# Effect of Stylo Grass (*Stylosanthes guianensis*) Supplement on Body Mass and Forage Intake of Khari Goats in the Mid-Hills of Nepal

Luma Nidhi Pandey[1], Michael Kam[2], Shambhu B. Pandey[3], Chet R. Upreti[3],
Netra P. Osti[3], A. Allan Degen*[2]

[1]Bandipur Research Station (Goat), Bandipur, Nepal

[2]Desert Animal Adaptations and Husbandry, Wyler Department of Dryland Agriculture, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Beer Sheva 84105, Israel

[3]Nepal Animal Science Research Institute (NASRI), Nepal Agricultural Research Council (NARC), Khumaltar, Lalitpur, Kathmandu, Nepal.

*degen@bgu.ac.il

***Abstract***

Tree fodders are the main constituent of livestock feed, especially for goats, in mid-hills of Nepal. Stylo grass (*Stylosanthes guianensis*) is also offered to livestock, but in much smaller quantities it can ease some of the pressure on tree fodder collection for livestock. It is grown in the mid-hills of Nepal, often on marginal land, and is also intercropped with trees. In this study, goats were offered up to 5 different tree fodders including khanayo (*Ficus semicordata*), sal (*Shorea robusta*), kabro (*Ficus lacor*), katus (*Catannopsis tribuloides*) and aanp (*Mangifera indica*) and stylo grass as supplement. We predicted that stylo grass (1) would have a greater effect on body mass of goats than any of the tree fodders and (2) would reduce the intake of tree fodders. Twenty castrated local Khari goats (initial mean body mass = 12.8±0.22 kg), 6 to 8 months of age, were selected and divided randomly into four groups of five goats each. The groups received tree fodders *randomly* and either 0, 120, 360 or 600 g fresh matter of stylo grass for 120 days. Stylo grass and all tree fodders significantly affected final body mass. Our predictions were partially confirmed. The total dry matter intake of tree fodders was reduced with an increase in stylo grass intake, as predicted. Furthermore, stylo grass intake significantly affected body mass, also as predicted, however, its effect was less than three of the tree fodders. Stylo grass orts were similar when the goats were offered with 120 and 360 g/d fresh matter, but increased when offered with 600 g/d fresh matter. This suggests that a level of less than 600 g/d fresh matter stylo grass should be offered to grow Khari goats.

***Keywords***

*Stylo Grass; Stylosanthes guianensis; Fodder Trees; Khari Goats; Mid-hills of Nepal*

## Introduction

About 70% of the total livestock in Nepal are raised in the hill region, which is mostly between 1,000 and 4,000 meters in altitude. More than 50% of Nepal's population live in this region, usually with small land holdings and, of these people, over 60% living below the poverty line (DFID, 2005). Livestock is an integral part of many subsistence households providing, in addition to income, draught power, milk, meat, fibre and manure. It generates 37% of the income of the hill people (NASA, 1991) and provides 55% of the on-farm income for small farmers (FAO, 1991). Furthermore, livestock contributes 32.3% of the total agricultural gross domestic products (Shrestha, 2005). The dominant farming system in the mid-hills of Nepal comprises three closely integrated components: crops, livestock and trees (Regmi, 2003). As expressed by Floyd *et al.*, (1999): "These components are closely linked, with the forest (trees) acting as the resource base by which crop and livestock production are sustained".

Goats are particularly important in subsistence livestock production in Nepal, and can play a significant role in improving the agricultural economy.

They are a major source of cash income for poorer Nepalese and are multipurpose animals, providing meat, hide, hair and manure. Goat meat is accepted by all ethnic groups. In addition, goats are used for sacrificial purposes at religious ceremonies and are given as gifts (*pewa*) to women. In the development of the Agriculture Prospective Plan in 1995, the goat was identified as a priority commodity and it was decided that the meat production should be increased from 3.38% of the total meat produced in 1994/95 to 7.15% by 2014/15 (Kuwar, Upreti and Pandey, 2001). However, this may be difficult to achieve because of feed shortage, mainly fodder leaves and green forage during winter (October to May). Otherwise, the cost of meat production from goats makes this enterprise non-viable (Upreti and Shrestha, 2006).

Tree fodder is the main constituent of livestock feed, especially for goats, in mid-hills of Nepal (Thapa *et al*., 1997; Pandey *et al*., 2009). There are more than 170 species of trees, shrubs and vines used for fodder, 40 of which are traditionally cultivated by farmers (Khanal and Subba, 2001). Fodder trees are particularly important during the dry winter which is from mid-January through mid-June, when feeds are scarce but the trees remain green and provide a nitrogen supplement to the poor quality crop remains (Thorne *et al*., 1999; Khanal and Upreti, 2008). Trees also provide lumber, fuel, fruit and leaves for bedding of livestock and the leaves mixed with livestock waste are an important source of fertilizer for the farmers (Acharya, 2009; Rabina and Ting 2012).

Communal property, consisting of forests, degraded forests and marginal lands, and private farmlands are the major sources for tree fodder. However, due to heavy population pressure, forests are continually being reconstructed for agricultural land. In addition, forest resources and land cover are declining at a rapid rate leading to an increase in soil erosion and lowered soil fertility. The existing situation demands the rejuvenation of degraded land and the maintenance of forests which can ultimately sustain goat production in rural areas (Malla, 1996; Floyd *et al*., 1999; Neupane, Sharma and Thapa, 2002). To accomplish these goals, Forest Users Groups have been formed to administer communal forests. These groups regulate the collection of fodder and use of the forests and are expected to invest part of the benefits to improve the forest resources (Brown *et al*., 2002; Pokharel and Suvedi, 2007).

Such a Community Forest User Group was established at Katteldanda, a village in Ghorka. Katteldanda includes 4880 rupani (20 rupani = 1 ha) of communal land in the vicinity of the village: 4636 rupani of forest (jungle) and 244 rupani of degraded forests and/or marginal land covered by dwarf shrubs and ferns (degraded former forest areas). To rejuvenate the forest, farmers were forbidden from cutting and carrying away branches for fodder from the community forest at the time of the study. They were allowed only to graze livestock in summer and to collect leaf litter in winter; So farmers did have access to the degraded shrub area throughout the year (Degen *et al*., 2010b).

FIG. 1 WOMEN CARRYING FODDERS TO THEIR HOUSEHOLDS

Furthermore, leaf collection was not allowed in government forests except for leaves from sal (*Shorea robusta*) trees for festive occasions such as weddings. Fodder leaves for livestock were harvested by lopping branches from trees on upland, rain-watered, private land (*bari*). Usually, women collected loads of fodder of about 30 kg each trip, however loads of more than 75 kg were also measured. The fodder loads were either placed in a basket or tied in a bundle and then secured with a belt which was placed on the forehead of the carrier, with much of the weight of the bundle on the carrier's back (Fig 1).

Introduction of forage legumes into the farming system has been suggested as one of the strategies for ensuring the sustainability of livestock and crop yields in the mid-hills of Nepal. Forage legumes not only provide quality feed for livestock but also contribute to sustaining soil fertility. Legumes have relatively high protein levels and also provide essential minerals and vitamins (Horne and Sturr, 2000). Stylo grass (*Stylosanthes guianensis*), which is often grown on marginal land in the mid-hills of Nepal and is also intercropped with trees, can ease some of the pressure on tree fodder collection for livestock. This perennial fodder legume is a sub-shrub, semi-erect or erect, with a strong tap root and small round root nodules. Phengsavanh and Phimphachanhvongsod (1997) reported that stylo 184 grew quickly and was well-adapted to a wide range of environmental conditions. Stylo grass has been used to supplement crop residues (Horne and Stur, 1999) and the macro-mineral bioavailability in grass planted with Verano-stylo legume can improve the utilization of Ca in goats (Bamikole, 2003).

In this study, local Khari goats were offered up to 5 different local tree fodders and stylo grass supplement. We hypothesized that stylo grass intake would affect (1) body mass of the goats and (2) the intake of tree fodders. We predicted that stylo grass would (1) have a greater effect on body mass of goats than any of the tree fodders and (2) reduce the intake of tree fodders.

## Materials and Methods

### *Study Site and Fodder Trees*

The study was conducted at Katteldana (28°00′N; 84°37′E), Gorkha, at the households of the farmers. Measurements were taken over 120 days, from June to September, 2006. Katteldanda, situated mid-way between Kathmandu and Pokhara, has 550 people and 78 households. It is about 1097 m above sea level, receiving an average annual rainfall of 1537 mm, usually between July and August, and having a minimum air temperature of about 8°C in winter (December – February) and a maximum air temperature of about 32°C in summer (April-August). According to the municipality at Ghorka Bazaar, at the time of our study, Katteldanda occupied 1478 rupani of privately owned land of which 397 rupani were used as paddies, 1049 rupani were non-irrigated but cultivated terraces, 20 rupani were gardens and 20 rupani were pasture. In addition, 8% of the households leased land. Most families in the village raised some goats for meat and 1 to 2 water buffalo for milk. The livestock were maintained in stalls or tethered near the household and were fed with mainly leaves collected from fodder trees and shrubs.

Five fodder trees were collected by farmers for goats at the time of the study: khanayo (*Ficus semicordata*), sal (*Shorea robusta*), kabro (*Ficus lacor*), katus (*Catannopsis tribuloides*) and aanp (*Mangifera indica*). Khanayo is used for fodder, in particular for dry and growing animals, fuel and fruit; sal for fodder, ritual plates, for timber and fuel; kabro for fodder, in particular for lactating and growing animals, fuel, soil conservation and pickle; katus for fodder and fuel and aanp for fodder, fruit and timber (Panday, 1982; Regmi, 2003; Magar, 2005). These fodder leaves, which the goats had experienced previous to the study, were collected daily and brought to the animals.

### *Goats Used and Fodder Selection*

Twenty castrated local Khari goats, 6 to 8 months of age, were selected and divided randomly into four groups of five goats each (initial mean body mass = 12.8±0.22 kg; among treatments $P = 0.613$). Khari is the most common breed in the mid-hills of Nepal, comprising about 56% of the goat population. On average, females first are bred at 311 days weighing 15.4 kg and produce 1.6 kids per kidding. Non-castrated male goats are usually slaughtered at 12 months of age, weighing about 16.3 kg, and castrated male goats at 24 months of age at about 30 kg. Generally, females are slaughtered only if they do not breed or are aged (Upreti and Khanal 1997; Neopane and Pokharel, 2008). The goats were divided among 20 households (that is, each household received one goat) and the offered tree fodder was that collected by each household in traditional manner, with as little interference as possible from the researchers. Up to five tree fodders being collected at Katteldanda at the time of the study were offered to the goats at any feeding. The choice of tree fodders was made by the household. Prior to the study, the goats were drenched against internal parasites and dipped against external parasites. Goats on trial were maintained in individual metabolic cages (length = 1.0 m; width = 0.5 m; height = 1.0 m; Fig. 1) that were produced locally from wood and bamboo. The cages, which allowed measurements of non-eaten forage that was offered, were built on 1 m stilts and roofed with corrugated sheeting.

Goats were offered water *randomly*, tree fodder *randomly*, 75 g/d fresh matter (FM) *khole* (a local concentrate mixture consisting of 60% maize grit, 23% rice bran, 15% mustard cake, 1% salt and 1% mineral) and one of four amounts of stylo grass: 0, 120, 360 and 600 g fresh matter. *Khole* was prepared by cooking the various ingredients with water till boiling forming a thickened porridge-like mixture. It offered the livestock at about 85% dry matter content. The goats were tethered in the cage and weighed tree fodder and stylo grass were hung in bundles for the goats to consume. The fodder and stylo grass were offered and refusals were weighed three times a day (07:30, 12:00 and 17:30) but the *khole* was offered only once in the morning. Each goat was weighed at the beginning, every 15 days thereafter, and at the end of the trial.

### *Composition and Metabolizable Energy Yield of Stylo Grass and Fodder Trees*

Dry matter content of the fodder and orts was determined by drying in an oven at 50°C until constant mass. Higher temperatures are not recommended because phenolics can be inactivated directly and/or can bind with fibre. In addition, drying samples at 50°C and 80 - 90°C was compared and no difference in dry matter content was found. Samples were analyzed for nitrogen (N) content by the Kjeldahl method and for ash by burning at 550°C (AOAC, 1984). Crude protein was calculated as 6.25 X N. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined as described by Van Soest, Robertson and Lewis (1991), applying a Fibertec System M6 (Tecator, Haganas, Sweden). Condensed tannins were determined using the butanol-HCl iron reagent and results were expressed as leucocyanidin equivalent (Porter *et al*., 1986; Makkar *et al*., 1993).

Metabolizable energy yield of the samples was estimated *in vitro* using the Hohenheim Gas method (Menke *et al*., 1979; Degen *et al*., 2010a). In this method the gas produced in anaerobic fermentation of substrate is used to predict the nutritive value. Rumen liquor and particulate matter were collected before morning feeding from two goats fed on a roughage diet of mainly poor quality wheat straw and some lucerne hay; And the liquor was homogenized, strained and filtered through glass wool. Incubation media was prepared as described by Menke *et al.* (1979). Samples, each of 200 mg DM, were incubated in triplicate in 100 ml calibrated glass syringes in which 30 ml of the incubation media was added. The glassware was kept at 39°C and flushed with $CO_2$ before use; And the mixture was continually stirred under $CO_2$ at 39°C. Gas production, as determined by piston movement, was measured over 24 h after correcting for gas production due to rumen fluid alone.

Gas production (GP, ml/200 mg DM) and crude protein (CP, g/kg DM) were used to estimate *in vitro* metabolizable energy (IVME) yield as follows (Menke *et al*., 1979; Menke and Steingass, 1988):

$$\text{IVME (kJ/g DM)} = 2.20 + 0.136\ \text{GP} + 0.0057\ \text{CP}$$

### *Statistical Analysis*

Regression analyses, with both single and multiple independent variables, were done using Statistica for Windows software (StatSoft, Inc., Tulsa, OK), and $p < 0.05$ was chosen as the minimal acceptable level of significance. Single regression analysis was used to test the effect of stylo grass on the intake of total tree forage. Multiple regression was used to determine the relationship between final body mass of the goats and each of the offered feeds. Collinearity statistics was used to evaluate the contribution of each of the independent variables in which tolerances, variance inflation factors, semi-partial correlations and beta coefficients were calculated. Tolerance measures the proportion of variance in the independent variable that is explained by other independent variables in the model. A tolerance of close to one indicates that the independent variable in question is not redundant with other independent variables in the equation. The variance inflation factor is the reciprocal of tolerance and measures the degree to which the interrelatedness of the variable with other independent variables inflates the variance of the estimated regression coefficient for that variable. A value of 1 is ideal in that there is no multicollinearity and a value of 4 is considered the minimum to indicate a possible multicollinearity problem. The squared semi-partial correlation was used to represent the per cent of total variance in body mass explained by stylo grass and each of the tree fodders. Beta coefficient is the regression coefficient after standardizing all the variables to a mean of zero and a standard deviation of one; thus beta coefficients represent the contribution of stylo grass and each of the tree fodders to the prediction of body mass.

## Results

The castrated male Khari goats stayed healthy and gained body mass throughout the 120 day study. Mean initial body mass of the goats was 12.8±0.22 kg and final body mass was 19.9 ± 0.40 kg. Stylo intake ranged between 0.0 and 0.24 of total dry matter intake and between 0.0 and 0.26 of total metabolizable energy intake. Stylo orts for goats offered with 600 g/d FM was higher than that for goats offered with 120 and 360 g/d FM ($P < 0.05$); And the latter two did not differ. Average daily gain was not significantly different among groups ($P > 0.05$) and ranged between 53.0 ± 8.1 for the 0 g/d FM stylo grass group and 64.6 ± 5.61 for the 360 g/d FM stylo grass group (TABLE 1).

TABLE 1 CRUDE PROTEIN (CP), NEUTRAL DETERGENT FIBRE (NDF) ACID DETERGENT FIBRE (ADF) ACID DETERGENT LIGNIN (ADL) CALCIUM (CA) PHOSPHORUS (P) AND CONDENSED TANNINS (CT) CONTENTS AND MEATABOLIZABLE ENERGY (ME) OF STYLO GRASS AND FIVE FODDER TREES OFFERED THE GOATS. COMPONENT VALUES ARE IN G/KG DRY MATTER AND ME IN KJ/G DRY MATTER.

| Species | CP | NDF | ADF | ADL | Ca | P | CT* | ME |
|---|---|---|---|---|---|---|---|---|
| Stylo (*Stylosanthes guianensis)* | 135.3 | 539 | 506 | 241 | 16.6 | 1.0 | 17.0 | 5.30 |
| Khanayo (*Ficus semicordata*) | 106.0 | 533 | 482 | 172 | 21.9 | 1.8 | 87.9 | 4.87 |
| Sal (*Shorea robusta*) | 77.2 | 642 | 619 | 260 | 3.1 | 1.5 | 106.0 | 4.83 |
| Kabro (*Ficus locar*) | 168.0 | 577 | 523 | 310 | 16.3 | 2.2 | 48.6 | 5.69 |
| Katus (*Castanopsis tribuloides*) | 109.9 | 764 | 753 | 342 | 4.2 | 1.7 | 63.0 | 4.59 |
| Aanp (*Mangifera indica*) | 73.6 | 548 | 532 | 215 | 13.6 | 1.3 | 74.0 | 5.12 |
| Mean (*S.E.*) | 112 ( 35.8) | 600 ( 89.6) | 569 (101.4) | 257 (62.1) | 12.6 (7.46) | 1.6 (0.42) | 66.1 (31.17) | 5.07 (0.392) |

*As leucocyanidin equivalent

TABLE 2. MEAN (*S.E.*) INITIAL AND FINAL BODY MASSES, AVERAGE DAILY BODY MASS GAIN (ADG) AND AVERAGE FODDER AND METABOLIZABLE ENERGY INTAKES IN KHARI GOATS (N = 5 PER TREATMENT) RECEIVING DIFFERENT FRESH MATTER (FM; G/D) LEVELS OF STYLO GRASS OVER 120 DAYS.

| FM stylo offered | 0 | 120 | 360 | 600 |
|---|---|---|---|---|
| *Body mass* | | | | |
| Body mass, initial (kg) | 13.0 (0.38) | 13.2 (0.36) | 12.6 (0.19) | 12.5 (0.73) |
| Body mass, final (kg) | 19.4 (0.74) | 20.7 (1.05) | 20.2 (0.45) | 19.3 (0.80) |
| ADG (g/d) | 53.0 (8.01) | 62.5 (6.30) | 64.6 (5.61) | 56.8 (5.04) |
| *Dry matter intake (DMI)* | | | | |
| Total DMI | 547.3 (13.18)$^{a}$ | 577.7 (16.00)$^{a}$ | 489.8 (13.15)$^{b}$ | 542.9 (16.10)$^{a}$ |
| *Khole* intake (g/d) | 62.4 (0.91) | 61.3 (0.93) | 63.8 (0.25) | 62.0 (1.07) |
| Stylo intake (g/d) | 0$^{a}$ | 25.8 (0.50)$^{b}$ | 85.8 (0.58)$^{c}$ | 131.8 (1.78)$^{d}$ |
| Stylo orts (g/d) | 0$^{a}$ | 4.5 (0.50)$^{b}$ | 4.7 (0.49)$^{b}$ | 19.6 (1.78)$^{c}$ |
| Tree fodder intake (g/d) | 484.8 (12.92)$^{a}$ | 490.6 (15.49)$^{a}$ | 340.2 (12.92)$^{b}$ | 349.2 (15.92)$^{b}$ |
| Khanayo intake (g/d) | 200.6 (11.69)$^{a}$ | 285.1 (16.83)$^{b}$ | 179.5 (12.3)$^{a}$ | 242.0 (16.44)$^{c}$ |
| Kabro intake (g/d) | 6.0 (2.93) | 4.5 (2.01) | 12.4 (4.43) | 7.3 (2.46) |
| Aanp intake (g/d) | 20.7 (7.68) | 25.9 (8.43) | 16.9 (8.95) | 7.7 (4.79) |
| Katus intake (g/d) | 133.0 (13.07)$^{a}$ | 90.9 (13.53)$^{b}$ | 80.8 (9.48)$^{b}$ | 59.3 (13.59)$^{b}$ |
| Sal intake (g/d) | 124.5 (14.08)$^{a}$ | 84.3 (11.82)$^{b}$ | 50.7 (7.10) $^{c}$ | 32.8 (6.33)$^{c}$ |
| *Metabolizable energy intake (MEI)* | | | | |
| Total MEI (MJ/d) | 2.67 (0.062)$^{a}$ | 2.84 (0.075)$^{a}$ | 2.45 (0.063)$^{b}$ | 2.73 (0.075)$^{a}$ |
| Khole (kJ/d) | 343.4 (5.01) | 337.3 (5.42) | 350.9 (1.39) | 340.7 (5.89) |
| Stylo (kJ/d) | 0$^{a}$ | 136.8 (2.67)$^{b}$ | 454.8 (3.10)$^{c}$ | 698.6 (9.44)$^{d}$ |
| Tree fodders (MJ/d) | 2.33 (0.060)$^{a}$ | 2.37 (0.072)$^{a}$ | 1.65 (0.061)$^{b}$ | 1.69 (0.074)$^{b}$ |

Values within rows with different superscripts are different from each other ($P<0.05$)

TABLE 3. THE EFFECT OF METABOLIZABLE ENERGY INTAKE OF *KHOLE*, STYLO, KHANAYO, KABRO, KATUS, AANP AND SAL (INDEPENDENT VARIABLES) ON FINAL BODY MASS (DEPENDENT VARIABLE) IN KHARI GOATS. INCLUDED IN THE TABLE ARE TOLERANCE, VARIANCE INFLATION FACTOR (VIF), BETA, SQUARED SEMI-PARTIAL CORRELATION (SP), $R^2$ AND P VALUES OF THE INDEPENDENT VARIABLES.

| Variable | Tolerance | VIF | $r^2$ | Beta | SP | t | *P* |
|---|---|---|---|---|---|---|---|
| *Khole* | 0.954 | 1.048 | 0.046 | 0.114 | 0.111 | 2.417 | 0.017 |
| Stylo | 0.585 | 1.709 | 0.415 | 0.505 | 0.386 | 8.418 | 0.000 |
| Khanayo | 0.591 | 1.692 | 0.409 | 0.657 | 0.505 | 11.013 | 0.000 |
| Kabro | 0.719 | 1.392 | 0.281 | 0.121 | 0.103 | 2.237 | 0.027 |
| Aanp | 0.686 | 1.458 | 0.314 | 0.392 | 0.325 | 7.088 | 0.000 |
| Katus | 0.690 | 1.450 | 0.311 | 0.704 | 0.584 | 12.747 | 0.000 |
| Sal | 0.529 | 1.890 | 0.471 | 0.756 | 0.550 | 11.990 | 0.000 |

### *Fodder Composition*

Crude protein of the plants ranged between 74 and 168 g/kg DM; And content of stylo was 135.3 g/kg DM, which was lower than kabro but higher than the other four tree fodders. Condensed tannins ranged between 48.3 and 106.0 g/kg DM in the tree fodders, with sal containing the highest content; content of stylo grass, 17.0 g/kg DM, was considerably lower than the tree fodders. Khanayo had the hightest calcium content, 21.9 g/kg DM, and sal the lowest, 3.1 g/kg DM. Metabolizable energy yield of the tree fodder leaves offered averaged 5.0 ± 0.21 kJ/g DM; that of stylo grass was 5.3 kJ/g DM (Table 2).

Multiple regressions of the effect of the independent variables, MEI of *khole*, stylo grass, khanayo, annp, katus and sal on the dependent variable body mass ($m_b$) were significant ($n = 158$; $r^2adj = 0.67$; SE of estimate = 1.435; $P<0.01$) and were used for further analyses. The tolerance of each independent variable ranged between 0.53 and 0.95 and the variance inflation factor ranged between 1.05 and 1.89 indicating very low collinearity among the offered feeds. The squared semi-partial correlation of stylo grass (0.39) and its beta (0. 50) were both lower than those of sal (0.55 and 0.76), katus (0.58 and 0.70 ) and khanayo (0.51 and 0.66) but above those of annp (0.32 and 0.39) and kabro (0. 10 and 0.12), indicating that its contribution to the total variance and its effect on $m_b$ was lower than the former three tree fodders and higher than the last two tree fodders (Table 3).

There was a significant negative correlation between stylo grass DM intake and tree fodder DM intake. The regression equation took the form:

Tree fodder DM intake (g/d) = 491.2 – 1.223 stylo grass DM intake (g/d) ($n = 158$; $r^2_{adj} = 0.30$; $S_a = 11.7$; $S_{y.x} = 95.9$; $P<0.001$), where $S_a$ = SE of the intercept and $S_{y.x}$ = SE of the estimate.

## Discussion

We predicted that stylo grass would have a greater effect on body mass of the goats than other tree fodders and that its intake would reduce the intake of tree fodders. Our predictions were partially confirmed. The dry matter intake of tree fodders was reduced with an increase in that of stylo grass, as predicted. Furthermore, dry matter intake of stylo grass significantly affected body mass, also as predicted, but its contribution to the variance of body mass and its effect on body mass were less than the dry matter intakes of three of the tree fodders.

Khari goats are generally reared under a poor nutritional regime, with tree fodders as the main dietary component. Consequently, average daily gain of castrated males in the mid-hills of Nepal is low, ranging between 44 g/d (Kuwar *et al*., 2001) and 50 g/d Pandey (2008). In this study, average daily gain among the four treatment groups was higher and ranged between 53.0 and 64.6 g/d. The tree fodder dry matter intake decreased with an increase of stylo grass DM intake. This is in contrast to the findings of Phengsavanh and Ladin (2003) who reported that growing goats consuming stylo 184 as a supplement to a medium quality grass diet increased feed intake.

There is evidence that there is an optimal amount of stylo grass that should be included in the diet for livestock. Phengsavanh and Ledin (2003) tested different levels of stylo grass supplementation (0, 20%, 30% and 40% of expected DM intake) in a diet of growing goats based on Gamba grass and suggested that 30% of stylo grass be included with the basal diet.

Lana *et al*. (1995) reported that live weight of Bali steers was higher with a 50:50 ratio of elephant grass:stylo grass mixture than with 100:0, 75:25, 25:75 and 0:100 elephant grass/stylo grass legume ratios. Furthermore, Pailan *et al*. (2005) concluded that a combination of buffel grass, stylo hay, and subabul leaf meal (40:40:20) improved nutrient utilization of sheep over that of only buffel grass or a mix of buffel grass with stylo hay (50:50). A supplement of 200g/d stylo hay increased in feed intake, utilization and weight gain yearling lambs raised by smallholder farmers (Njwe and Kona, 1994). In this study, the difference in rates of average daily gain among the four treatment groups was not significant, although the goats that received 120 g FM of stylo grass tended to consume the highest amount of metabolizable energy and have the highest body mass at the end of the experiment.

Tree fodders generally have high crude protein but are also high in condensed tannins, which reduce dry matter intake and digestibilities of forages and can be toxic to herbivores (Clausen *et al*., 1990; Degen 1995, 1997). They form precipitates with proteins, resulting in the formation of indigestible tannin-protein complexes (Makkar, 1993) and form complexes with carbohydrates, cellulose, hemicellulose and amino acids reducing their digestibilities (Makkar, Singh and Dawra, 1987). In addition, tannins react with the protein of the outer cellular layer of the gut, thereby reducing the permeability of nutrients, interacting with digestive enzymes rendering them ineffective (Silanikove, Nitsan and Perevolotsky, 1994) and inhibiting microorganisms resulting in a reduction in the production of gases and volatile fatty acids (Makkar, Blümmel and Becker, 1995). However, low levels of tannins can be handled by ruminants and can have some beneficial effects (Villalba *et al*., 2002). They can enhance intakes and digestibilities of other dietary components and can reduce the internal parasite load, at least in small ruminants (Min *et al*., 2004). Sal had the highest condensed tannin and the lowest crude protein contents but its intake was intermediate among all feeds, which was not avoided. Moreover, it explained more of the variance and had the greatest effect on body mass of all fodders, including stylo grass.

The goats in this study were offered with stylo grass and at least two other fodder leaves at each feeding. Several hypotheses have been proposed to explain dietary selection by ruminants (Jansen *et al*., 2007). Some researchers have proposed "minimization" hypotheses: ruminants minimize the intake of plants with high tannins and/or other secondary compounds that have detrimental effects and the intake of plants with high lignin content that lower the digestibility (Skarpe *et al*., 2007). Apparently, this did not occur in this study, at least in the goats offered with 600 g/d FM Stylo grass. It seems that the stylo grass orts of 4.5 to 4.7 g/d DM for goats offered with 120 and 360 g/d FM may have been the minimal amount that could be left due to such reasons as trampling. Stylo grass orts for goats offered with 600 g/d FM was 19.6 g/d and was higher ($P > 0.05$) than these two groups; consuming more stylo grass by these goats which would have reduced tannin intake. Other authors have proposed "maximization" hypotheses: ruminants maximize nutrient intake (Behmer *et al*., 2002) and/or energy intake (van Wieren, 1996). Yet another proposed option is the satiety hypothesis which, according to Jansen *et al*. (2007), can take into account both the minimization and maximization approaches. In this hypothesis, animals stop eating highly toxic or highly nutritious food due to aversion to food that has just been eaten, and then select other feeds (Provenza *et al*., 2003). This may have occurred in this study with the stylo grass at the highest level. It is also possible that some fodders are consumed or avoided because of minerals such as calcium, which was the highest in khanayo and phosphorus, which was the highest in kabro. Therefore, slotting dietary intakes according to these hypotheses can be difficult, as they are not exclusive from each other.

## Conclusion

Dry matter intake of tree fodders decreased with an increase in that of stylo grass, and all feeds were able to explain 0.67 of the variance in body mass. Stylo grass orts increased when 600 g/d fresh matter was offered, suggesting that a lower level should be offered to growKhari goats.

### ACKNOWLEDGEMENTS

We thank Nhuchhe Lal Tamraker for help on all aspects of the research, Raj Kumar Jha for fodder sample analyses, Basanta Shrestha, Sanjeev Panday and Prajwal Regmi for data collection and Bishnu Rana Magar and Roger W. Benjamin for helpful discussions. Special thanks to the farmers who participated in the study and their warm hospitality. The research was funded by the US-Israel Cooperative Development Research Program, Office of the Science Advisor, United States Agency for International

Development (USAID), under Grant Number TA-MOU-00-C17-014.

## REFERENCES

Acharya, K.P. “Utilization and statys of plant resources in northern part of Pokhara valley, central Nepal”. Ethnobotanical Leaflets 13 (2009): 116-122.

AOAC. “*Official methods of analysis*, 14th ed. Association of Official Analytical Chemists”. Arlington, VA, 1984.

Bamikole, M.A. “Macro-minerals bioavailability study in goats fed forages of nitrogen fertilized Guinea grass and Guinea grass-Verano stylo mixture”. Livestock Research for Rural Development 15 (2003): 12.

Behmer, S.T., Simpson, S.J., and D. Raubenheimer, “Herbivore foraging in chemically heterogenous environments: nutrients and secondary metabolites”. Ecology 83 (2002): 2489-2501.

Brown, D., Malla, Y., Schreckenberg, K., and O. Springate-Baginski. “From supervisiong 'subjects' to supporting 'citizens': Recent developments in community forestry in Asia and Africa”. Overseas Development Institute (ODI), London, UK, 2002.

Clausen, T.P., Provenza, F.D., Burritt, E.A., Reichardt, P.B., and J.P. Bryant. “Ecological implications of condensed tannin structure: a case study”. Journal of Chemical Ecology 16 (1990): 2381-2392.

Degen, A.A., Becker, K., Makkar, H.P.S. and N. Borowy. “*Acacia saligna* as a fodder tree for desert livestock and the interaction of its tannins with fibre fractions”. Journal of the Science of Food and Agriculture 68 (1995): 65-71.

Degen, A.A., Blanke, A., Becker, K., Kam, M., Benjamin, R.W. and H.P.S. Makkar. “The nutritive value of *Acacia saligna* and *Acacia salicina* for goats and sheep”. Animal Science 64 (1997): 253-259.

Degen, A.A., Kam, M., Pandey, S.B., Upreti, C.R., El-Meccawi, S., and N.P. Osti. “*In vitro* gas production of leaves from fodder trees and shrubs from mid-hills of Nepal using cow, sheep and goat rumen liquor”. Journal of Agricultural Science, Cambridge 148 **(**2010a**)**: 445-451.

Degen, A.A., Pandey, L.N., Kam, M., Pandey, S.B., Upreti, C.R., and N.P. Osti. “Goat production and fodder leaves offered by local villagers in the mid-hills of Nepal”. Human Ecology 38 (2010b): 625-637.

DFID (2005). *Integrating indigenous and biological knowledge to implement improved dry season feeding strategies on farms in the hills of Nepal*, Department for International Development (DFID), London, UK. http://www.research4development.info/SearchResearchDatabase.asp?ProjectID=2475.

FAO (1991). *Food and Agriculture Organization of the United Nations FAOSTAT: the statistical database of FAO*, http://www.fao.org/waicent/portal/statistics_en.asp

Floyd, C.N., Harding, A.H., Paddle, K.C., Rasali, D.P., Subedi, K.D., and P.P. Subedi. “The adoption and associated impact of technologies in the western hills of Nepal”. Network Paper No. 90, Agricultural Research and Extension Network (AgREN), Department for International Development (DFID), Overseas Development Institute (ODI), London, UK, 1999.

Horne, M.P., and W.W. Sturr. “Developing forage technologies with smallholder farmers. How to select the best varieties to offer farmers in Southeast Asia”. (Published by ACIAR and CIAT). ACIAR Monograph 62 (2000): 80.

Jansen, D.A.W.A.M., van Langevelde, F., de Boer, W.F., and K.P. Kirkman. “Optimization or satiation, testing diet selection rules in goats”. Small Ruminant Research 73 (2007): 160-168.

Lana, K., Nitis, I.M., Sukanten, W., Suarna, M., and S. Putra. “Effect of stylo legume supplement to elephant grass diet on the performance of Bali steer”. In Enhancing sustainable livestock-crop production in small holder farming systems. Proceedings of the Fourth Regional Working Group on Grazing and Feed Resuorces in Southeast Asia, Nha Trang, Vietnam, edited by C.C. Wong and Le Viet Ly, 171-182. FAO, Rome, 1995.

Khanal, R.C., and D.B. Subba. “Nutritional evaluation of leaves from some major fodder trees cultivated in the hills of Nepal”. Animal Feed and Science Technology 92 (2001): 17-32.

Khanal, R.C., and C.R. Upreti. “Evaluation of selected species of tree fodders cultivated for feeding ruminants in the hills of Nepal”. Pakistan Journal of Nutrition 7 (2008): 297-302.

Kuwar, B.S., Upreti, C.R., and S.B. Pandey. “Development of suitable feeder and feeding package for goats”.

Agriculture Research Station (Goat), Bandipur, Nepal, 27 pp., 2001.

Magar, B.R. "Study on the nutritive value of major fodder tree leaves available in Bandipur Village Development Committee of Tanahun *District*, Nepal". M.A. thesis, Tribhuvan University, Kirtipur, Kathmandu, Nepal, 2005.

Makkar, H.P.S. "Antinutritional factors in foods for livestock". In Animal production in developing countries, edited by M. Gill, E. Owen, G.E. Pollott and T.L.J. Lawrence, 69-85. Occasional publication, British Society of Animal Production, no. 16, 1993.

Makkar, H.P.S., Blümmel, M., and K. Becker. "Formation of complexes between polyvinyl pyrrolidone and polyethylene glycol with tannins and their implications in gas production and true digestibility in *in vitro* techniques". British Journal of Nutrition 73 (1995): 897-913.

Makkar, H.P.S., Blümmel, M., Borowy, N.K., and K. Becker. "Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods". Journal of the Science of Food and Agriculture 61 (1993): 161-165.

Makkar, H.P.S., Singh, B., and R.K. Dawra. "Tannin-nutrient interactions". International Journal of Animal Sciences 2 (1987): 127-140.

Malla, Y. "Tree management and household strategies in a changing rural economy: Beyond the use of direct incentives". The University of Reading, UK, 1999. http://www.mtnforum.org/resources/library/mally99b.htm.

Menke, K.H., and H. Steingass. "Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid". Animal Research and Development 28 (1988): 7-55.

Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D., and W. Schnider. "The estimation of the digestibility and metabolizable energy content of ruminant feedstuffs from the gas production when they are incubated with rumen liquor in vitro". Journal of Agricultural Science, Cambridge 93 (1979): 217-222.

Min, B.R., Pomroy, W.E., Hart, S.P., and T. Sahlu. "The effect of short-term consumption of a forage containing condensed tannins on gastro-intestinal nematode parasite infections in grazing wether goats". Small Ruminant Research 51 (2004): 279-283.

NASA. "Proceedings of the First National Animal Science Convention". Nepal Animal Science Association (NASA), Kathmandu, Nepal, 1991.

Neopane, S., and P.K. Pokharel. "Indigenous goats of Nepal Publication". Nepal Agricultural Research Council, Animal Breeding Division, Khumaltar, lalitpur, Nepal, 2008.

Neupane, R.P. Sharma, K.R., and G.P. Thapa. "Adoption of agroforestry in the hills of Nepal: a logistic regression analysis". Agricultural Systems 72 (2002): 177-196.

Njwe, R.M., and B. Kona. "Comparative evaluation of stylo (*Stylosanthes guianensis*) hay and concentrate as protein supplement for West African Dwarf sheep fed basal diet of elephant grass (*Pennisetum purpureum*)". In Small Ruminant Research and Development in Africa, Proceedings of the Third Biennial Conference of the African Small Ruminant Research Network, edited by S.H.B. Lebbie and E. Kagwini, 326. UICC, ILRI, Nairobi, Kenya, 1994.

Pailan, G.H., Verma, N.C., Sharma, D.K., and J.N. Gupta. "Supplementation of Caribbean stylo hay and Subabul leaf meal to buffel grass hay for sheep". Animal Nutrition and Feed Technology 5 (2005): 31-38

Panday, K.K. "Fodder trees and tree fodder in Nepal". Swiss Federal Institute of Forestry Research, Birmensdorf, Switzerland, 1982.

Pandey, L.N. "Fodder selection, dietary intake and effect of stylo (Stylosynthes guianensis) supplementation in goats". M.Sc. thesis, Ben-Gurion University of the Negev, Beer-Sheva, Israel, 2008.

Pandey, L.N., Kam, M., Pandey, S.B., Upreti, C.R., Osti, N.P. and A.A. Degen. "Indigenous knowledge of fodder tree selectivity by local goats in the mid-hills of Nepal". Journal of Enterprising Communities: People and Places in the Global Economy 3 (2009): 241-255.

Phengsavanh, P., and V. Phimphachanhvongsod. "Environmental adaptation of forage in Lao PDR". In Proceedings of the Third Regional Mmeeting of the Forages for Smallholders Project, Agency for Liveswtock Services of East Kalimantan, Indonesia, edited by W.W. Stur, 41- 52. CIAT Working Document No. 188, 1998.

Phengsavanh, P., and I. Ledin. "Effect of Stylo 184 (*Stylosanthes guianensis* CIAT 184) and Gamba grass (*Andropogon gayanus* cv. Kent) in diets for growing goats". Livestock Research for Rural Development 15 (2003): 10.

Pokharel, R.K., and M. Suvedi. "Indicators for measuring the success of Nepal's community forestry program: A local perspective". Research in Human Ecology 14 (2007): 68-75.

Porter, L.J., Hrstich, L.N., and B.C. Chan. "The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin". Phytochemistry 25 (1986): 223-230.

Provenza, F.D., Villalba, J.J., Dziba, L.E. Atwood, S.B., and R.E. Banner. "Linking herbivore experience, varied diets, and plant biochemical diversity". Small Ruminant Research 49 (2003): 257-274.

Rabina G., and Z. Ting. "Effect of managing methods of the community forests on the expression of forest products extracted from them in Lalitpur and Dhadhing districts from Nepal". African Journal of Agricultural Research 7 (2012): 4465-4473.

Regmi, B.N. "Contribution of agroforestry for rural livelihoods: A case of Dhading District, Nepal". The International Conference on Rural Livelihoods, Forests and Biodiversity, Bonn, Germany, 2003.

Shrestha, N.P. "Importance of different feed resources in livestock improvement in Nepal". Proceeding of the workshop on Fodder Oats, Fodder Technology and Small Farm income Generation, pp. 111-119, Kathmandu, Nepal, 2005.

Silanikove, N., Nitsan, Z., and A. Perevolotsky. "Effect of a daily supplementation of polyethylene glycol on intake and digestion of tannin-containing leaves (*Ceratonia siliqua*) by goats". Journal of Agricultural and Food Chemistry 42 (1994): 2844-2847.

Skarpe, C., Jansson, I., Seljeli, L., Bergstrom, R. and E. Roskaft. "Browsing by goats on three spatial scales in a semi-arid savanna". Journal of Arid Environments 68 (2007): 480-491.

Thapa, B., Walker, D.H., and F.L. Sinclair. "Indigenous knowledge of the feeding value of tree fodders". Animal Feed and Science Technology 67 (1997): 97–114.

Thorne, P.J., Subba, D.B., Walker, D.H., Thapa, B., Wood, C.D., and F.L. Sinclair. "The basis of indigenous knowledge of tree fodder quality and its implication for improving the use of tree fodder in developing countries". Animal Feed and Science Technology 81 (1999): 119-131.

Upreti, C.R., and R. Khanal. "Comparative performance of seven goats breeds at ARS Bandipur". In Proceeding of 2nd National workshop in Livestock and Fisheries Workshop, 24-28, Kathmandu, Nepal, 1997.

Upreti, C.R., and B.K. Shrestha. "Nutrient content of feeds and fodder in Nepal". Animal Nutrition Division, Nepal Agricultural Research Council (NARC), Khumaltar, Lalitpur, Nepal, 2006.

Van Soest, P.J., Robertson, J.B., and B.A. Lewis. "Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition". Journal of Dairy Science 74 (1991): 3583-3592.

Van Wieren, S.E. "Do large herbivores select a diet that maximizes short-term energy intake rate?". Forest Ecology and Management 88 (1996): 149-156.

Villalba, J.J., Provenza, F.D., and J.P. Bryant. "Consequences of the interaction between nutrients and plant secondary metabolites on herbivore selectivity: benefit or detriments for plants?". Oikos 97 (2002): 282-292.

# Terminal Residues of β-Cyfluthrin in Cotton

Reena Chauhan*[1], Krishna Rolania[2] and Beena Kumari[3]

*Department of Soil Science, Department of Entomology
CCS Haryana Agricultural University,
Hisar 125 004, India
beena@hau.ernet.in

***Abstract***

Degradation behavior of β–cyfluthrin in cotton seed, lint and soil under crop was studied, by conducting a field experiment at the Research Farm of CCS HAU, Hisar. The mean deposits were found to be 0.005 and 0.022 mg $kg^{-1}$ in cotton lint at the time of harvest for minimum effective (18 g a.i $ha^{-1}$) and double effective dose (37.5 g a.i $ha^{-1}$), respectively. Average residues in cotton lint were found below MRL value at single dose and nearly equal to MRL value at double dose application. In cotton seed oil, the residues were observed to be below MRL at both the doses. In soil the *β* –cyfluthrin residues maintain little amount at harvest time.

***Keywords***

*Residues; β –Cyfluthrin; Cotton Seed; Lint; Soil; MRL*

## Introduction

Cotton termed as "The King of Fibres and a crop of prosperity", having a great impact on men and matter, is an industrial commodity of worldwide importance. It is a variety of plants of the genus Gossypium, belonging to the Malvacae family. Out of about 50 species of cotton plants in the world, only four have been domestically cultivated for cotton fibres.

The areas under cotton production in the world are estimated at around 30-31 million hectares. India is the largest area under cotton production. China is the largest producer of cotton in the world whereas India stands at second position. Interestingly, although China with almost half the area under cotton production compared to India, but produces more than 2½ times yield (kg per hectare) of cotton as compared to India.

Cotton is one of the principal crops of India and plays a vital role in the country's economic growth by providing substantial employment and making significant contributions to export earnings. The cotton cultivation sector not only is engaged in around 6 million farmers, but also involved in another about 40 to 50 million people relating to cotton cultivation, cotton trade and its processing (Anonymous, 2012). Low productivity of cotton may be attributed to both biotic and abiotic stresses. Among the biotic stresses, insect pests are known to cause heavy loss to cotton resulting in drastic reduction in yield. Unfortunately, the pest spectrum of cotton is quite complex and is attacked by 1,326 species of insect pests from sowing to maturity (Santhum, 1997). Crop losses in cotton have been reported due to several pests.Among these nine are key pests in India out of which the tobacco caterpillar, *Spodoptera litura* (Fab.) is apolyphagous lepidopterous pest causing damage to 112 crop species throughout the country such as cotton, tobacco, chilli, groundnut, castor etc. (Lefroy, 1908). Other chief species include *Earias vittella* (30-40%), *Pectinophora gossypiella* (20-95%) (Panwar, 1995) and *Helicoverpa armigera* (20-80%) (Monga and Jeyakumar, 2002). Several potent pesticides have been recommended for managing these pests on this crop which consumes around 50% of pesticides in India Singh *et al.*(2004) and accounts for 40% of the total production cost and ranks first in terms of pesticide consumption, Dudani and Sengupta (1992).

Indiscriminate use of pesticides to combat insect pests led to resistance and also development of resurgence. To overcome these problems,several new insecticides with new chemistry have been tested in various parts of the world. The compound β-cyfluthrin, [cyano(4-fluoro-3-phenoxyphenyl) methy l3-(2,2- dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate] belonging to synthetic pyrethroid group, acts as a contact and stomach poison. It gives wide spectrum activity against lepidoptera, coleoptera and hemiptera pests and various workers have studied residues of β-cyfluthrin in rice , Naini *et al.* (2003), cotton Battu *et al.* (1999), Mukherjee *et* *al*.( 2002), brinjal (Dikshit, 2001; Sinha and Gopal (2002) and Mandal *et al*. 2010), okra (Dikshit *et al*. 2002) and tomato (Dikshit *et al*. 2003 and Dharumarajan *et al*. 2009). As very scanty work has been carried out to know the behaviour of β -cyfluthrin on cotton under Indian conditions, Therefore the present study was carried out to evaluate the residues of β -cyfluthrin in cotton lint, seed oil and soil under crop at harvest.

## Materials and Methods

All the solvents were of analytical grade and glass distilled before use. β –cyfluthrin was purchased from local market. Standard stock solution (1,000 μg/ml) was prepared in acetone. Lower dilutions were prepared by taking required aliquot from the stock solution and diluting it with n-hexane. An aqueous solution of NaCl (10%) was partitioned twice with dichloromethane to remove the impurities. Sodium sulfate was washed with acetone, dried at room temperature, and then activated at 110°C for 4 h before use.

The cotton crop (variety H-1226) was raised at the research farm of CCS Haryana Agricultural University, Hisar during *kharif* season of year 2010 following recommended agronomic practices. The Plot size was taken as 25 $m^2$ each. There were three replications for each treatment (i.e control, recommended and double the recommended dosages) the recommended dosages arranged in a randomized block design (RBD). β –cyfluthrin was applied in the field at the time of flower initiation stage at two different doses. Before spraying, cotton plants in all plots/replicates were tagged and sprayed with recommended doses ($T_1$) consisting of 18.75 g a.i.$ha^{-1}$ and the doubled recommended dose ($T_2$) consisting of 37.50 g a.i.$ha^{-1}$ and a control where no pesticide was applied. Cotton lint, seeds and soil samples from top 15 cm of soil profile were collected at the time of harvest and then processed.

The cotton seed samples were air dried and delint to get cotton seed and lint to analyze them separately. Representative cotton seed sample (10 g) and cotton lint (5g), was extracted by Soxhlet apparatus using 200 ml acetonitrile for 8 h. The extract was then filtered, transferred in separatory funnel and diluted it with 10% sodium chloride solution. The extract was partitioned twice with hexane (100 and 100 ml) followed by partitioning twice with dichloromethane (100 and 100 ml) by vigrous shaking. The organic phases were combined and then concentrated to about 5 ml on a rotary vacuum evaporator at 50-55°C. For clean–up, glass column (60 cm x 22 mm i.d) was packed compactly with silica gel in between two layers of anhydrous sodium sulphate. The column was prewetted with hexane firstly and then the concentrated extract was loaded in the column. The column was eluted with 125 ml solution of dichloromethane: acetone (1:1 v/v). The cleaned extract was evaporated to dryness and finally dissolved in 2ml n-hexane for analysis by GC.

Soil samples were extracted as per method of Kumari et al.(2008). To the well-ground, sieved and representative soil sample (20 g), 0.5 ml ammonia solution was added and kept for half an hour. Ten grams of anhydrous sodium sulfate, 0.3 g Florisil and 0.3 g activated charcoal were added and mixed properly. The homogenized sample was packed compactly in a glass column (60 cm × 22 mm i.d.) in between two layers of anhydrous sodium sulfate. The column was eluted with 150 ml solution of hexane: acetone (1:1 *v/v).* Eluate was concentrated to 5 ml on a rotary vacuum evaporator at 40°C followed by gas manifold evaporator to dryness. Final volume of the concentrated extract was reconstituted by adding n-hexane up to 2 ml and analyzed by GC.

The cleaned extracts were analysed on GC Shimadzu 2010, Model, equipped with capillary column using a $Ni^{63}$ electron capture detector (ECD). Operating conditions were: fused silica column: 30 m x 0.32 mm i.d, coated with 5% diphenyl/ 95% dimethyl silicone, 0.25 μm film thickness (supelco SPB-5), with split injection system.

$N_2$ was used as carrier gas at a linear gas velocity of 2 mL min−1 throughcolumn and made up gas 60 mL $min^{-1}$. The injection port maitained at 280°C and the oven temperature was 150°C for 5 min. then at 8°C $min^{-1}$ to 190°C for 2 min, and finally at 15°C $min^{-1}$ to 280°C for 10 min. The detector temperature, 300°C was used for estimation. Retention time was 20.72 min. Limit of detection and determination/quantitation were 0.002 and 0.005 mg kg−1, respectively.

The efficiency of the method was evaluated by carrying out recovery experiments. The percent recoveries of β -cyfluthrin in cotton lint were 81.60 and 83.77 and in cotton seed oil were 81.05 and 82.31 at 0.25 and 0.50 mg $kg^{-1}$ level respectively (TABLE 1).

TABLE 1 PERCENT RECOVERY OF Β –CYFLUTHRIN IN COTTON LINT AND SEED OIL

| Substrate | Level of Fortification ($mgkg^{-1}$) | % Recovery |
|---|---|---|
| Cotton Lint | 0.25 | 81.60 ± 4.85 |
| | 0.50 | 83.77 ± 2.16 |
| Cotton Seed Oil | 0.25 | 81.05 ± 1.95 |
| | 0.50 | 82.31 ± 1.80 |

*Mean±SD of three replicates

## Results and Discussion

TABLE 2 RESIDUES (mg $kg^{-1}$) OF Β –CYFLUTHRIN IN SOIL, COTTON LINT AND COTTON SEED OIL AT SINGLE DOSE

| Commodity | Residues ($mgkg^{-1}$) | |
|---|---|---|
| | Single Dose (18.75 g a.i $ha^{-1}$) | Double Dose (37.50g a.i $ha^{-1}$) |
| Cotton Lint | 0.005 ± 0.004 | 0.022 ± 0.005 |
| Cotton Seed Oil | BDL | 0.010± 0.004 |
| Soil (Harvest) | 0.052 ± 0.013 | 0.127 ± 0.022 |

MRL: Cotton seed 0.020 mgkg-1, BDL: 0.005 mgkg-1

The overall results of the analysis of cotton lint and seed following the application of β -cyfluthrin @ 18.75 g a.i. $ha^{-1}$ and 37.50 g a.i. $ha^{-1}$ are presented in TABLE 2. The average deposits of β -cyfluthrin were found to be 0.005 and 0.022 mg $kg^{-1}$ on cotton lint on harvest at minimum effective and the doubled effective dosages, respectively. In case of seed oil,the residues were found below detectable level for minimum effective dose while 0.010 mg $kg^{-1}$ for the doubled effective dosages. In soil 0.052 and 0.127 mg $kg^{-1}$ residues were found at single and double dose, respectively. The result revealed that the applied dose persisted in soil at the time of harvest. As the residues reached below MRL value hence safe for human consumption. The results were similar be similar to findings of Raj *et al*.1990, who reported that deltamethrin did not leave any residues in cotton seed, oil and lint. Fenvalrate and cypermethrin residues were below the maximum residue limit of 0.2 ppm in cotton seed. Residues were considerably lower in second picking for all the insecticides. Residues of synthetic pyrethroid were much lower than those of carbaryl and levels fell sharply during second picking of bolls. Residues levels were also found below MRL value for all the insecticide treatments and posed no toxicological hazard when presented in cotton seed oil by Gupta *et al*. (1990). Battu *et al*. (1999) estimated that residues of synthetic pyrethroids in cotton seed and lint. No residues were found in case of cotton seed but residues of cypermethrin and fenvalrate were detected in case of lint. The residues dissipated with the half-life of 2.4 and 2.6 days persisted for 5 days only. The mean initial deposits of β-cyfluthrin were 0.12 and 0.23 mg $kg^{-1}$ on the okra fruits following 3rd application with respect to β-cyfluthrin at 18 and 36 g a.i. $ha^{-1}$. These deposits were disspated to 0.02 and 0.06 mg $kg^{-1}$ after 1 day at single and double dosages respectively, thereby showing a loss of about 83.33 and 73.91 per cent. These residues of β-cyfluthrin reached below its determination limit of 0.01 mg $kg^{-1}$ in 3 and 5 days at single and double dosages, respectively (Sahoo et al.2012). Mature mango fruits at harvest were free from residues of β -cyfluthrin by Mohapatra *et al*. (2011). Similar results were shown by Singh and Singh (2007).

Mandal *et al*. (2010) reported that soil samples under brinjal crop did not show the presence of β -cyfluthrin at their detection limit of 0.01 mg $kg^{-1}$ when collected at the time of harvest. Half-life ($T_{1/2}$) values of β -cyfluthrin were observed to be 1.74 and 1.39 days, respectively, when applied @ 18 and 36 g a.i. $ha^{-1}$. Vig *et al*. (2001) observed that cypermethrin was more persistent as compared to deltamethrin under cotton crop in soil and up to 63–67% of cypermethrin residues were detected in 1995 after 15 days and up to 54.9% in 1998 between 2–10 days after treatment. No residues were detected on 105 day. Similar results were reported by Dikshit et al.(2002).

## Conclusion

The applied dose is effective for the pests as the residues are persisting in the soil. As the residues are below MRL value hence safe for the human consumption. Residues are presented in Cotton lint at higher dose only at higher dose, but its safety couldn't be assessed due to non availability of MRL value.

## ACKNOWLEDGMENT

The authors wish to express their gratitude to the Head, Department of Entomology for providing research facilities.

## REFERENCE

Anonymous." www.indiantextilejournal.com". 2012.

Battu RS., Joia BS., Singh B., Kang BK., and Chahal KK. "Residues of β-cyfluthrin in cotton seed and cotton lint". Pestic. Res. J 11: 222–224,1999.

Battu R S., Joia BS., Dhawan AK. and Udeaan AS. "Harvest-time residues of different insecticides in cotton seed and lint in Punjab". Pestic. Res. J. 15(2): ,2003.

Dharumarajan S., Dikshit AK. and Singh SB. "Persistence of combination-mix (β-cyfluthrin + imidacloprid) on tomato (*Lycopersicon esculntum*)". Pestic. Res. J. 21: 83–85, 2009.

Dikshit AK., Lal OP., Sinha SR. and Srivastava YN. "Safety evaluation, persistence and bioefficacy of imidacloprid and betacyfluthrin on okra": PartII. Pestology 26: 30–37, 2002.

Dikshit AK., Pachauri DC. and Jindal T. "Maximum residue limitand risk assessment of beta-cyfluthrin and imidacloprid on tomato (*Lycopersicon esculentum* Mill)". Bull. Environ. Contam. Toxicol. 70: 1143–1150, 2003.

Dikshit AK., Srivastava YN. and Lal OP. "Residue studies and bioefficacy of beta cyfluthrin and lamda-cyhalothrin in brinjal (*Solanum melongena* L.) fruits". Pestology 25: 27–32, 2001.

Dudani AT and Sengupta S. "In intensive use of agricultural chemicals in India and their ecological and environmental impacts". In "Third Agricultural Science Congress", PAU, Ludhiana, India, 1992.

Gupta G P. ,Agnihotri NP., Katiyar KN. and Jain HK. "Bioefficacy and persistence of synthetic pyrethroids in cotton". Pestic. Res. J. 2(2):115-122, 1990.

Kumari B., Madan VK., and Kathpal T S. "Status of insecticide contamination of soil and water in Haryana, India". *Environ.Monit. and Assess.* 136: 239–244, 2008.

Lefroy HM. "The tobacco caterpillar, *Prodenia littoralis*". *Memoirs of the Department of Agriculture in India, Entomological Series,* 2, 79–93, 1908.

Mandal K., Chahil GS., Sahoo SK., Battu RS. and Singh B." Dissipation kinetics of β-cyfluthrin and imidacloprid in brinjal and soil under subtropical conditions of Punjab, India". Bull. Environ.Contam. Toxicol. 84: 225–229, 2010.

Mohapatra S., Deepa M. and Jagdish GK." Behavior of beta- cyfluthrin and imidacloprid in/on mango (Mangifera indica L.)". Bull. Environ. Contam. Toxicol. 87(2): 202-207, 2011.

Monga D. and Jeyakumar P. "Cotton field ecology-Introduction to insect pest and diseases in relation to growth and development". In 'National Training course on IPM in cotton, rice and vegetable crops', NCIPM, New Delhi, 2002.

Mukherjee I., Gopal M. and Kusum." Evaluation of residues of β-cyfluthrin on cotton". Bull. Environ. Contam. Toxicol. 69:54–58, 2002.

Naini T., Jaykumar R., Kuttalam S., Chandrasekaran S. and Premalatha R. "Harvest time residues of betacyfluthrin on rice". Pestic. Res. J. 15: 58–59, 2003.

Panwar VPS . "Pests of fiber crops". In "Agricultural insect pests of crops and their control". pp: 87-104, 1995.

Raj MF., Shah PG., Patel BK., Talati JG. and Patel AS. "Residues of synthetic pyrethroids in cotton seed, oil and lint". Indian J of Plant Prot. 18(2):191-195, 1990.

Sahoo SK., Chahil GS., Mandal Kousik, Battu RS. and Singh B." Estimation of β-cyfluthrin and imidacloprid in okra fruits and soil by chromatography techniques" .J Environ. Sci .and Health, Part B: Pestic. Fd. Contam. Agri. Waste 47(1):42-50, 2012.

Santhum V. *Cotton—Indian Council of Agricultural Research Publication*. pp. 35–50, 1997.

Singh A., Trivedi TP., Sardana HR., Sabir N., Dhandapani A., Singh J., Sohi AS., Sharma PD., Chauhan MS., Kharab SS., Bhosle BB., Lavekar RC. and Gawas VR. "Validation and promotion of IPM in cotton- A participatory approach". Natural Symposium on Cotton: 232- 242, 2004.

Singh NK. and Singh SP. "Dissipation of β -cyfluthrin residues on chickpea". Pestic. Res. J 19:104–105, 2007.

Sinha S. and Gopal M. “Evaluating the safety of *β*-cyfluthrin insecticide for usage in eggplant (*Solanum melongena* L.) crop”. Bull.Environ. Contam. Toxicol 68: 400–405, 2002.

Vig K., Singh DK., Agarwal HC., Dhawan AK. and Dureja P. “Insecticide residues in cotton crop soil”. Pestic. Fd. Contam. Agri. Waste. 36 (4):421-434, 2001.