

Original Research Article

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Bioefficacy of Animal Urine Based Formulations against *Spodoptera litura* (Fabricius) and *Spilarctia obliqua* (Walker) at Pantnagar, Uttarakhand – A Novel Approach

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ABSTRACT

To evaluate the bioefficacy of animal urine based formulations *i.e.* Cow urine collected from Desi, Holstein and Jersey breeds, Buffalo urine, Ox urine, Goat urine and Horse urine @ 20% and animal urine based neem leaf extracts @5% against *Spodoptera litura* and *Spilarctia obliqua*, laboratory experiments were conducted for the first time in Department of Entomology, GBPUAT, Pant Nagar, Uttarakhand during 2017. The growth and development studies clearly showed the efficacy of Desi cow urine, Goat Urine, Horse urine and all animal urine based neem leaf extracts against 7days old larvae of *S. litura* and *S. obliqua* as all larvae died in treated castor leaves. Among the other treatments, significantly less survival and reduce weights of larvae, pupae and adults were recorded with extended developmental period and very less growth index values in comparison to untreated control. Antifeedant activity of animal urine based formulations showed that Goat urine, Desi cow urine neem leaf extract(DCUNLE) and Goat urine neem leaf extract (GUNLE) were found with strong antifeedant action with preference index between (0.46-0.48) against 4th instar larvae of *S. litura* whereas Goat urine, DCUNLE, GUNLE, Ox urine neem leaf extract (OUNLE), Buffalo urine neem leaf extract (BUNLE) and Horse urine neem leaf extract (HUNLE) showed strong antifeedant activity with preference indices (0.26 to 0.49) against 4th instar larvae of *S. obliqua*. Thus, these preliminary studies clearly demonstrated the potential of animal urine based formulations for eco-friendly management of noxious pests, *S. litura* and *S. obliqua*.

Keywords

Animal urine, Antifeedant, Cow urine, Growth parameters, *Spodoptera*, *Spilarctia*

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Introduction

The Tobacco caterpillar, *Spodoptera litura* (Fabricius) (Noctuidae: Lepidoptera) a key pest amongst the polyphagous pests distributed throughout tropical and subtropical parts of the world and causing damage to more than 150 species of host plants (Rao *et al.*,

1993 and Murugesan and Dhingra, 1995). It has been reported to feed on 112 cultivated crop plants belonging to 44 families out of which about 40 species are known from India (Mallikarjun *et al.*, 2004). It has been reported to cause heavy yield loss throughout India (Ferry *et al.*, 2004). The Bihar hairy caterpillar, *Spilarctia obliqua* (Walker)

(Lepidoptera: Arctiidae), is a sporadic pest, widely distributed in India and can attack 126 plant species belonging to 25 families and thrives on about 25 weed species (Mandal and Bhattacharya, 2003).

The chemical pesticides have been used for several decades in controlling these noxious pests as they have a quick knock down effect. However, their indiscriminate use resulted in development of resistance in *S. litura* to insecticides, particularly pyrethroids and carbamates (Kranthi *et al.*, 2002, Huang and Han, 2007 and Whalon *et al.*, 2008). *S. obliqua* also showed certain levels of behavioral resistance to different class of insecticides (Mandal *et al.*, 2013) hence successful control of these pests is to some extent is difficult, but traditional synthetic pesticides have been the most effective means of pest control (Pavela, 2012).

Several work has been conducted on different types of plant materials such as neem, jatrophia, onion, ginger, turmeric, lantana etc. against *S. litura* and *S. obliqua* under field and laboratory conditions (Patel and Gajjer, 2001, Purwar and Yadav, 2003, Getanjaly and Tiwari, 2014a,b). Indigenous products such as plant extracts and animal excreta such as cow urine, cow dung, bio gas etc. have emerged as an alternative to the chemicals pesticides in recent times. Several workers reported the use of cow urine and plant extracts for the control of insect pests of field crops (Purwar and Yadav, 2004, Gupta, 2005; Akash and Tiwari, 2010a,b; Tiwari *et al.*, 2016). Therefore, considering the economic importance of these pests and to reduce the poisonous effects of pesticides in an agro-ecosystem, a novel study was carried out to evaluate the growth inhibitory and antifeedant effects of some animal urine based eco-friendly formulations against *S. litura* and *S. obliqua* under laboratory conditions.

Materials and Methods

The bioassay technique of leaf dip method was followed to find out the growth regulatory effects and antifeedant activity of different animal urine based formulations against *S. litura* and *S. obliqua* based on various growth parameters on treated castor leaves, The studies were conducted under laboratory conditions during 2017 in the Department of Entomology, GB Pant University of Agriculture and Technology, Pant Nagar, Uttarankhand.

Culture of the test insects

The cultures of *S. litura* and *S. obliqua* were raised in glass jars on leaves of castor, *Ricinus communis* Linn. The nucleus culture of the test insect larvae were collected from university premises and brought to the laboratory, were reared on fresh castor leaves till pupation and healthy pupae were procured for the next generation. The cultures were maintained at 27C and 70 ± 5 % RH. The adults obtained from above culture were released in separate glass jars (21x15 cm) the walls of which were lined with white paper for egg laying. White paper strips were also kept in the jars for egg laying and muslin cloth and strips were checked daily for egg laying and eggs were removed and placed in a separate jar on fresh succulent castor leaves for hatching. The neonate larvae were reared on fresh castor leaves to maintain the test culture of both the tested insects.

Collection of animal urine and preparation of plant extracts

Different animal urine based formulations were prepared using Cow urine collected from Desi, Holstein and Jersey breeds, Buffalo urine, Ox urine, Goat urine and Horse urine @ 20% and animal urine based neem leaf extracts @5% and evaluated against larval

stages of *S. litura* and *S. obliqua*. The animal urine was collected from the dairy farm of the University while the cow urine based plant extracts were prepared by collecting the neem leaves from the University campus of Pant nagar. To prepare 5% concentrations of animal urine based neem extracts, 50 grams of neem leaves were weighed by using a top balance, washed, dried under shade and macerated in the electrical grinder and dipped in 1000ml of water separately in the containers and were kept for fermentation for 24 hrs and then filter by using muslin cloth.

Bioassay to study growth and development of tested larvae

The growth and development of 7days old larvae of *S. litura* and *S. oblique* on different treated leaf discs was evaluated by using no-choice test method as suggested by (Singh *et al*, 1995). Fresh castor leaf discs (4x4 cm²) were treated with cow urine and plant extracts, separately, with the help of atomizer and placed in tilted orientation under ceiling fan air dried at room temperature in the laboratory to evaporate water from the leaf and kept in a petridishes lined with moist filter paper to maintain the humidity treatment wise separately. Thereafter, 10 larvae / replication of both insects were released separately, into each petri dish (90mm dia.) containing treated leaf disc and allowed to feed till pupation. Treated leaves were provided *ad libitum* till pupation. Each treatment was replicated thrice. In control, the leaf discs were dipped in distilled water and drip dried before being given to larvae. Observations were recorded on the different growth parameters such as larval weight, larval survival, larval and pupal period, pupal survival, per cent adult emergence and growth index using following formula:

$$\text{Growth index} = \frac{\text{Per cent adult emergence}}{\text{Total developmental period}}$$

Bioassay for Feeding Inhibition (Antifeedant activity)

The feeding inhibition and antifeedant activity of animal urine based formulations on freshly molted 4th instar larvae was investigated through no-choice bioassay method using 4.0cm² leaf discs. The 4th instar larvae were subjected to bioassay using leaf disc residue technique. All experiments were conducted at 29 ± 0.5°C and 75 ± 5% RH. The areas of leaf consumed by the larvae in 24 hours were recorded with the help of graph paper. The preference for the treated leaves by the tested larvae was examined by the leaf disc choice test (Jermy, 1961). For this purpose the leaf disc (4 cm²) of standard plant (castor) untreated or treated with different animal urine based formulations were used. The 6 h starved 4th instar larvae were released in triplicated per treatment. The per cent feeding in each treatment over control was worked out using the following formula:

$$\text{Per cent feeding} = \frac{\text{Leaf area fed by the larvae}}{\text{Initial leaf area given for feeding}}$$

% Feeding inhibition (FI) (Antifeedant activity) was calculated following Pande and Shrivastav (2003)

$$\% \text{ Feeding inhibition (FI)} = \frac{C-T}{C+T} \times 100$$

Where C = Consumption of control leaves
T = Consumption of treated leaves

Preference index was calculated according to Kogan and Geoden, (1970)

$$\text{Preference index (C)} = \frac{2A}{M+A}$$

Where C = Preference index
A = area eaten on the treated leaf
M = area eaten on the untreated leaf

The antifeedant activity of each plant extracts will be worked out on the basis of preference indices (C-values) according to the following scale:

C –value	Class
0.1-0.25	Extremely antifeedant
0.26-0.50	Strong antifeedant
0.51-0.75	Moderately antifeedant
0.76-0.99	Slightly antifeedant
>1	Preferred plant extract

Statistical analysis

Data will be subjected to Complete Randomized Design (CRD) after suitable transformations. Mean data is analyzed by using square root transformation with adding factor 0.5 and per cent values were analyzed by using angular transformed values.

Results and Discussion

Effect of animal urine based formulations on growth and development of 7 days old larvae of *S. litura*

The data in Table 1 represented the efficacy of various animal urine formulations against 7 days old larvae of *S. litura* with the lowest larval survival (10.00%) was observed in Goat urine followed by Horse urine (13.33%) and Desi cow urine (16.67%) with 90.00% larval survival in untreated control. A significant increase in larval period was observed in all treatments which were ranged from 9.54days in Jersey Cow urine to 11.73days in Ox urine in comparison to significantly less mean larval period (7.33 days) in untreated control. The mean larval weight was recorded the least in Desi cow urine (148.40mg) which was at par with Goat urine (149.67mg) followed by Horse urine (154.75mg with the maximum larval weight (600.88mg) recorded in untreated control. No pupation was observed in Goat urine whereas very less pupation was

observed in Horse urine (10.00%) and Desi cow urine (13.33%) with significantly maximum pupation in untreated control (90.00%). The extended pupal period was observed among the treatments ranged from (9.50 days) in Jersey cow urine to 11.00 days in Desi cow urine with 8.29days recorded in control. No adult emergence was observed in Desi cow urine, Goat urine and Horse urine leaf discs. The mean pupal weight was ranged from 124.00mg in Horse urine to 170.38mg in Holstein cow urine with 301.37mg in untreated control. Only (6.67%) adult emergence was observed in Ox urine followed by Holstein cow urine and Buffalo urine (20.00%, each) and Jersey cow urine (26.67%) in comparison to 90.00% adult emergence recorded in untreated. The mean adult weight was recorded the least in Ox urine (144.00mg) followed by Buffalo urine (153.50mg), Holstein cow urine (196.00mg) and Jersey cow urine (200.12mg) with the maximum adult weight (218.70mg) observed in untreated control. The growth index values couldn't be recorded for Desi cow urine, Goat urine and Horse urine treatments due to total larval mortality whereas very less growth index values were calculated for other animal urine treatments *i.e.* Ox urine (0.30), Buffalo urine (0.95), Holstein cow urine (1.00) and Jersey cow urine (1.40) with highest growth index value was calculated (5.76) in untreated control.

Effect of animal urine based neem leaf extractson growth and development of 7days old larvae of *S. litura*

The data based on the effect of neem leaf extracts prepared in various animal urine against 7 days old larva of *S. litura* is given in Table 2 which clearly represented the high efficacy of GUNLE with only(3.33%) larval survival followed by HUNLE (6.67%), DCULE and OUNLE (10.00% each),with significantly highest larval survival (90.00%)

recorded in untreated control. A significant increase in larval period was observed in all animal based neem leaf extracts treatments i.e. ranged from 10.00days in DCUNLE to 11.00 days in GUNLE with the least larval period of 7.33 days were observed in untreated control. The larval weight was found significantly very less, ranged from (118.00mg) in GUNLE to (174.67mg) in HCUNLE with maximum larval weight (600.88mg) in untreated control. The all released 7 days old larvae were died so no pupation was observed in GUNLE followed by only 3.33% pupation occurred in DCUNLE and HUNLE with significantly highest pupation observed in untreated control (90.00%). The pupal duration was found more in animal urine based neem leaf extracts treatments i.e., 10.00days in OUNLE to 11.50 days in DCUNLE with 8.29 days in control. The mean pupal weight was again observed significantly very less ranged from 120.00mg in DCUNLE (120.00mg) to 182.67mg in JCUNLE with maximum pupal weight (301.37 mg) recorded in untreated control. All the pupae reared in castor leaves treated with DCUNLE, GUNLE, OUNLE and HUNLE were deformed and dead so no adult emergence was observed, The mean adult weight was calculated very less ranged from BUNLE(152.00mg) to JCUNLE (193.50mg) with maximum adult weight (218.70mg) in untreated control. The growth index values were found very less in BUNLE (0.15) followed by JCUNLE (0.48) and HCUNLE (0.62) with highest growth index value (5.76) in untreated control.

Effect of animal urine on growth and development of 7 days old larvae of *S. obliqua*

The data shown in Table 3 demonstrated that Ox urine gave the lowest larval survivability (16.67%) followed by Goat urine (20.00%), Desi cow urine (23.33%), Horse urine (26.67%) and Buffalo urine (30.00%) with

93.33% larval survival in untreated control. A significant increase in larval period was observed in all treatments ranged from 16.66days in Holstein Cow urine to 19.17.days in Goat urine with significantly less larval period (13.00 days) recorded in untreated control. In animal urine treated castor leaves, larval weight was observed very less which was ranged from 151.71mg in Desi cow urine to 209.81mg in Jersey cow urine with the maximum larval weight (572.25 mg) recorded in untreated control. Similarly, a least pupation was recorded in Goat urine treated leaves (3.33%) followed by Desi cow urine and Ox urine (13.33% each), Buffalo urine (16.67%). with maximum pupation (93.33%) occurred in untreated control. The mean pupal period was found extended in all animal urine formulations which was ranged from 10.00days in Jersey cow urine to 11.38days in Goat urine treatments with the least pupal period (9.36days) recorded in untreated control. The mean pupal weight was also very less ranged from 101.00mg in Goat urine to 205.54mg in Jersey cow urine with maximum pupal weight (299.85mg) obtained in untreated control. No adult emergence was recorded in Goat urine treated leaves with the least adult emergence observed in Ox urine (3.33%), Desi cow urine (6.67%), Buffalo urine (10.00%) and Horse urine (16.67%) with comparatively higher adult emergence was recorded in untreated control (90.00%). The adult weight was calculated very less in Desi cow urine (155.00mg) followed by Horse urine (163.00mg), Ox urine (172.00mg) and Buffalo urine (183.00mg) with whereas comparatively more mean adult weight (223.50mg) was recorded in untreated control. The growth index values were calculated very less for animal urine treatments which was ranged from Ox urine (0.12) to Jersey cow urine (1.38) in comparison to highest growth index value was calculated (4.03) in untreated control.

Effect of animal urine based neem leaf extracts on growth and development of 7 days old larvae of *S. obliqua*

The data given in Table 4 clearly represented the high efficacy of GUNLE with only (6.67%) larval survival followed by OUNLE (10.00%) with significantly highest larval survival (93.33%) recorded in untreated control. An extended larval period was again recorded from 17.25days in HCUNLE to 19.73days in GUNLE with minimum of 13.00days in untreated control. The significantly less mean larval weight was calculated in GUNLE (139.00mg) followed by OUNLE (150.00mg), DCUNLE (158.50mg), HUNLE (164.00) with maximum mean larval weight (572.25mg) in untreated control. A very less pupation ranged from (3.33%) in GUNLE to (23.33%) in HCUNLE was recorded with significantly highest pupation observed in untreated control (93.33%). The mean pupal weight was recorded very less in GUNLE (73.00mg) followed by OUNLE (118.33mg), DCUNLE (151.50mg), BUNLE (177.00mg) with maximum pupal weight (299.85mg) observed in untreated control. A significantly very less adult weight ranged from (149.00mg) in DCUNLE to (194.20mg) in HCUNLE with maximum weight (223.50mg) of adult moths was calculated in untreated control. Similarly, the mean pupal weight was recorded the least in Goat urine (73.00mg) followed by Ox urine (118.33mg) and Desi cow urine (151.50mg) with maximum pupal weight (299.85mg) observed in untreated control. It has been clearly observed that the all pupae in DCUNLE, OUNLE and GUNLE were deformed and dead so no adult emergence was observed, while the adult emergence in other treatments was again significantly less which was ranged from (6.67%) in HUNLE to (20.00%) in JCUNLE with 90.00% in untreated control. A significantly very less adult weight ranged from 149.00mg in DCUNLE to 194.20mg

HCUNLE was recorded with maximum weight adult (223.50mg) in untreated control. The growth index values were calculated again very less in HUNLE (0.22) followed by BUNLE (0.36), HCUNLE (0.61) and JCUNLE (0.68) with highest growth index value (4.03) in untreated control.

Antifeedant activity of animal urine against 4th instar larvae of *S. litura* and *S. obliqua*

A glance at Table 5 showed that *S. litura* larvae fed very less (4.77 cm²) in Goat urine to 12.66 cm² in Holstein cow urine treated castor leaf discs in comparison to untreated control where leaf discs were almost completely eaten (15.26 cm²). The significantly lowest mean per cent feeding was recorded in castor leaf discs treated with Goat urine (29.81%) followed by Desi cow urine (56.50%). The highest antifeedant activity 68.74% was recorded for Goat urine and 40.76% in Desi cow urine treated castor leaves. Similar trend was observed for feeding inhibition of tested larvae which was calculated the maximum (54.87%) in Goat urine to 19.59% in Ox urine treated castor leaves. Overall mean preference index indicated that none of the animal urine were found to belong extremely strong antifeedant category but the preference indices calculated for Goat urine treated castor leaf discs were significantly less (0.48) which showed its strong antifeedant activity followed by Desi cow urine (0.74) and Buffalo urine (0.77) showed moderately antifeedant activity as compared to slightly antifeedant activity observed on other treated castor leaf discs treated with Ox urine, Horse urine, Jersey cow urine and Holstein cow urine (0.80, 0.83, 0.85 and 0.90 respectively).

The data depicted in Table 5 clearly showed that larvae of *S. obliqua* also fed very less in Goat urine (5.18 cm²) followed by Ox urine (7.03 cm²) and Desi cow urine (8.36 cm²) in comparison to control (16.00 cm²). The

significantly lowest larval feeding on treated castor leaves was recorded in Goat urine (32.38%) followed by Ox urine (43.93%) and Desi cow urine (52.25%) with (100.00%) larval feeding was obtained in untreated control. Goat urine showed the highest antifeedant activity (67.63%) followed by Ox urine (56.06%), Desi cow urine (47.77%) and Buffalo urine (39.06%) whereas larval feeding inhibition was more in castor leaves treated with Goat urine (51.09%) and Ox urine (38.94%) followed by Desi cow urine (31.36%) whereas In untreated control, leaf discs were almost completely eaten by the larvae. Overall mean preference index values clearly indicated that none of the animal urine formulations were found to belong extremely strong antifeedant category but the preference indices was significantly less in Goat urine (0.49) which showed its strong antifeedant activity whereas Ox urine (0.61) and Desi cow urine (0.68) showed moderately antifeedant activity as compared to slightly antifeedant activity observed on other treated castor leaf discs treated with Buffalo urine, Jersey cow urine, Horse urine and Holstein cow urine are 0.75, 0.85, 0.86 and 0.96 respectively.

Antifeedant activity of animal urine based neem leaf extracts against 4th instar larvae of *S. litura*

The data presented in Table 6 showed that the consumption of leaf discs by *S. litura* larvae was very less in DCUNLE (4.63cm²) followed by GUNLE (4.93 cm²) and HUNLE (6.83cm²) with maximum consumption in untreated control (15.26 cm²).

The significantly lowest mean larval feeding was recorded in DCUNLE (28.93%) followed by GUNLE (30.81%), HUNLE (42.68%) with highest larval feeding in untreated control (95.37 %). The highest antifeedant activity 65.69% and 66.44% was recorded DCUNLE and GUNLE The maximum larval feeding

inhibition (53.44%) was calculated in DCUNLE followed by GUNLE (51.16%), Overall mean preference index values indicated that none of the animal urine formulations were found to belong extremely antifeedant category but a strong antifeedant activity was noticed in DCUNLE (0.46) and GUNLE (0.48) whereas HUNLE (0.61) and BUNLE (0.63) and OUNLE (0.69) showed moderately antifeedant activity as compared to slightly antifeedant activity observed in JCUNLE (0.78) and HCUNLE (0.79) against 4th instar larvae of *S. litura*.

The leaf areas consumed by the larvae of *S. obliqua* is given in Table 6 which showed that larvae fed very less leaf areas in Desi cow urine (2.40cm²) followed by Horse urine (2.83cm²) and Goat urine (3.47cm²) treated castor leaves in comparison to control (16.00 cm²).

The significantly less mean larval feeding was observed in DCUNLE (15.00%) and HUNLE (17.68%) followed by GUNLE (21.68%) with 100.00% larval feeding recorded in untreated control.

The highest antifeedant activity was calculated in DCUNLE (85.00%) and HUNLE 82.31%) followed by GUNLE (78.31%).

The maximum per cent feeding inhibition of larvae was recorded in DCUNLE (73.91%) followed by HUNLE (69.94%), GUNLE (64.36%). The overall mean preference index values indicated that none of the animal urine was belong to extremely antifeedant category but the strong antifeedant activity was noticed in DCUNLE (0.26), HUNLE (0.30), GUNLE (0.35), BUNLE (0.48), OUNLE (0.49), moderately antifeedant activity was shown by HCUNLE (0.72) whereas JCUNLE with preference index value (0.81) showed the slight antifeedant activity against 4th instar larvae of *S. obliqua*.

Table.1 Effect of animal urine on growth and development of 7 days old larvae of *Spodoptera litura*

Treatments	Conc. (%)	Larval survival %	Mean larval period (days)	Mean larval weight (mg)	Pupation (%)	Mean pupal period (days)	Mean pupal weight (mg)	Adult emergence (%)	Mean adult weight (mg)	Growth index (G.I.)
Desi cow urine	20	16.67 (24.09)	10.80 (3.36)	148.40 (12.20)	13.33 (21.41)	11.00 (3.39)	129.25 (11.39)	0.00 (0.79)	0.00 (0.72)	0.00
Holstein cow urine	20	36.67 (28.49)	10.27 (3.28)	268.45 (16.40)	26.67 (31.09)	9.67 (3.19)	170.38 (13.07)	20.00 (26.57)	196.00 (14.02)	1.00
Jersey cow urine	20	43.33 (41.17)	9.54 (3.17)	237.38 (15.43)	30.00 (33.21)	9.50 (3.16)	165.00 (12.86)	26.67 (31.09)	200.12 (14.16)	1.40
Buffalo urine	20	30.00 (33.21)	10.80 (3.36)	179.00 (13.40)	23.33 (28.88)	10.20 (3.28)	0.00 (0.73)	20.00 (26.57)	153.50 (12.40)	0.95
Ox urine	20	50.00 (45.00)	11.73 (3.50)	173.47 (13.19)	20.00 (26.57)	10.43 (3.31)	0.00 (0.72)	6.67 (14.96)	144.00 (12.02)	0.30
Goat urine	20	10.00 (18.43)	11.00 (3.39)	149.67 (12.25)	0.00 (0.60)	0.00 (0.72)	0.00 (0.73)	0.00 (0.65)	0.00 (0.74)	0.00
Horse urine	20	13.33 (21.41)	10.75 (3.54)	154.75 (12.46)	10.00 (18.44)	10.00 (3.24)	124.00 (11.16)	0.00 (0.46)	0.00 (0.74)	0.00
Untreated Control	-	90.00 (71.56)	7.33 (2.80)	600.88 (24.52)	90.00 (71.57)	8.29 (2.97)	301.37 (17.37)	90.00 (71.56)	218.70 (14.81)	5.76
SEM+		1.550	0.002	0.004	0.107	0.002	0.005	0.154	0.003	
CD at 5%		4.648	0.005	0.013	0.324	0.006	0.014	0.460	0.010	
CV		7.581	0.081	0.128	0.645	0.123	0.092	1.232	0.067	

Mean values are analyzed by square root transformation with 0.5 adding factor
 Per cent values are angular transformed

Table.2 Effect of animal urine based neem leaf extracts on growth and development of 7 days old larvae of *Spodoptera litura*

Treatments	Conc. (%)	Larval survival %	Mean larval period (days)	Mean larval weight (mg)	Pupation (%)	Mean pupal period (days)	Mean pupal weight (mg)	Adult emergence (%)	Mean adult weight (mg)	Growth index (G.I.)
Desi Cow Urine Neem Leaf Extract (DCUNLE)	5	10.00 (18.44)	10.00 (3.24)	150.67 (12.29)	3.33 (10.52)	11.50 (3.46)	120.00 (10.99)	0.00 (0.60)	0.00 (0.72)	0.00
Holstein Cow Urine Neem Leaf Extract (HCUNLE)	5	20.00 (26.57)	10.83 (3.37)	174.67 (13.24)	20.00 (26.57)	10.75 (3.35)	167.17 (12.95)	13.33 (21.41)	192.75 (13.90)	0.62
Jersey Cow Urine Neem Leaf Extract (JCUNLE)	5	13.33 (21.41)	10.50 (3.32)	165.00 (12.86)	10.00 (18.43)	10.50 (3.32)	182.67 (13.53)	10.00 (18.44)	193.50 (13.93)	0.48
Buffalo Urine Neem Leaf extract (BUNLE)	5	16.67 (24.10)	10.60 (3.33)	150.00 (12.27)	16.67 (24.10)	11.00 (3.39)	134.40 (11.62)	3.33 (10.50)	152.00 (12.35)	0.15
Ox Urine Neem Leaf Extracts (OUNLE)	5	10.00 (18.44)	10.67 (3.34)	138.00 (11.77)	6.67 (14.96)	10.00 (3.24)	125.00 (11.20)	0.00 (0.60)	0.00 (0.73)	0.00
Goat Urine Neem Leaf Extract (GUNLE)	5	3.33 (10.51)	11.00 (3.39)	118.00 (10.90)	0.00 (0.52)	0.00 (0.72)	0.00 (0.72)	0.00 (0.79)	0.00 (0.73)	0.00
Horse Urine Neem Leaf extract (HUNLE)	5	6.67 (14.97)	10.75 (3.35)	140.00 (11.85)	3.33 (10.51)	11.00 (3.39)	122.00 (11.07)	0.00 (0.52)	0.00 (0.73)	0.00
Untreated Control	-	90.00 (71.56)	7.33 (2.80)	600.88 (24.52)	90.00 (71.53)	8.29 (2.96)	301.37 (17.37)	90.00 (71.57)	218.70 (14.80)	5.76
SEM+		0.006	0.001	0.005	0.103	0.003	0.006	0.188	0.006	
CD at 5%		0.019	0.004	0.014	0.308	0.008	0.019	0.566	0.017	
CV		0.042	0.069	0.061	0.806	0.156	0.096	2.097	0.132	

Mean values are analyzed by square root transformation with 0.5 adding factor
 Per cent values are angular transformed

Table.3 Effect of animal urine on growth and development of 7 days old larvae of *Spilarctia oblique*

Treatments	Conc. (%)	Larval survival (%)	Larval period (days)	Final mean larval weight (mg)	Pupation (%)	Pupal period (days)	Mean pupal weight (mg)	Adult emergence (%)	Mean adult weight (mg)	Growth index (G.I.)
Desi cow urine	20	23.33 (28.88)	18.57 (4.37)	151.71 (12.34)	13.33 (21.41)	11.00 (3.39)	158.00 (12.59)	6.67 (14.98)	155.00 (12.48)	0.23
Holstein cow urine	20	40.00 (39.24)	16.66 (4.14)	198.67 (14.11)	30.00 (33.21)	10.25 (3.42)	194.33 (14.46)	30.00 (33.20)	208.78 (14.47)	1.08
Jersey cow urine	20	53.33 (46.91)	17.68 (4.26)	209.81 (14.50)	43.33 (41.16)	10.00 (3.45)	205.54 (14.40)	40.00 (39.24)	206.83 (14.40)	1.38
Buffalo urine	20	30.00 (33.22)	17.80 (4.28)	188.44 (13.75)	16.67 (24.10)	10.40 (3.30)	186.40 (13.55)	10.00 (18.44)	183.00 (13.55)	0.35
Ox urine	20	16.67 (24.09)	18.11 (4.31)	174.20 (13.22)	13.33 (21.42)	11.22 (3.28)	179.25 (13.13)	3.33 (10.51)	172.00 (13.15)	0.12
Goat urine	20	20.00 (26.57)	19.17 (4.44)	154.50 (12.45)	3.33 (10.52)	11.38 (3.24)	101.00 (0.73)	0.00 (0.90)	0.00 (0.73)	0.00
Horse urine	20	26.67 (31.09)	18.37 (4.34)	166.88 (12.94)	20.00 (26.57)	10.67 (3.34)	160.33 (4.75)	16.67 (24.10)	163.00 (12.79)	0.57
Untreated Control	-	93.33 (7506)	13.00 (3.67)	572.25 (23.93)	93.33 (75.06)	9.36 (3.14)	299.85 (14.97)	90.00 (71.59)	223.50 (14.97)	4.03
SEM_±		0.007	0.001	0.006	0.008	0.001	1.42	0.062	0.007	
CD at 5%		0.021	0.004	0.019	0.025	0.004	4.26	0.185	0.021	
CV		0.032	0.060	0.035	0.046	0.068	22.23	0.401	0.099	

DCUNLE-Desi Cow Urine Neem Leaf Extract JCUNLE- Jersey Cow Urine Neem Leaf Extract HCUNLEE-Holstein Cow Urine Neem Leaf Extract
 BUNLE-Buffalo Urine Neem Leaf extract OUNLE-Ox Urine Neem Leaf Extracts GUNLE-Goat Urine Neem Leaf Extract HUNLE- Horse Urine
 Neem Leaf extract; Mean values are analyzed by square root transformation with 0.5 adding factor
 Per cent values are angular transformed

Table.4 Effect of animal urine based neem leaf extracts on growth and development of 7 days old larvae of *Spilarctia oblique*

Treatments	Conc. (%)	Larval survival (%)	Mean larval period (days)	Mean larval weight (mg)	Pupation (%)	Pupal period (days)	Mean pupal weight (mg)	Adult emergence (%)	Mean adult weight (mg)	Growth index (G.I.)
Desi Cow Urine Neem Leaf Extract (DCUNLE)	5	13.33 (21.41)	19.22 (4.44)	158.50 (12.60)	6.67 (14.97)	12.00 (3.54)	151.50 (12.33)	0.00 (0.84)	149.00 (12.24)	0.00
Holstein Cow Urine Neem Leaf Extract (HCUNLE)	5	30.00 (33.21)	17.25 (4.21)	202.67 (14.25)	23.33 (28.88)	10.22 (3.27)	197.86 (14.07)	16.67 (24.10)	194.20 (13.95)	0.61
Jersey Cow Urine Neem Leaf Extract (JCUNLE)	5	23.33 (28.87)	18.60 (4.37)	205.57 (14.35)	20.00 (26.57)	11.38 (3.45)	200.50 (14.18)	20.00 (26.56)	184.00 (13.58)	0.68
Buffalo Urine Neem Leaf extract (BUNLE)	5	20.00 (26.57)	17.33 (4.22)	176.17 (13.29)	16.67 (24.09)	10.75 (3.35)	177.00 (13.32)	10.00 (18.44)	175.67 (13.27)	0.36
Ox Urine Neem Leaf Extracts (OUNLE)	5	10.00 (18.44)	18.90 (4.41)	150.00 (12.27)	10.00 (18.44)	11.25 (3.42)	118.33 (10.90)	0.00 (0.79)	164.50 (12.84)	0.00
Goat Urine Neem Leaf Extract (GUNLE)	5	6.67 (14.97)	19.73 (4.50)	139.00 (11.81)	3.33 (10.51)	12.70 (3.64)	73.00 (8.57)	0.00 (0.98)	0.00 (0.72)	0.00
Horse Urine Neem Leaf extract (HUNLE)	5	16.67 (24.09)	18.50 (4.36)	164.00 (12.86)	13.33 (21.41)	11.67 (3.49)	155.75 (12.50)	6.67 (14.96)	172.25 (13.14)	0.22
Untreated Control	-	93.33 (75.03)	13.00 (3.68)	572.25 (23.93)	93.33 (75.03)	9.36 (3.14)	299.85 (17.33)	90.00 (71.57)	223.50 (14.97)	4.03
SEM+		0.003	0.003	0.009	0.010	0.002	0.003	0.081	0.006	
CD at 5%		0.010	0.008	0.026	0.030	0.005	0.008	0.243	0.019	
CV		0.018	0.104	0.104	0.063	0.078	0.035	0.709	0.093	

Mean values are analyzed by square root transformation with 0.5 adding factor
Per cent values are angular transformed

Table.5 Comparative antifeedant activity of animal urine against 4th instar larvae of *Spodoptera litura* and *Spilartia obliqua* (leaf area provided= 16 cm²)

Treatments	Co nc. (%)	<i>Spodoptera litura</i>						<i>Spilartia obliqua</i>					
		Mean leaf area consumed (cm ²)	Feeding (%)	Antifeedant activity (%)	Feeding Inhibition (%)	Preference index*	Antifeedant Class	MLA C (cm ²)	Feeding (%)	Antifeedant activity (%)	Feeding Inhibition (%)	Preference index	Antifeedant Class
Desi cow urine	20	9.04 (3.09)	56.50	40.76	25.59	0.74	Moderately antifeedant	8.36 (2.98)	52.25	47.77	31.36	0.68	Moderately antifeedant
Holstein cow urine	20	12.66 (3.63)	79.12	17.03	9.31	0.90	Slightly antifeedant	14.90 (3.93)	93.12	6.97	3.55	0.96	Slightly antifeedant
Jersey cow urine	20	11.30 (3.44)	70.62	25.95	14.90	0.85	Slightly antifeedant	11.94 (3.53)	74.62	25.37	14.53	0.85	Slightly antifeedant
Buffalo urine	20	9.75 (3.20)	60.93	36.10	22.03	0.77	Slightly antifeedant	9.75 (3.20)	60.93	39.06	24.27	0.75	Moderately antifeedant
Ox urine	20	10.26 (3.28)	64.12	32.76	19.59	0.80	Slightly antifeedant	7.03 (2.75)	43.93	56.06	38.94	0.61	Moderately antifeedant
Goat urine	20	4.77 (2.50)	29.81	68.74	54.87	0.48	Strong antifeedant	5.18 (2.60)	32.38	67.63	51.09	0.49	Strong antifeedant
Horse urine	20	10.88 (3.37)	68.0	28.70	16.75	0.83	Slightly antifeedant	12.08 (3.54)	75.50	24.50	13.96	0.86	Slightly antifeedant
Untreated Control		15.26 (3.97)	95.37	-	-	-		16.00 (4.06)	100	-	-	-	
SEM+		0.001						0.002					
CD at 5%		0.002						0.007					
CV		0.034						0.120					

Preference index values - 0.1-0.25 =Extremely antifeedant, 0.26-0.50 = Strong antifeedant 0.51-0.75= Moderately antifeedant 0.76-0.99 = Slightly antifeedant >1 = Preferred host

Table.6 Comparative antifeedant activity of animal urine neem leaf extracts against 4th instar larvae of *Spodoptera litura* and *Spilartia obliqua* (leaf area provided= 16 cm²)

Treatment s	Conc. (%)	Spodoptera litura						Spilartia obliqua					
		Mean leaf area consumed (cm ²)	Feeding (%)	Antifeedant activity (%)	Feeding Inhibition (%)	Preference index	Antifeedant Class	MLAC (cm ²)	Feeding (%)	Antifeedant activity (%)	Feeding Inhibition (%)	Preference index	Antifeedant Class
(DCUNLE)	5	4.63 (2.27)	28.93	69.65	53.44	0.46	Strong antifeedant	2.40 (1.71)	15.00	85.00	73.91	0.26	Strong antifeedant
(HCUNLE)	5	10.08 (3.25)	63.00	33.94	20.44	0.79	Slightly antifeedant	9.08 (3.10)	56.75	43.25	27.59	0.72	Moderately antifeedant
(JCUNLE)	5	9.94 (3.23)	62.12	34.86	21.11	0.78	Slightly antifeedant	10.94 (3.38)	68.37	31.62	18.78	0.81	Slightly antifeedant
(BUNLE)	5	7.11 (2.75)	44.43	53.40	36.43	0.63	Moderately antifeedant	5.11 (2.37)	31.93	68.06	51.59	0.48	Strong antifeedant
(OUNLE)	5	8.16 (2.94)	51.00	46.52	30.31	0.69	Moderately antifeedant	5.16 (2.38)	32.25	67.75	51.23	0.49	Strong antifeedant
(GUNLE)	5	4.93 (2.44)	30.81	66.44	51.16	0.48	Strong antifeedant	3.47 (1.99)	21.68	78.31	64.36	0.35	Strong antifeedant
(HUNLE)	5	6.83 (2.70)	42.68	55.24	38.16	0.61	Moderately antifeedant	2.83 (1.82)	17.68	82.31	69.94	0.30	Strong antifeedant
Untreated Control		15.26 (3.97)	95.37	-	-	-		16.00 (4.06)	100	-	-	-	
SEM+		0.001						0.003					
CD at 5%		0.003						0.010					
CV		0.061						0.227					

DCUNLE-Desi Cow Urine Neem Leaf Extract JCUNLE- Jersey Cow Urine Neem Leaf Extract HCUNLEE-Holstein Cow Urine Neem Leaf Extract
 BUNLE-Buffalo Urine Neem Leaf extract OUNLE-Ox Urine Neem Leaf Extracts GUNLE-Goat Urine Neem Leaf Extract HUNLE- Horse Urine Neem Leaf extract

*Preference index values - 0.1-0.25 =Extremely antifeedant, 0.26-0.50 = Strong antifeedant 0.51-0.75= Moderately antifeedant 0.76-0.99 = Slightly antifeedant >1 = Preferred host

Schoonhoven (1982), Sadek (2003) classified antifeedant substances into repellents (which repels an insect without making contact with the material), suppressants (which suppress biting activity after contact) or deterrents (which deter an insect from further feeding after ingestion of the plant material). Patel and Gajjar (2001) reported that neem leaves extract @5% suspension gave 76.77 and 71.70 per cent larval mortality of *S. litura*. Purwar and Yadav, (2003, 2004) reported that the cow urine and decoction of neem leaves in cow urine were found promising to control *S. litura* and *S. obliqua* under field conditions at Pant nagar, India (Gupta, 2005). Neem in combination with cow urine was found effective in reducing the mustard aphid population with no adverse effect on coccinellid predators (Boomathi *et al.*, (2006) conducted laboratory studies on the effect of neem seed kernel extract with cow urine and cow dung against Gram pod borer, *Helicoverpa armigera* and reported the toxicity of NSKE 5%+cow urine 5%+cow dung extract 5% against eggs and larvae of *H. armigera* with low growth index values and high antioviposition and antifeedant index under laboratory conditions. Panwar and Chibber (2006) studied the effect of plant extracts on feeding potential of *Spilarctia obliqua*. They found leaf extracts of *A. indica* and Eucalyptus globule and green foliage of *Lantana camara* have suppressants and deterrents properties. However, strong repellent activity was noticed in case of neem only. Mala and Muthalagi (2008) reported that the larval duration of Spodoptera was increased when the fifth instar larvae were fed with 0.8% neem oil extract treated leaves. Sharma *et al.*, (2009) reported that the aqueous extract of *A. indica* and *M. azedarach* resulted in statistically higher larvicidal (killing 20.9% and 19.2% larvae of *S. litura*). Pandey (2013) evaluated the antifeeding activity of leaf and seed extracts of the plant, *Azadirachta indica* against fourth instar larvae of *S. litura* and maximum antifeedant activity was observed in seed extract of *A. indica* at 5% extract with antifeedant index of 100 per cent. Indiati (2014) reported that the neem seed, sugar apple leaf, and sugar apple seeds extract

at a concentration of 50 g/l (w/v) were effective to suppress armyworm, *S. litura* populations (47-49%) higher than chemical insecticides 2 cc/l (43%). Akash Chand and Tiwari (2010) reported the effect of cow urine and different indigenous plant leaf extracts on food consumption and body weight of 10 days old *S. Litura* larvae and revealed that the larvae consumed significantly less leaf area when especially fed on neem extract treated leaves (8.20cm²). Alexander *et al.*, (2010) reported the efficacy of DDVP (.025%) + NSKE+cow urine 2% against *S. litura* with higher larval mortality after 72 h of exposure. Geetanjal and Tiwari (2014) conducted laboratory experiments to study the antifeedant and growth regulatory effects of cow urine, neem leaf extracts prepared in water and cow urine@ (5% and 10%) against different stages of *Spilarctia obliqua*. The cent per- cent mortality of neonate larvae of *S. obliqua* was observed with less mean leaf area consumed by 4th instar larvae at higher concentration @10%. Chaudhary *et al.*, (2016) reported the efficacy of methanolic extracts of *C. juncea*, *C. quinquefolia*, curry leaf and garlic recorded above 80.00% feeding inhibition of *S. obliqua* and larval mortality of 20.00%-44.44%.

In conclusion, the experimental results of the present novel study clearly showed the superiority of all treatments to untreated control. Desi cow urine, Goat urine, Horse urine and animal urine based neem leaf extracts were showed growth regulatory and strong antifeedant activity against 7 days old and 4th larvae of *S. litura* and *S. obliqua*. The present studies demonstrated that Desi cow urine, Ox urine, Goat urine, Buffalo urine and Horse urine alone and in combination with 5% neem leaf extracts can be used against insect pests as they are easily available, cost effective eco- friendly natural products. Therefore, it can be finally concluded that the animal urine based formulations has tremendous potential and possibilities to be included in overall pest management programme for the management of *S. obliqua* and *S. litura* but as being a preliminary and novel study made for the first

time at Pantnagar, Uttarakhand, further in depth studies are essentially required to reach to the final conclusions.

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