposition deterrent and ovicidal effects, together can restrict the population build-up of *S. litura* on host plant. In the present study NKAS was found to be highly effective though was not as potent as NKME in terms of oviposition deterrent and ovicidal activity. This indicates that NKAS can be used to manage this pest for its of immense practical utility for resource poor farmers in developing countries. The aqueous suspension can be produce readily by the local farmers using locally available raw material. The synergistic effect of diverse bioactive component in crude extract will also mitigate the development of resistance which is very common in case of insecticides based on single or few bioactive component(s). There is a need to promote this natural insecticide for an economic and environmentally safe pest management.

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