Research Article

Preliminary Bioassay Studies on the Efficacy of Animal Urine against *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae)

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Abstract

The laboratory studies on the effect of animal urine formulations on growth and development of *H. armigera* neonates and their antifeedant activity against 4th instar larvae were conducted at IPM laboratory, Department of Entomology, GBPUA&T Pantnagar, Uttarakhand during April-May 2018. This novel study was consists of seven different domestic animal's urine each used at 20% concentration. No choice bioassay was used with leaf dip method. Significantly lower growth indices of *H. armigera* were recorded in different animal urine formulations (0.00 to 0.447) than untreated control (2.631). Preference indices were significantly less in goat urine (0.35), Desi cow urine (0.45) and horse urine (0.50) which showed their strong antifeedant action. The treatments such as buffalo urine (0.62), ox urine (0.63) showed moderately antifeedant action. Therefore, animal urine formulations were found to have higher growth and development inhibition of test insect with relative antifeedant action and can be incorporated in integrated pest management of *H. armigera*.

Keywords: larval survival, *H. armigera*, growth indices, animal urine and antifeedant

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Introduction

The gram pod borer, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) is a polyphagous, prolific and wide spread pest known to feed on several economically important crops such as cotton, sorghum, groundnut, pigeon pea, chickpea, black gram, tomato and most of the vegetable [1]. The losses were reported up to Rs 10, 000 million solely due to this pest in these crops [2]. Globally, it attacks over 200 crop species belonging to 45 families, thus leading to yield loss tune to USD 2 billion annually. In India, the loss tune to 200 million USD on pigeon pea and chickpea [3]. It is distributed to geographical range from Europe, Africa, Asia and Australasia to the New World [4]. The biological attributes that makes this pest as noxious are high degree of polyphagy, high mobility, facultative diapause, high fecundity and multi-generations [5].

Pest management in the developing countries like India is mainly depends on the use of chemical pesticides as they are the most reliable and economical but indiscriminate use of them resulted in a series of problems in the agro-ecosystem, mainly the development of resistance in insects to the insecticides, resurgence of treated population, outbreak of secondary pests into primary nature, destruction of natural enemies, increase in input cost on chemicals, environmental pollution and pesticide residues *etc.*[6]. Insecticide application for pod borer is uneconomical under subsistence farming and is largely beyond the means of resource poor farmers. The *H. armigera* was reported to have developed resistance against organophosphates and carbamates in many countries of Asia [7] and Australia [8].

The failure of modern tactics has compelled the scientific community to go back to the traditional and indigenous products for tackling the pest problem [9]. There is a vast potential in the traditional methods practiced in rural India that can be included for combating the pest problems. The cow urine and cow dung were reported to be effective for insect control as reported by Peries [10] and Rankin [11], respectively. The plant extracts prepared in cow urine were found very effective against *H. armigera* [12]. According to the literature searched so far, there was no any research work conducted on the laboratory bio-efficacy of different types of animal urine against this obnoxious insect pest. Thus, the present field study is preliminary and novel in context to evaluate the efficacy of different animal's urine formulations against *H. armigera* with the following objectives. 1) Evaluating the growth inhibitory effects of animal urine against *H. armigera* neonates and 2) Determination of antifeedant activity of animal urine against test insect larvae.

Material and Methods

The details regarding the materials used and methods adopted under laboratory experiments for carrying out the

"Preliminary Bioassay Studies on the Efficacy of Animal Urine against *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae)" during the course of investigation are described below.

Rearing of test insect under laboratory conditions

Gram pod borer larvae were collected from chickpea fields, NEBCRC, GBPUA&T, Pantnagar. Further insect culture was maintained on artificial diet as per the standard procedure prescribed by Koul *et al.* [13]. The obtained larvae were used for laboratory studies on antifeedant and growth inhibitory effects of animal urine formulations.

Laboratory studies on the effect of biorationals on growth and development of H. armigera

The growth and development of neonate staged larvae were evaluated by using no-choice test method [14]. Fresh tomato leaf discs (4x4 cm) were treated with eco-friendly formulations as mentioned above in treatment details separately, with leaf dip method and placed in tilted orientation to evaporate water from the leaf at room temperature. Such air dried leaf discs were kept in a pertidishes lined with moist filter paper for maintaining the humidity. Thereafter, 30 larvae per replication released into each petridish separately (90 mm diameter) containing treated tomato leaf disc and allowed to feed till pupation. Treated leaves are provided *ad libitum* till pupation. Each treatment was replicated thrice. In control, the leaf discs were dipped in distilled water and dried before being given to larvae. Observations were recorded on the different growth parameters *viz.* Per cent survival of larvae, pupae and adults, Mean weight of larvae, pupae and adults, Per cent adult emergence, Mean adult weight, Larval, pupal and adult deformities, and Growth indices (per cent adult emergence/total development period).

Feeding Inhibition and Antifeedant activity of Biorationals on H. armigera

The feeding inhibition and antifeedant activity of different animal urine on freshly molted 4^{th} instar larvae were investigated through no choice bioassay method using 16 cm^2 tomato leaf discs. The 4^{th} instar larvae were subjected to bioassay using leaf disc residue technique. All experiments were conducted at 29 ± 0.5^0C and 75 ± 5% RH. Similar procedure as given above was followed with single fourth instar larvae starved for six hours with known weight were subjected to bioassay using treated leaf discs separately in triplicates. The leaf discs dipped in water was used as untreated control. The area of leaf consumed by the larvae on treated and untreated leaves was recorded till pupation at the interval of 24 h with the help of graph paper.

The per cent feeding in each treatment over control was worked out using the following formula:

$$\text{Per cent feeding} = \frac{\text{Initial area given for feeding} - \text{leaf area left after feeding}}{\text{Initial area given for feeding}} \times 100$$

Per cent feeding inhibition (FI) (Antifeedant activity) was calculated from the following formula [15].

$$\text{Per cent feeding inhibition (FI)} = \frac{C-T}{C+T} \times 100$$

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

Preference index was calculated according to the formula give by Kogan and Geoden, [16].

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

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

The antifeedant activity of animal urine formulation was 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 animal urine

Results and Discussion

Effect of animal urine formulations on growth and development of neonate larvae (0-24h) of H. armigera

The data regarding the effect of animal urine formulations @ 20 per cent on different parameters of growth and development of neonate larvae of *H. armigera* fed with treated tomato leaf discs is presented in **Table 1**. The least larval survival was recorded in Desi cow urine (3.33%) followed by horse urine (6.67%), goat urine (10.00%), buffalo urine (16.67%) and ox urine (20.00%). Whereas, Jersey cow urine (33.67%) and Holstein cow urine (26.67%) recorded with maximum and untreated control (96.67%) with the highest larval survival. The extended larval period observed in all animal urine treatments which were ranged from 23.87 days in HF cow urine to 25.20 days in buffalo urine treated tomato leaves fed to neonate larvae of *H. armigera* with the least larval period in untreated control (22.34 days). The mean larval weight of survived larvae was very less in Desi cow urine treated tomato leaves (132.25 mg) followed by horse urine (150.00 mg) and goat urine (169.00 mg). Among the treatments, Holstein cow urine and Jersey cow urine treated tomato leaves fed to larvae gave more larval weights of 209.75 mg and 207.64 mg, respectively with a maximum larval weight of 537.25 mg in untreated control.

Table1 Effect of animal urine on growth and development of neonate larvae (0-24h) of *H. armigera*

Treatments	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
Desi cow urine @ 20%	3.33 (10.49)*	24.34 (4.98)**	132.25 (11.52)**	0.00 (0.00)*	0.00 (0.05)**	0.00 (0.05)**	0.00 (0.00)*	0.00 (0.05)**	-
Holstein cow urine @ 20%	26.67 (31.08)	23.87 (4.94)	209.75 (14.50)	13.33 (21.40)	12.13 (3.55)	154.60 (12.45)	10.00 (18.44)	176.33 (13.29)	0.277
Jersey cow urine @ 20%	33.67 (35.46)	24.75 (5.02)	207.64 (14.43)	20.00 (26.57)	12.50 (3.61)	151.83 (12.34)	16.67 (24.09)	183.60 (13.57)	0.447
Ox urine @ 20%	20.00 (26.57)	24.35 (4.98)	191.00 (13.84)	16.67 (24.09)	12.33 (3.58)	139.33 (11.82)	13.33 (21.40)	150.50 (12.42)	0.363
Buffalo urine @ 20%	16.67 (24.09)	25.20 (5.07)	181.60 (13.49)	10.00 (18.44)	13.50 (3.74)	142.50 (11.96)	6.67 (14.95)	136.00 (11.68)	0.185
Goat urine @ 20%	10.00 (18.44)	25.00 (5.50)	169.00 (11.02)	0.00 (0.00)	0.00 (0.05)	0.00 (0.05)	0.00 (0.00)	0.00 (0.05)	0.00
Horse urine @ 20%	6.67 (14.94)	24.86 (5.04)	150.00 (12.27)	0.00 (0.00)	0.00 (0.05)	0.00 (0.05)	0.00 (0.00)	0.00 (0.05)	0.00
Untreated Control	96.67 (79.45)	22.34 (4.79)	537.25 (23.19)	90.00 (71.58)	11.86 (3.51)	224.00 (14.98)	90.00 (71.58)	206.07 (14.37)	2.631
SEM±	0.001	0.014	0.002	0.006	0.118	0.012	0.043	0.112	
CD @5%	0.002	0.041	0.005	0.017	0.354	0.008	0.072	1.098	
CV	0.052	0.098	0.075	0.059	2.609	0.115	0.008	1.095	

*****Figures in the parentheses are angular transformed values
**Figures in the parentheses are square root transformation values with adding factor 0.5

No pupation was observed in Desi cow urine, goat urine and horse urine treated tomato leaves. Whereas, on the other hand only 10.00 per cent larvae entered into pupation in buffalo urine treated tomato leaves followed by Holstein cow urine (13.33%), ox urine (16.67%) and Jersey cow urine (20.00%) with highest pupation of 90.00 per cent in untreated tomato leaves. The extended pupal period was observed in animal urine treated tomato leaves such as buffalo urine (13.50 days), Jersey cow urine (12.50days) followed by ox urine (12.33 days) and Holstein cow urine (12.13 days) which found significantly superior to untreated control (11.86 days). A significantly very less mean pupal weight (139.33 mg) was observed in ox urine followed by buffalo urine (142.50 mg), Jersey cow urine (151.83 mg) and Holstein cow urine (154.60 mg) whereas untreated control recorded with highest pupal weight of 224.00 mg.

A very less adult emergence was observed in buffalo urine (6.67%) followed by Holstein cow urine (10.00%), ox urine (13.33%) and Jersey cow urine (16.67%) treated tomato leaves with highest of 90.00 per cent in untreated control. The mean adult weight was recorded least in buffalo urine (136.00 mg) followed by ox urine (150.50 mg), Holstein cow urine (176.33 mg), Jersey cow urine (183.60 mg) with the maximum adult weight of 206.07 mg in untreated control. The larval, pupal and adult deformities were observed at non significant differences in larvae fed on tomato leaves treated with different animal urine especially Desi cow urine, goat urine, buffalo Urine and horse urine**.**

Similarly, significantly lower growth indices of *H. armigera* larvae were observed when they fed on tomato leaves treated with different animal urine formulations than untreated control (2.631) (Table 1). Among animal urine's, the treatments Desi cow urine, horse urine and goat urine were yielded zero growth index of test insect. Whereas, buffalo urine adversely affected the larval growth and gave the least growth index value (0.185) followed by Holstein cow urine (0.277), ox urine (0.363) and Jersey cow urine (0.447). The larval, pupal and adult deformities were observed at non significant differences in larvae fed on tomato leaves treated with different animal urine especially Desi cow urine, goat urine, buffalo urine and horse urine.

Bioassay studies on the antifeedant activity of animal urine formulations on H. armigera

The data presented in **Table 2** regarding the different parameters of antifeedant activity, feeding inhibition and preference index of 4th instar larvae of H. armigera fed on tomato leaf discs treated with animal urine formulations clearly showed that the mean leaf area consumption was very less in goat urine (3.20 cm2) followed by Desi cow urine (4.36 cm2), horse urine (5.01 cm2), ox urine (6.03 cm2) and buffalo urine (6.75 cm2). Whereas, high mean leaf areas were consumed by the larvae in Jersey cow urine (10.94 cm^2) and Holstein cow urine (9.90 cm^2) treated tomato leaves to mean leaf area was consumed by larvae in untreated control. The untreated control (15.00 cm^2) differed significantly from the treatments however; significant differences were also existed between the treatments (Table 2). No larval mortality was recorded within 24 hours of observed period.

The significantly lowest mean per cent feeding was observed in goat urine (20.00) followed by Desi cow urine (27.25), horse urine (31.31) and ox urine (37.39) whereas, higher per cent larval feeding (68.38) and (61.88) observed on tomato leaves treated with Jersey cow urine and Holstein cow urine, respectively with a highest per cent feeding of 93.75 in untreated control**.** The antifeedant activity was more in goat urine (78.67) and Desi cow urine (70.93) followed by horse urine (66.66) with a lower antifeedant activity recorded for Jersey cow urine -(27.07) and Holstein cow urine (34.00) treatments. The order of efficacy of antifeedants in a decreasing order was goat urine>Desi cow urine>horse urine>ox urine>buffalo urine>Holstein cow urine>Jersey cow urine.

Table 2 Comparative antifeedant activity of animal urine against 4th instar larvae of *H. armigera* (leaf area provided= 16 cm^2)

Treatments	MLAC (cm^2)	Feeding (%)	Antifeedant activity (%) (Protection over control)	Feeding Inhibition (%)	Preference index	Antifeedant Category
Desi cow urine @20%	4.36	27.25	70.93	54.96	0.45	Strong antifeedant
Holstein cow urine @20%	9.90	61.88	34.00	20.48	0.79	Slightly antifeedant
Jersey cow urine @20%	10.94	68.38	27.07	15.65	0.84	Slightly antifeedant
Ox urine @20%	6.03	37.39	59.80	42.65	0.63	Moderatly antifeedant
Buffalo urine @20%	6.75	42.19	55.00	37.93	0.62	Moderatly Antifeedant
Goat urine @20%	3.20	20.00	78.67	64.84	0.35	Strong antifeedant
Horse urine @20%	5.01	31.31	66.66	49.92	0.50	Strong antifeedant
Untreated control	15.00	93.75	-	-	-	

The maximum per cent feeding inhibition of larvae was recorded in goat urine (64.84) followed by Desi cow urine (54.96), horse urine (49.92), ox urine (42.65), buffalo urine (37.93), Holstein cow urine (20.48) and Jersey cow urine (15.65). Overall mean preference index values clearly indicated that none of the animal urine formulations were found to belong under extremely antifeedant category but the preference indices were significantly less in goat urine (0.35), Desi cow urine (0.45) and horse urine (0.50) which showed their strong antifeedant action. The treatments such as, buffalo urine (0.62), oxurine (0.63) showed moderately antifeedant action whereas, Holstein cow urine (0.79) and Jersey cow urine (0.84) recorded with slightly antifeedant action against 4th instar of test insect.

The laboratory studies on growth and development of neonates and antifeedant activity of animal urine against 4th instar larva proved their effectiveness by reducing growth index and antifeedant activities and is supported by the following findings. Barapatree and Lingappa [17] reported that cow urine + half dose of quinalphos and cow urine + half dose of endosulfan were proved superior by causing maximum larval mortality of *H. armigera* (88.89%). Boomathi *et al.* [18] found neem seed kernel extract @ 5% + cow urine + cow dung extract @5% treatment exhibiting more toxic effect on eggs and larvae of *H. armigera* with low larval growth index. Chandrashekharaiah *et al.* [19] reported that cow urine @25% had 39.07 per cent antifeedant effect and caused 10 per cent reduction in

weight gained over control in fourth instar larva of *P. xylostella*. Danish *et al.* [20] recorded the maximum mortality (26.32%) of third instar *H. armigera* treated with cow urine + cow dung @ 5%.

Conclusion

The laboratory bioassay experiments were proved the growth regulatory and antifeedant activity of different animal urine against test insect. By having strong growth inhibitory and antiffedant effects animal urine *viz.* Desi cow urine, Goat urine and Horse urine could be employed in integrated management of *H. armigera* and other caterpillar pests under field conditions. Further extensive field studies are required to determine the field efficacy of these animal urine formulations against insect pests and natural enemies.

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