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Research Article

Validation of the usefulness of bovine urine in the control of *Oryctes Rhinoceros* grub

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ABSTRACT: Experienced farmers traditionally applied bovine urine in compost pits and cow dung pits for preventing the larval development of *Oryctes rhinoceros*, (Linn.), a serious pest of coconut palms. This traditional knowledge was validated in the laboratory by mixing 100 ml of bovine urine with 500 gm cow dung and it was found that presence of urea at a concentration of 1.0 to1.5 % in the bovine urine was the reason behind the insecticidal activity. Aqueous solution of urea at a concentration of 0.3 to 0.4 % in cow dung has resulted 100% mortality of *Oryctes rhinoceros* larvae in 3-5 days. Toxicity has resulted in hyperproteinemia, elevated total free amino acid and uric acid of hemolymph. Activity of hemolymph aspartate amino transferase, alanine amino transferase and two proteolytic enzymes such as leucine amino peptidase, cathepsin D were decreased, indicating that urea had affected protein turn over and aminoacid metabolism. The carbohydrate metabolism was also affected through elevated activity of trehalase and subsequent decrease in trehalose content. The polysaccaride glycogen showed sharp decrease but the larvae were unable to utilize glucose which resulted hyperglycaemia. SDS-PAGE of hemolymph of urea intoxicated larvae showed sharp changes in the protein profile which was attested through GEL-DOC analysis. Intoxicated larvae showed decrease in total hemocyte count together with selective increase in the population of granulocyte. Urea was the major dissolved constituent of bovine urine and the presence of it was the reason behind its pest control value.

KEY WORDS: Bovine urine, GEL-DOC, hyperproteinemia, *Oryctes rhinoceros*, SDS-PAGE, urea

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INTRODUCTION

Oryctes rhinoceros Linn. (Order: Coleoptera, Family: Scarabidae) is a serious pest of coconut palm *Cocos nucifera* L. The adult beetle bores into the unopened fronds and inflorescence of the palm. Decaying organic matter and cattle dung are the breeding sites of the adult beetle. The emerging grub grows by feeding on the organic matter and takes about 100-250 days to become an adult (Nirula, 1955). Treatment of breeding sites with insecticides and application of pesticides on the infested region of coconut are the control measures at present but this method is problematic not only because of highly secluded nature of grubs which exists deep in the cow dung pits and the negative effect of pesticides on the environment. Bovine urine is basically an excellent germicide and a potent antibiotic. (Jerald *et al.,* 2008). Bovine urine has proved to be an effective pest controller and larvicide when used alone and also in combination with different plant preparations by enhancing the efficacy of different herbal preparations. Bovine urine is believed to have therapeutic value and used in many drug formulations (Chawla, 2010). Bovine urine is not a toxic effluent as 95 % of its content being water, 2.5% urea and the remaining 2.5% a mixture of minerals, salts, hormones and enzymes (Bhadauria, 2002). Presence of urea, creatinine, Swarn kshar (aurum hydroxide), carbolic acid, phenols, calcium and manganese have strongly explained for exhibition of antimicrobial and germicidal properties of cow urine (Jain *et al.,* 2010). Urea is the major dissolved constituent of bovine urine. It is generally assumed that urea is nontoxic and that even the high concentration present in uremia are in nocuous and do not contribute to the symptoms encountered in this condition to support this elasmo branch fishes contain 2% of urea. Urea does not appear to target any specific biochemical process, but instead have general cytotoxic effects. Urea is a protein denaturant (Somero and Yancey, 1997) and larvae reared on urea containing media have increased levels of proteins (David *et al.,* 1999). As bovine urine has been successfully used traditionally in compost pits to control the development of *O. rhinoceros* beetle by experienced farmers and urea is the major single dissolved material in bovine urine, it was proposed to undertake a preliminary study on the toxic effect of urea with an assumption that

toxic effect bovine urine on the *O rhinoceros* larvae may be due to the presence of high concentration of urea in it.

MATERIALS AND METHODS

Rearing of larvae

The larvae of *Oryctes rhinoceros* was collected from cow dung pits at Punchakari, Thiruvananthapuram (2015-2016).Larvae were reared in autoclaved cow dung, to make it free from infectious mites and other parasites. Cow dung of healthy cows fed with grass and dry paddy straw were used for the study as described by Seekumar and Prabhu (1998).

Mixing of Urea with cow dung

Urea (Nice chemicals) was dissolved as appropriate concentration of aqueous solution.100 ml solution of urea was mixed evenly with 500 gm cow dung in such a way that the final effective concentration of urea in cow dung ranged between 0.5 to 1.5% and duration of five days was considered as limit of activity.

Bovine urine

100 ml of Bovine urine of non-lactating cow (around 1 year age) collected freshly was mixed with 500 gm cow dung and into it, two larvae were released.100 ml pond water mixed with cow dung served as control. For all experiments actively feeding third instar larvae of body weight 10gm±1.0gm were used and only two larvae were maintained in 500gm cow dung, kept in a rectangular container of 30x30x6 cm

Assay of biochemical constituents

Biochemical investigations were carried out by standard protocol, Protein (Lowry *et al.,* 1951), Free Amino Acid (Spice, 1957), Uric acid (Biolabs supplied standard kit).Assay of Transaminases (Reitman and Frankel, 1957), Leucine Amino Peptidase (Amador and Wacker, 1967), Cathepsin D (Mycek, 1970), Glucose (Span Diagnostic manufactured kit), Trehalose (Roe,1955),Trehalase (Friedmann,1966) Glycogen (Sciefter *et al.,* 1951) Electrophoresis (Laemmlli, 1970) Total Hemocyte Count (Jones, 1962), Differential Hemocyte Count (Wiggles worth, 1972).Urea content of Bovine urine was estimated by Diacetyl monooxime method(Harold, 1955).

Statistical Analysis was done by using SPSS 21 software (Daniel, 2006). The results were considered statistically significant if P≤0.05.

RESULTS AND DISCUSSION

Urea dissolved in water, which was mixed with cow dung has resulted dose dependent mortality in *O rhinoceros* larvae. Urea at a concentration of 0.3 to 0.4% in cow dung was lethal to grubs and 100% mortality was resulted within 5 days. The larvae reared in urea treated cow dung showed a significant decrease in body weight on the 5th day. Larvae reared in cow dung mixed with bovine urine showed significantly low body weight than control and became immobile on the fifth day of experiment. The result will be clear from Table I. Concentration of urea in bovine urine showed wide range between individuals and also from season to season but its concentration was 1.0 to 1.5% when tested during March to May 2016 which are summer months in Kerala.

Table 1. Body weight of larvae after the fifth Day of maintenance in urea containing cowdung

Concentration of urea (%)	Body weight larvae in gm	
	Initial	after 5 days
Control	11.14 ± 0.02	11.81 ± 0.02
0.5	11.14 ± 0.02	9.86 ± 0.025*
1.0	11.14 ± 0.02	8.16 ± 0.03*
1.5	11.14 ± 0.02	7.04 ± 0.025*
Bovine Urine	11.14±0.02	9.61±0.03

*Values are significantly higher than their control values. Each value is mean ± S.D. All values are significantly different at P ≤ 0.01, with respect to control n=6

Table 2. Effect of urea on the content of uric acid, glucose, trehalse, total hemocyte count in the hemolymph

Quantity of urea in 500gm cow dung (%)	uricacid (mg/100ml)	glucose (mg/dL)	trehalase activity *	total hemocyte count (THC)
Control	4.83 ±0.8	77.4 ± 5	19.07 ± 1.21	9927 ± 60
0.5	14.69 ± 0.7*	111.5 ± 7*	24.89 ± 1.5*	6441± 24
1.0	17.39 ± 0.6*	182.3 ± 10*	28.44 ± 2.0*	5356 ± 20
1.5	21.70 ±0.13*	214.3 ± 18*	33.86 ± 2.34*	4054 ± 15
Bovine Urine	13.51±0.8	107.0±8	23.75±1.9	7056±30

*Values are significantly higher than their control values. Each value is mean ± S.D. All values are significantly different at P ≤ 0.01, with respect to control, n=6

O. rhinoceros larvae when treated with urea showed elevation of total free amino acid and hyperproteinaemia in the hemolymph (Fig 1). Hyperproteinaemia in *O. rhinoceros* larvae was also reported under experimental infection by *Bacillus thuringiensis* and also in cold shock (Adhira and Evans, 2014). Wantanabe (1976) suggested that increase in the amino acid content indicates either low Transaminase activity or high proteolytic activity of enzymes moreover this increase may be due to low food intake, reduction in protein synthesis or higher mobilization of silkworm. Uric acid

content of the hemolymph of healthy grubs was 4.83± 0.8 mg/100ml. Urea intoxicated larvae showed elevated in uric acid on a dose dependent way (Table 2). Wolf *et al.* (1972) pointed out that increase uric acid due to degradation of purines or increase of uric acid level caused by an inability to excreate by the urinary system. Toxicity by bovine urine also caused hyperproteinemia, hyperaminacidemia and elevation of uric acid in *O. rhinoceros* larvae.

Protein Content of the the Hemolymph is expressed in micrograms/ml. Total Free Amino Acid Content of the hemolymph is expressed in micrograms/ml.

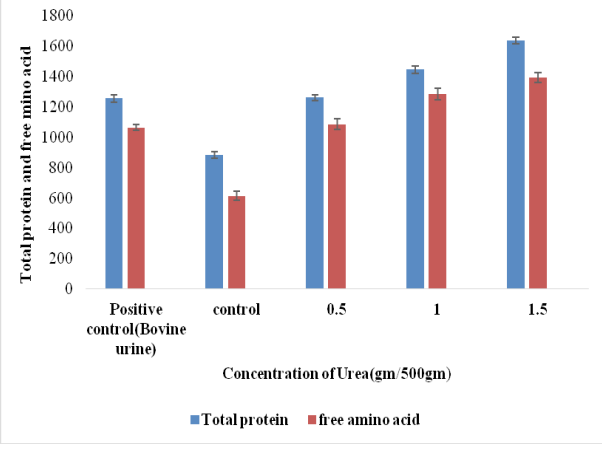
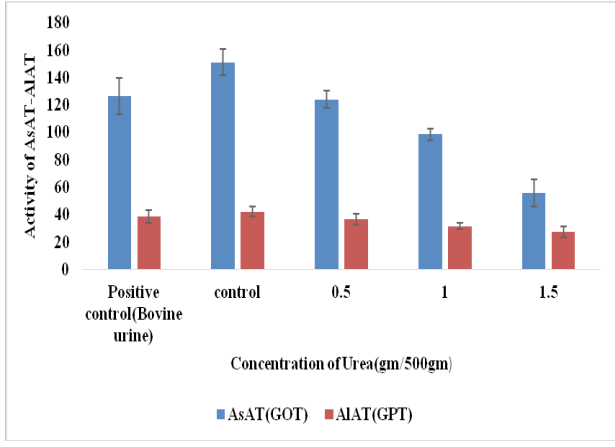


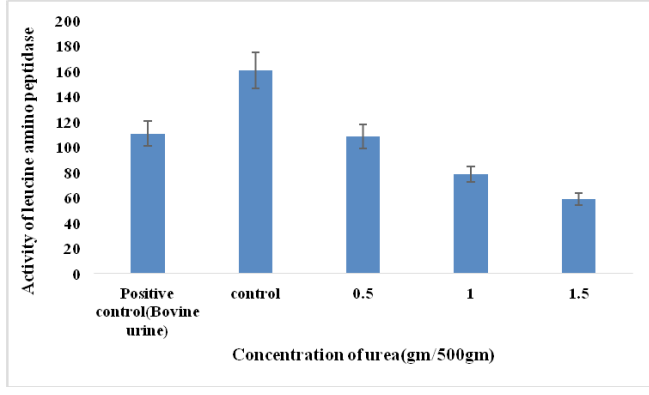
Fig. 1. Effect of urea on the protein and amino acid content of hemolymph.

Activity of Transaminases, both AsAT and AlAT showed a dose dependent decrease in the larvae maintained in cow dung mixed with different concentration of urea. Results are shown in Fig 2. Quassin, a mosquito larvicide isolated from *Quassia amara* induced dose dependent inhibition of both transaminases in *Culex quinqnefasualis* larvae (Evans and Kaleysaraj 1992). Both the above enzymes were inhibited by urea intoxication and ratio of AlAT/ AsAT was elevated. This is an indication of imbalance in amino acid metabolism. Leucine amino peptidase belongs to a group of proteolytic enzymes. Urea intoxicated larvae showed gradual decrease on the activity of leucine amino peptidase on a dose dependent way (Fig. 3). Honey bees visiting on plants contaminated with insecticidal spray also showed inhibition of leucine amino peptidase activity. Winston (1987). Activity of Cathepsin D treated larvae showed gradual decrease and these results are shown in (Fig. 2) Inhibition of proteolytic activity such as LAP and CA can also arrest protein turn over and accumulation of proteins in hemolymph. Glucose level of urea treated larvae showed hyperglycemia (Table 2). This observation very well agreed with the result of Abdel Aziz (2002) who suggested that methomyl caused a significant increase in glucose content of rat's blood serum. Larvae kept in cow dung mixed with bovine urine showed imbalance on the activity of transaminases, inhibition of leucine aminopeptidase and cathepsin D activity which is a clear indication that it is affecting the amino acid and protein turn over in larvae.



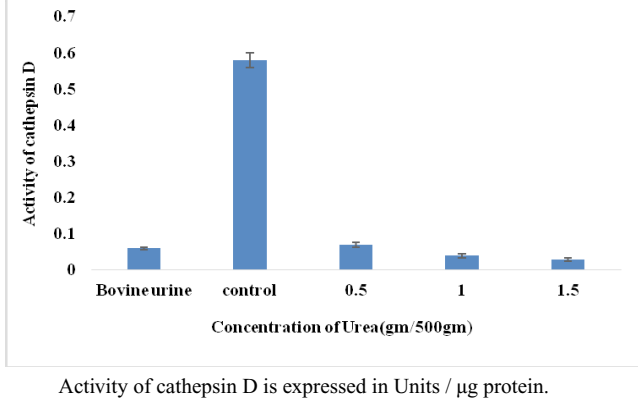
Activity of transaminases is expressed in Units/L

Fig. 2. Effect of urea on the activity of transaminases (AsAT/ GOT & AlAT/GPT) of hemolymph.



All values are mean ± S.D
All values are significantly different at P ≤ 0.01, with respect to control.
Activity of leucine Amino Peptidase is expressed in –naphthylamine liberated / hour/µg protein.

Fig. 3. Effect of Urea on the activity of leucine amino peptidase in larvae.



Activity of cathepsin D is expressed in Units / µg protein.

Fig. 4. Effect of urea on the activity of cathepsin D.

Insect hemolymph usually contains high concentration of trehalose and it ranged from 4-20mg/ml (Wyatt, 1976). In our present study decrease in trehalose content can be correlated to the increased activity of trehalase and accumulation of glucose in the hemolymph (Fig. 5 and Table 2).

Similarly, *Metarhizium anisopliae* a fungi on experimental infecting *Locusta migratoria* also showed a similar decrease in hemolymph trehalose content together with increased activity of trehalase enzymes (Zhao *et al,* 2007) Urea intoxicated larvae also showed significantly low content of glycogen in larval fat body (Fig. 5). Intoxication by bovine urine has also resulted elevation of treahalase activity with subsequent decrease in the trehalose content.

SDS-PAGE of hemolymph protein showed urea have adverse effect on certain proteins. Molecular weight of 36.14 kda, 21.23kDA was seen in the control larvae. These are absent in high concentration of urea treated larvae. Significance of this disappearance of band having molecular weight of 36.14 kDa and 21.23 kDa was not known (Fig. 6).

THCs of *O. rhinoceros* were significantly affected by urea treatment (Table 2). THCs of the treated group showed a tendency to decrease compared with the control groups. Similar results were reported by Gupta (1985) who injected a juvenoid into the last nymphal instar of cockroach and found a 50% reduction of hemocytes in adult. The differential hemocyte count of the *O. rhinoceros* larvae treated with urea a sharp increase in the count of granulocyte (Grs) whereas all other hemocyte showed a significant decrease (Fig 7). Similarly Geroge and Ambrose (2004) also recorded the greatest increase in the number of granulocytes. Intoxication by bovine urine has resulted imbalance on the proportion of different hemocytes which was equivalent to toxicity by urea at a concentration of 0.5%.

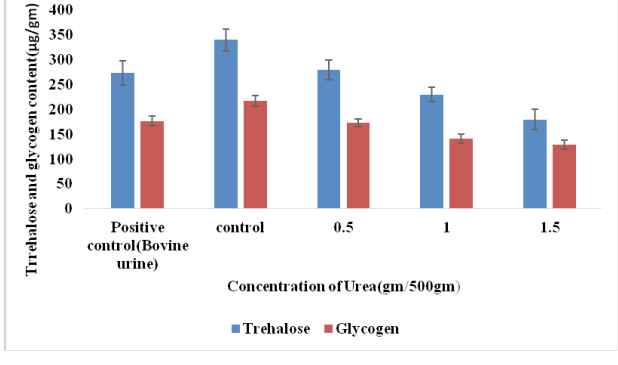
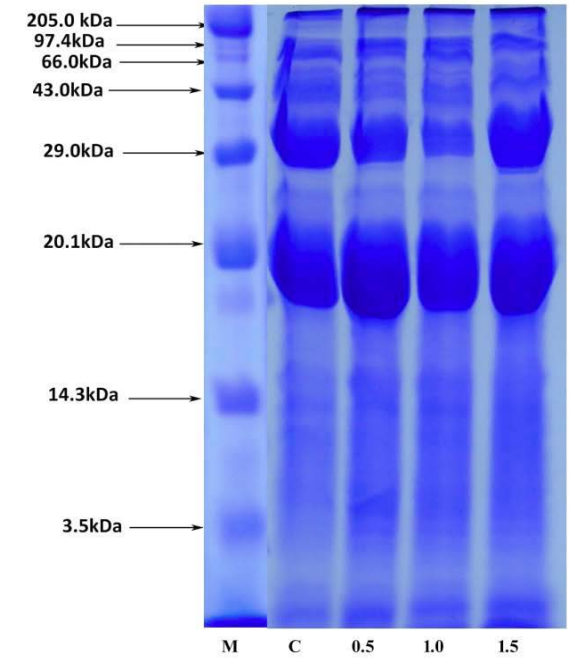


Fig. 5. Effect of urea on the trehalose and glycogen content of larvae.



M- Marker , 0.5 - 0.5 % of Urea, 1.0 - 1.0 % of Urea, 1.5. - 1.5 % of Urea

Fig. 6. Electrophorogram of *Oryctes rhinoceros* grubs on exposure to various concentrations of urea.

Our present study proved that urea has adversely altered metabolism of amino acid and carbohydrate of the hemolymph together with change in the cellular constituents of hemolymph urea is the major dissolved constituent of bovine urine. Hence bovine urine can be used as an effective and safe method for pest control as a part of integrated pest management.

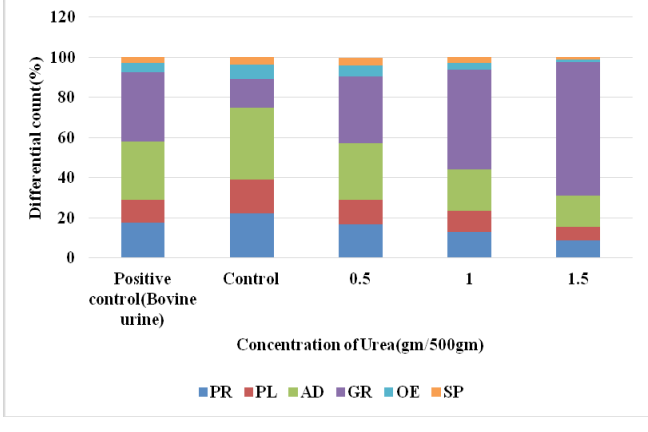


Fig. 7. Graphical representation of differential hemocyte count on treatment with urea.

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