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CONTENTS OF THE VOLUME

- i. Copyright Notice
 - ii. Editorial Board Members
 - iii. Chief Author and Dean
 - iv. Table of Contents
 - v. From the Chief Editor's Desk
 - vi. Research and Review Papers
-
- 1. Biogas Production from Anaerobic Digestion of Manure Waste: Moroccan Case. ***1-3***
 - 2. Effect of Varying NDF, ADF and Digestible Energy Levels on Growth Performance, Nutrient Digestibility, Caecal Fermentation, Caecal and Faecal Microflora of Growing Rabbits. ***5-13***
 - 3. Leaching of Bifenthrin in two Different Types of Soil under Laboratory Condition. ***15-18***
 - 4. Evaluation of Shoot Fly Resistance through SSR Markers in Sorghum [Sorghum Bicolor (L.) Moench]. ***19-22***
-
- vii. Auxiliary Memberships
 - viii. Process of Submission of Research Paper
 - ix. Preferred Author Guidelines
 - x. Index



Biogas Production from Anaerobic Digestion of Manure Waste: Moroccan Case

By Afilal M.E, Belkhadir N & Merzak Z

Mohamed Premier University

Résumé - Le biogaz issu de la fermentation anaérobie des substrats organiques animaux ou végétaux est une énergie renouvelable qui suscite un intérêt croissant. L'exploitation de cette ressource énergétique présente en effet de nombreux avantages au niveau économique et environnemental.

En revanche cette technologie reste très peu connue au Maroc par les institutions, les décideurs et les acteurs privés (gérants, bureau d'études, personnel);

Dans cette étude nous dressons une présentation cartographique de la densité en déchets d'élevages au niveau des différentes régions du Maroc, nous présentons également une estimation du potentiel énergétique pouvant être produit par fermentation méthanique.

Notre étude montre qu'à l'échelle des différentes régions du Maroc, nous avons la possibilité de récupérer plus de 87 millions de tonnes de déchets et résidus organiques fermentescibles de fumier de bétail et de volailles ceci est équivalent à plus de 4,5 Mtep/an.

Keywords : biomasse, énergie, méthanisation, carto-graphie, déchets organiques.

GJSFR-C Classification : FOR Code: 850599, 660502p



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Afilal M.E^α, Belkhadir N^σ & Merzak Z^ρ

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Les avantages de cette technologie et ces applications seront discutés en perspective d'une large diffusion dans le monde rural.

Motclés : biomasse, énergie, méthanisation, cartographie, déchets organiques.

Summary - Biogas from the anaerobic fermentation of organic substrates, animals or vegetal, is a renewable energy that requires a growing interest. The exploitation of this energy has indeed many advantages in economic and environmental terms.

However this technology is still very little known in Morocco by the institutions, policy makers and private actors (managers, engineering, personnel);

In this study we make a cartographic presentation of the density of livestock waste in the various regions of Morocco, we also present an estimation of the potential energy that can be produced by methane fermentation.

Our study shows that across the different regions of Morocco, we are able to recover more than 87 million tons of fermentable organic waste and residues of livestock manure and poultry this is equivalent to more than 4.5 MTep / year. An integrated energy policy taking into account this factor is necessary for optimal use of this renewable energy source.

The advantages of this technology and applications will be discussed in view of the wide dissemination in rural areas.

Keywords : biomass, energy, methanation, cartography, organic waste.

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I. INTRODUCTION

Morocco as a developing country, and not a producer of fossil fuels, is subjected to a strong energy constraint illustrated by an energy deficit that has worsened over time; it was about 73% in 1970 rising to 83% in 1980 and is currently at 97% [1, 2].

Against this backdrop, Morocco seeks other energy resources more cleaner, such as renewable energies. Among these sources, biogas technology, that is renewable, non-polluting and can be applied on the farm as well as in the food industry or in municipal landfills

Currently, a considerable number of biogas installations of different types and sizes are operational in several countries.

In this context, our study aims to cite the advantages and applications of biogas technology, to give an assessment of national potential for the development of this technology, and to enhance energy performance of manure waste in particular.

II. THE BIOGAS

Biogas is a mixture containing mainly methane (50-70%), carbon dioxide, water of the hydrogen sulfide. Due to a high concentration of methane, biogas is very rich in energy ($> 7 \text{ kWh / m}^3$).

Biogas is produced by methanation (also called "anaerobic digestion") is the transformation of the organic matter into biogas by microbial consortium operating in the absence of oxygen [3].

The composition of biogas and its quality depend on the nature of the organic matter used and the fermentation conditions. In general, the total fermentation time varies between 35 and 60 days [4].

The exploitation of this energy resource indeed presents many advantages:

- **Energetically:** biogas is an energy resource that can be exploited in various ways:
 - As fuel for heating or hot water
 - As fuel to generate electricity
 - As an alternative fuel in vehicles.
- **Environmentally:** Biogas production is a way to valorise organic waste, thus avoiding pollution and nuisances with a significant reduction of CO₂ and CH₄ emissions (GHG), in addition, the residual

products from this fermentation may be used for the production of compost fertilizer in agriculture.

- **Economically:** Biogas presents advantages at local level as additional activity for the farmers who can develop economically and energetically their agricultural waste (vegetal or animal source) and the introduction of this technology will contribute to sustainable development and economic autonomy for biogas producers.
- **Technically:** biogas technology seems simple and can be easily installed on a small scale for a better adaptation to the national context, as is the case in several countries (China, India, Senegal) especially during the last thirty years.

III. THE ENERGETIC POTENTIAL OF LIVESTOCK WASTES IN MOROCCO

The study of potential biogas will contribute to the awareness of the public concerned and would draw attention to this very promising new energy, at both economically and ecologically level.

It is noteworthy that a detailed cartography of solar and wind potentials is available. However, few studies on the potential of biomass have been developed. In this context, we focus on agricultural biomass, urban biomass and agribusiness. Our goal is to estimate the amount of this resource in Morocco and its energy potential of biogas and translate the results into a mapping on the scale of Morocco.

IV. MATERIALS AND METHODS

The general method for estimating the biomass potential involves estimating the maximum quantity or theoretical and adjust it to reflect the performance of recoveries. [2].

The available potential is expressed, in general, according to the formula below,

$$aB \text{ (t / year)} = tB \text{ (t / year)} \cdot f$$

aB = potential in organic waste (available biomass)

tB = the set of organic waste produced (theoretical biomass)

f = percent recovery (rate available for methane fermentation), varies depending on the substrate and the site.

a) Livestock waste potential

The potential production in manure is estimated according to official figures published in the Statistical Yearbook and the journal of the poultry sector concerning the number of head in different herds and the yield per year for each waste type [5, 6].

$$tB = N_h \times Y_w$$

N_t = number of heads

Y_w = waste in ton / head / year

b) Slaughterhouse Waste

$$Q_{sw} = a_w \times f_a \times N_h$$

Q_{sw}: quantity of slaughterhouse waste (t / year)

N_h = number of heads

a_w: average weight of the animal

f_a: fraction of waste (liquid and solid) depending on the type of animal (14% cattle, sheep, goats, poultry 35%.

c) Estimation of the energetic potential

The total energetic potential, by type of waste, is given by:

$$Pe_a = P_d \times P_b \times P_{CI} \times R_t$$

Pe_a = energy potential of waste "a" in kWh / year

P_d = potential in available waste "a" tons / year

P_b = potential in biogas waste "a" m³/ton of fresh matter for each type of waste [7, 8, 9, 10]

P_{CI} = based on methane % of the biogas considered in this study as 60% [1m³ of Biogas = 6 kWh
1Tep = 11628KWh

R_t = for electrical conversion = 1.7 kWh / m³ biogas and 2 kWh / m³ biogas in the case of thermal combustion

V. RESULTS

a) Livestock Waste

Our study shows that in 2007 on the scale of different regions of Morocco, we were able to recover more than 87 million tons per year, which could generate biogas with an energy value more than 4.5 Mtep / year.

It should be noted that this potential has been estimated by assuming that the animals spend 24 hours in the breeding local and this is equivalent to a coefficient of manure recovery of 100%. In our study of northern Morocco [2] we considered the time spent outside the local livestock at 12 hours. The recovery coefficient is therefore adjusted to 50% except in the case of poultry, this factor is maintained at 100% [2].

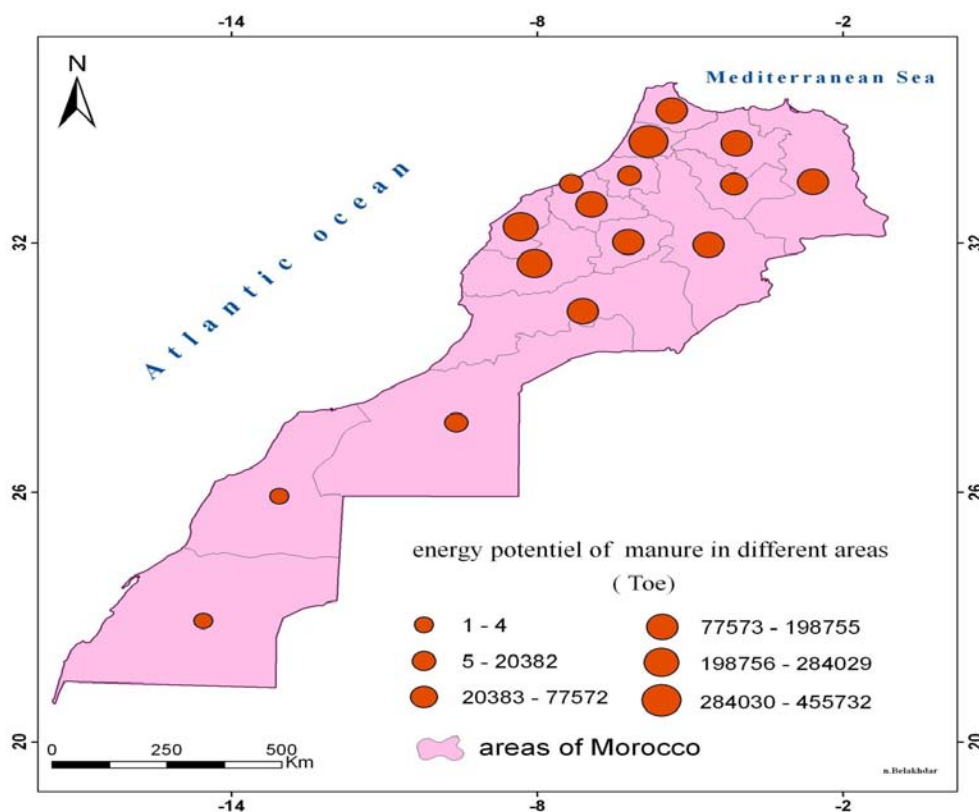
The map below illustrates the geographical distribution of biomass potential and its potential energy in Tep, at the different regions of Morocco, by making use of software Arc Gis (9 .3) to edit the versions map 1: 100 000.

VI. CONCLUSION

The management of these resources must be part of an integrated energy policy taking into account the energy needs for rural development.

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Effect of Varying NDF, ADF and Digestible Energy Levels on Growth Performance, Nutrient Digestibility, Caecal Fermentation, Caecal and Faecal Microflora of Growing Rabbits

By S.O. Osho, A.O. Oso, I.E. Akpan, T.A. Ayanniyi, A.O. Fafiolu, A.V. Jegede, O.A. Isah, R.Y. Aderinboye, P. Dele, V.O.A. Ojo, I.M. Ogunade, S.O. Durosaro, D.A. Ekunseitan, A.A. Ayoola & O.M.O. Idowu.

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Abstract - A total of 135 weaner rabbits were used in a 70 days feeding trial to investigate the effect of Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and digestible energy (DE) levels on growth performance, nutrient digestibility, caecal fermentation, caecal and faecal microflora. The experiment was laid out in a 3×3 factorial arrangement of 3 levels of fibre components (low, optimum and high) and 3 levels of DE (low, optimum and high). There were 9 dietary treatments with 15 rabbits allotted to each diet. Final liveweight ($P < 0.01$) and weight gain ($P < 0.05$) of rabbits increased with increasing dietary fibre.

Keywords : growing rabbits, dietary fibre, caecal fermentation, total bacterial count, lactobacillus count.

GJSFR-C Classification : FOR Code : 970106, 279999



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Effect of Varying NDF, ADF and Digestible Energy Levels on Growth Performance, Nutrient Digestibility, Caecal Fermentation, Caecal and Faecal Microflora of Growing Rabbits

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Keywords : growing rabbits, dietary fibre, caecal fermentation, total bacterial count, lactobacillus count.

I. INTRODUCTION

Fibre is regarded as one of the main constituents of rabbit feed ranging from 150 g/kg to 500 g/kg (Gidenne, 2003). A dietary fibre level of 335 - 400 g/kg NDF, 170 g/kg ADF (De Blas and Mateus, 1998) and a daily intake of 6 g lignin (Dalle Zotte, 2002) were recommended for a growing rabbit. In rabbit nutrition, to formulate a ration that is balanced in fibre mostly leads to a compromise in energy. This is because fibrous ingredients are mostly characterized with low energy

(Gidenne *et al.*, 2000). Excessive fibre intake has been reported to cause reduced feed efficiency and poor nutrient digestibility (Nicodemus *et al.*, 1999). On the other hand, intake of high energy diets (mostly at the expense of fibre) resulted in incidence of digestive disturbances, loss of appetite and reduced feed conversion ratio (Bennegadia *et al.*, 2001). An appropriate balance between dietary fibre and energy is therefore crucial to optimal growth and health of rabbits. Dietary fibre level were reported in previous studies to affect growth (Tao and Li, 2006), nutrient digestibility, caecal fermentation, fibrolytic activities (Falcao-e-Cunha *et al.*, 2004) and gut microflora population (Fortun-Lamothe and Boullier, 2007). The gut microbial activity determines the concentration of volatile fatty acids (VFA) generated in the rabbit caecum (Bellier and Gidenne, 1996). Intake of fibrous diet was reported to affect gut microflora count in rabbits (Oso *et al.*, 2011) and lead to high bacterial shedding in the faeces of pigs (Chen *et al.*, 1998). Research studies aimed at investigating the interactive effect of fibre components and digestible energy (DE) levels on caecal fermentation, gut and faecal microflora of growing rabbits are rare. This study therefore seeks to investigate the main effect, interaction effect of varying fibre components (NDF, ADF) and digestible energy on growth performance, nutrient digestibility, caecal fermentation, gut and faecal microflora of growing rabbits.

II. MATERIALS AND METHODS

a) Experimental diets and Management

The study was carried out at the Rabbit Unit of the Teaching and Research Farm of the University of Agriculture, Abeokuta, Nigeria, West Africa. It is located 76 m above sea level and falls within latitude 7°15' North and longitude 3°21' East. Nine experimental diets were formulated in a 3 × 3 factorial arrangements having three levels of fibre components {low (249 - 258 g/kg NDF, 149 - 157 g/kg ADF), optimum (349 - 381 g/kg NDF, 188 - 193 g/kg ADF) and high (430 - 456 g/kg NDF, 249 - 253 g/kg ADF)}, and in each case with three

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DE levels {low (8 – 8.5 MJ/kg), optimum (10.5 – 11 MJ/kg) and high (12 – 12.30 MJ/kg)} (Table 1). Dietary NDF and ADF levels regarded as low (Gidenne *et al.*, 2004; Pinheiro *et al.*, 2009) and optimum (De Blas and Mateous, 1998; Chao and Li, 2008) in the current study were as reported in previous findings. Digestible energy levels of diets similar or close to values recommended for maximum rabbit meat production was considered as optimum (INRA, 1989; Lebas, 1991).

b) Growth response and metabolic trial

A total of one hundred and thirty five, 5 weeks old, male weaner rabbits, of mixed breeds with a mean body weight of 448.14 ± 16.05 (SEM) g were randomly allotted to 9 dietary groups of 15 rabbits each. The feeding trial lasted for 70 days. A total of 27 fabricated wired rabbit hutches (each of dimension 200 cm × 80 cm × 80 cm (LWH)) were used in all for the study. Each hutch was partitioned into 5 equal cage units. A total of 3 hutches were assigned to a treatment. Feed intake and live weight were recorded per rabbit housed in each cage unit. The total feed intake, weight gain and the feed to gain ratio were also computed. At the end of the feeding trial, 6 rabbits per treatment were randomly selected and housed in separate metabolic cages to measure apparent nutrient digestibility. The rabbits were acclimatized for 3 days prior to the commencement of the trial. Selected rabbits were fed with diet at quantity which matched their previous mean daily intake. Sample collection was done for 4 days. Excreta (free of feed particles and other contaminants) were collected daily from each cage and dried overnight (at 60°C for 12 hours). Feed samples and dried excreta samples (n=5 per treatment) were analyzed for proximate constituents according to the Official Methods of Association of Analytical Chemists (AOAC 1990). The fibre fraction (NDF, ADF, ADL) of the samples were determined as described by Van Soest *et al.* (1991). The gross energy of the samples was determined using the ballistic bomb calorimeter.

c) Caecal Fermentation

At 70 days of age, 6 rabbits were selected at random without prior fasting from each treatment and slaughtered between 5–7 hours after the beginning of daily light period for the collection of caecal content. About 10g of caecum content was collected from each rabbit. The caecal content collected was divided into two, half was immediately used for caecal fermentation while the rest used for microbial analysis. The caecal content meant for microbial analysis were placed under a stream of CO₂, stored in separate plastic bottles and immediately transported to the laboratory for microflora count. For the determination of VFA concentration, a solution of 5% orthophosphoric acid (v/v) plus 1% mercury chloride (w/v) was added to the caecal content samples. Volatile fatty acids were measured by gas chromatography, with a gas column: free fatty acids and

phenols 10% H₃PO₄, 1% acid-washed chromosorb W, 100 - 120 mesh. The carrier gas was N₂ with a flow rate of 40 ml/min: H₂ and air flows to the detector were 60 ml/min. Injector and detector temperatures were 250°C while the oven temperature was 150°C.

d) Faecal and caecal microbial count

At 60 days of age, light plastic collars were put around the neck of 6 rabbits randomly selected from each treatment at 08:00. These collars were taken out after 2 set samples of 4 - 5 g of ceacotrophs had been collected per rabbit. The fresh ceacotroph samples collected were placed under a stream of CO₂, stored in separate plastic bottles for microflora count. Microbial counts of the faecal sample and caecal content were determined according to the methods of Xia *et al.* (2004). Approximately 1 g of fresh sample (faecal and caecal content) was dispersed in 9 ml phosphate buffered saline solution supplemented with 0.5 g/l Cysteine-hydrochloride, thoroughly mixed and further diluted to factor 10⁻⁸. From this material, each 0.1 ml was spread on sterile Petri dishes containing selective media and incubated for analysis of total microbial colony count using the Plate-count agar incubated at 30°C, for 72 hours, Coliform (ES agar, 37°C, 24 hours, Merck, Darmstadt), lactobacilli (MRS-agar 37°C, 72 hours, Merck, Darmstadt), Clostridia (Wilkins-Chalgren Agar + Novobiocin (8 mg/l) + Colistin Sulfate (8 mg/l), 37°C, 72 hours). Colony forming units (CFU) were counted. Colony counts were calculated on the base of 1 g of sample and then transformed as log₁₀ of viable bacteria per gram of fresh matter.

e) Statistical Analysis

Data obtained were analysed using SAS (2003) Software Package. Analysis was done to determine the main effects of fibre levels, digestible energy levels and combined interaction effect of dietary fibre and digestible energy levels. Microbial counts initially expressed as coliform units per gram (CfU/g) were transformed into logarithm (log₁₀). Mean values showing significant ($P < 0.05$) differences in the ANOVA were compared using Duncan's multiple range test as described by Duncan (1955).

III. RESULTS

a) Growth response and apparent nutrient digestibility

The main effect of dietary fibre and DE level on growth response and apparent nutrient digestibility of the rabbits is shown in Table 2. Final liveweight ($P < 0.01$) and weight gain ($P < 0.05$) increased with increasing dietary fibre level. Rabbits fed with high fibre recorded the highest feed intake ($P < 0.05$), final liveweight ($P < 0.01$) and weight gain ($P < 0.05$). However, rabbits fed with low fibre had the worst ($P < 0.05$) feed to gain ratio. Highest ($P < 0.05$) apparent dry matter, ether extract digestibility, NDF and ADF digestibility were

obtained with rabbits fed diet containing optimum fibre. Main effect of digestible energy level showed highest ($P<0.05$) weight gain with rabbits fed with high DE levels. Rabbits fed with low DE had the least ($P<0.05$) apparent dry matter, ash, crude protein, NDF and ADF digestibility. Rabbit fed optimum digestible energy diets recorded the highest ($P<0.05$) apparent dry matter, ash, crude protein, ether extract, NDF and ADF digestibility values. Interaction of dietary fibre and DE levels showed highest final liveweight ($P<0.001$), weight gain ($P<0.001$) and feed intake ($P<0.05$) with rabbits fed high fibre + low DE (Table 3). Rabbits fed diet containing low fibre + low DE had the least ($P<0.001$) final liveweight, weight gain, apparent dry matter and ADF digestibility. Highest ($P<0.05$) apparent NDF digestibility was obtained with rabbits fed diets containing optimum fibre + optimum DE while highest ($P<0.001$) ADF digestibility obtained with rabbits fed with high fibre + high DE.

b) *Ceacal fermentation, caecal and faecal microflora*

The main effect of dietary fibre and DE level on caecal fermentation, caecal and faecal microflora of rabbits is as shown in Table 4. The concentration of total VFA and acetic acid increased ($P<0.05$) with increasing dietary fibre level. Rabbits fed with low DE recorded the least ($P<0.05$) total volatile fatty acid. Main effect of dietary fibre also showed that *Lactobacillus* counts of the caecum content increased ($P<0.01$) with increasing level of dietary fibre. Rabbit fed high fibre diets recorded the highest caecal ($P<0.01$) *lactobacillus* and total bacterial count. The least faecal ($P<0.05$) *lactobacillus* count was obtained from caecotroph samples of rabbits fed low fibre diets. The faecal samples of rabbits fed with low DE diets also showed the highest ($P<0.05$) *lactobacillus* count.

Interaction effect of dietary fibre and digestible energy level on caecal fermentation, caecal and faecal microfloral of rabbits is shown in Table 5. Irrespective of the DE level of the diets, all rabbits fed high fibre diets showed the highest ($P<0.05$) total VFA, acetic acid concentration and high ($P<0.01$) caecal total bacterial counts. Rabbits fed low fibre + high DE and those fed high fibre + high DE showed similar *lactobacillus* count in the caecal content. Rabbit fed low fibre + optimum DE, low fibre + high DE, optimum fibre + optimum DE and high fibre + high DE showed no presence of *Lactobacillus* spp.

IV. DISCUSSION

Improved growth response obtained in the current study with rabbits fed diet containing high fibre + low DE agreed with previous findings that rabbits can be sustained on high fibre diets (De Blas *et al.*, 1995; Tao and Li, 2006). The findings of this study also agreed with Pinheiro *et al.* (2009) who reported improved growth

performance with growing rabbits fed diets containing 233 g/kg ADF and 73 g/kg ADL. Improved feed conversion ratio obtained with rabbits fed high fibre + low DE diet also agreed with the report of Nguyen and Nguyen (2008) who concluded that dietary inclusion of up to 410 g/kg NDF in rabbits gave higher growth rates and better benefits for rabbit producers. Highest feed intake obtained with rabbits fed high fibre + low DE diet agreed with previous findings that rabbits have the ability to adjust its voluntary intake in response to changes in dietary energy concentration (Patridge *et al.*, 1989). Rabbits fed high fibre diets tend to consume more to meet its energy requirement. Poor growth noticed with rabbits fed low fibre diets in this study agreed with previous findings (Bennegadia *et al.*, 2001).

The improved apparent dry matter, ash and NDF digestibility obtained with rabbits fed diet containing optimum fibre + optimum DE could be due to the fact that dietary fibre and DE at this level lied within the recommended level (Lebas, 1991; Gidenne *et al.*, 2001). The proportion of the low digestible fibre (cellulose, lignin) and high digestible fibre (hemicellulose) at this level was also considered to be adequate for growing rabbits (Gidenne and Perez, 2003). The degree of lignification (Gidenne and Perez, 1994) and lignocellulose ratio (Gidenne *et al.*, 2001) of the diet containing optimum fibre + optimum DE diet were also adequate for growing rabbits and could be contributory to the improved nutrient digestibility obtained.

Volatile fatty acids (VFAs) produced as a result of caecal fermentation of digestible fibre or undigested nutrients provide a vital source of energy for the rabbits (Nicodemus *et al.*, 1999). High total VFA and acetic acid concentration generated from the caecum content of rabbits fed high fibre diets resulted from greater quantity of fibre fermentation (Chao and Li, 2008). Gidenne *et al.*, (2000) reported that the concentration of caecal total VFA and proportion of acetic acid produced increased as the dietary fibre level is enhanced. Tao and Li (2006) also confirmed that fibrolytic activities in the caecum of growing rabbits increased as the dietary NDF increased resulting in higher production of energy. Increased fibrolytic activities have been reported to improve growth and the health of the rabbits as Peters *et al.* (1995) found a high caecal VFA in rabbits resulting in a protective effect against experimental enteropathogenic *E. coli* infection.

Bacteria constituted the most dominant of the gut microbiota in rabbits (Boulharouf *et al.*, 1991). A stable gut microflora is reported to be responsible for increased caecal fermentation, improved growth and reduced digestive disorders (Gidenne, 1996). High caecal *lactobacillus* and total bacterial count obtained from rabbits fed high fibre diets could be linked with the NDF level and botanical source (Belenguer *et al.*, 2000).

Dietary NDF was reported as one of the most crucial factors regulating microbial activity within the caecum of rabbits (De Blas *et al.*, 1999). Increased lactobacillus and bacterial count obtained with high fibre intake favoured high fibrolytic activity (Gidenne *et al.*, 2004) and improved the health status of rabbits (Debray *et al.*, 2002; Oso *et al.*, 2011). Previous findings also reported increased fibrolytic activity of caecal bacteria when dietary crude fibre increased from 110 g/kg to 170 g/kg in rabbits (Boulahrouf *et al.*, 1991). However, low enteric microbial population was reported to be implicative of impaired growth of animals (Davis *et al.*, 2003).

High lactobacillus count obtained from the faecal samples of rabbits fed high fibre diets resulted from high digesta passage rate created as a result of high fibre intake. High fibre intake lowers the duration of contact between digesta and the intestinal bacteria for effective digestion hence more of the bacteria will be shed in the faeces. Excessively fibrous diets have been reported to reduce microbial activity by increasing the digesta transit rate (Robertson, 1988).

Table 1 : Gross composition of experimental diets

Fibre level	Low			Optimum			High		
Digestible energy level	Low	Optimum	High	Low	Optimum	High	Low	Optimum	High
Maize	225	190	130	235	195	135	204	164	124
Maize starch	90	110	145	90	115	140	80	100	120
Vegetable oil	5	20	45	5	20	55	10	30	50
wheat offal	230	230	230	140	140	140	60	60	60
Rice bran	90	90	90	130	130	130	195	195	195
Rice husk	60	60	60	100	100	100	151	151	151
Fixed ingredients including vitamin/mineral premix ^a	300	300	300	300	300	300	300	300	300
Determined composition (in g/kg)									
Dry matter	901.4	910.4	901.1	909.2	899.7	908.8	906.2	900.9	899.7
Organic matter	898.8	897.0	898.5	901.1	890.2	889.5	901.8	892.2	900.5
Crude ash	91.20	92.50	94.50	88.90	90.40	88.80	86.20	84.20	90.60
Crude protein									
NDF	257.71	252.11	249.70	380.11	370.79	350.22	445.70	440.41	431.24
ADF	157.00	154.20	149.70	192.40	189.61	188.00	252.10	250.09	249.44
ADL	46.67	46.10	45.82	56.40	55.91	55.34	71.90	69.71	67.82
Gross energy (MJ/Kg)	12.03	16.09	17.62	12.67	16.95	19.13	14.19	18.56	21.29
Digestible energy (MJ/Kg)	8.17	10.97	12.11	8.06	10.84	12.27	8.01	10.52	12.09
Ca	12.90	13.00	12.55	13.00	12.72	13.10	13.00	14.10	13.90
P	8.10	7.99	7.55	8.00	9.55	8.95	8.20	8.00	8.11

^a Contained : 250 g/kg soyabean meal (440 g/kg crude protein), 25.0 g/kg bone meal, 15.0 g/kg oyster shell, 2.0 g/kg lysine- HCL, 2.0 g/kg DL- methionine, 3.0 g/kg salt (NaCl) and 3.0 g/kg vitamin/mineral premix (providing the following per kg of diet 0.04g manganese, 0.034 g zinc, 0.023 g iron, 0.0026 g copper, 2.48 mg retinol, 0.003 mg cholecalciferol, 5.55 mg riboflavin, 0.70 mg thiamin, 0.70 mg pyridoxine, 2.80 mg niacin, 0.35mg calcium pantothenate and 0.70 mg cyanacobalamin. ^b Calculated using deBlas *et al.*, (1992) ($DE=GE \times (0.867-0.0012ADF)$)

Table 2 : Main effect of dietary fibre and digestible energy levels on growth response and apparent nutrient digestibility of rabbits

Parameters	Main effect of Fibre			Main effect of digestible energy		
	Low	Optimum	High	Low	Optimum	High
Final live weight (g/rabbit)	903.93±24.50 ^c	1087.13±41.90 ^b	1144.83±74.10 ^a	1053.90±43.2	1036.00±42.1 ⁹	1046.00±44.80
Feed Intake (g/rabbit)	1716.67±61.00 ^b	1691.67±35.00 ^c	1749.90±29.70 ^a	1758.33±28.20 ^a	1661.00±52.4 ^c	1738.90±42.70 ^b
Weight gain (g/rabbit)	463.77±25.60 ^c	650.00±19.9 ^b	739.37±34.50 ^a	603.30±23.70 ^b	599.97±23.4 ^b	649.87±46.4 ^a
Feed to gain ratio	3.70±0.13 ^a	2.60±0.16 ^b	2.37±0.27 ^b	2.91±0.39	2.77±0.33	2.68±0.22
Apparent nutrient digestibility (%)						
Dry matter	76.9±3.57 ^b	78.50±2.96 ^a	74.35±1.45 ^c	70.23±2.17 ^c	81.23±2.02 ^a	78.32±2.79 ^b
Ash	64.83±5.43 ^a	65.70±6.62 ^a	58.66±2.69 ^b	52.95±3.15 ^c	69.81±3.35 ^a	66.42±4.56 ^b
Ether extract	91.43±9.98 ^b	94.30±2.86 ^a	92.14±7.63 ^b	89.32±1.56 ^b	94.51±6.35 ^a	66.42±4.56 ^c
Crude protein	81.26±2.20	81.12±3.21	80.21±2.07	78.88±0.67 ^c	82.73±8.67 ^a	80.98±1.15 ^b
NDF	55.12±9.83 ^b	57.88±4.13 ^a	53.31±1.61 ^c	48.96±2.68 ^c	60.36±7.97 ^a	56.99±2.96 ^b
ADF	44.60±7.62 ^b	46.92±4.55 ^a	41.63±2.11 ^c	34.82±3.50 ^c	51.51±5.83 ^a	46.81±4.44 ^b

^{a,b,c} Means on the same row with different superscripts are different significantly ($P<0.05$)

Table 3 : Interaction effect of varying dietary fibre and digestible energy on performance characteristics and apparent nutrient digestibility of rabbits

Fibre level	Low			Optimum			High		
	Low	Optimum	High	Low	Optimum	High	Low	Optimum	High
Digestible energy level									
Final weight (g/rabbit)	811.80±26.60 ^h	850.00±22.43 ^g	1050.00±82.	1125.00±92.	1070.70±86.	1065.70±81.	1224.90±92.1	1187.30±99.69 ^b	1022.30±88.19 ⁱ
Feed Intake (g/rabbit)	1750.00±54.40 ^c	1700.00±44.4	1700.00±63.	1650.00±44.	1700.00±62.	1725.00±48.	1875.00±68.8	1583.00±67.29 ^g	1791.70±66.43 ^b
Weight gain (g/rabbit)	366.30±26.70 ⁱ	412.50±43.30 ^e	612.50±32.1	668.80±25.2	668.60±39.6	612.60±43.3	774.80±37.12 ^h	718.80±33.51 ^b	724.50±28.50
Feed to gain ratio	4.78±0.83 ^a	4.12±0.41 ^a	2.78±0.37 ^b	2.47±0.09 ^b	2.54±0.13 ^b	2.82±0.10 ^b	2.42±0.39 ^b	2.20±0.49 ^b	2.47±0.44 ^b
Apparent nutrient digestibility (%)									
Dry matter	63.40±1.98 ^e	79.70±0.91 ^b	87.67±0.21 ^a	78.30±1.99 ^b	88.87±1.01 ^a	68.34±1.01 ^d	69.00±2.01 ^d	75.11±0.90 ^c	78.95±0.72 ^b
Ash	44.38±0.78 ^e	68.67±0.77 ^b	81.43±0.85 ^a	65.23±1.00 ^c	81.94±2.20 ^a	49.94±1.02 ^d	49.23±1.03 ^d	38.82±0.99 ^f	67.90±3.01 ^b
Ether extract	83.67±2.01	77.88±3.01	86.62±1.93	78.44±2.77	97.22±4.01	91.25±5.03	89.85±3.02	92.36±4.04	94.22±2.20
Crude protein	76.64±2.96	82.28±2.09	84.85±4.03	81.24±2.05	85.25±3.03	76.88±2.01	78.77±1.99	80.67±2.44	81.20±2.55
NDF	40.85±2.22 ^e	57.88±3.01 ^b	46.62±2.00 ^d	58.78±2.33 ^b	68.08±1.55 ^a	46.57±1.75 ^d	47.04±1.99 ^d	55.11±2.02 ^d	59.77±3.01 ^b
ADF	23.34±0.76 ^g	48.85±0.89 ^c	41.60±0.72 ^d	47.49±1.59 ^c	62.39±1.51 ^b	30.88±0.88 ^f	33.64±0.21 ^e	42.30±0.78 ^d	67.96±2.02 ^a

^{a,b,c,d,e,f,g} Means on the same row with different superscripts are different significantly ($P<0.05$)

Table 4 : Main effect of varying dietary fibre and digestible energy on caecal fermentation, caecal and faecal microflora of rabbits

Parameters	Main effect of fibre			Main effect of digestible energy		
	Low	Optimum	High	Low	Optimum	High
Caecal VFA (mmol/100ml)						
Total	4.53±0.12 ^c	6.33±0.19 ^b	8.17±0.97 ^a	5.80±0.22 ^b	6.60±0.59 ^a	6.63±0.26 ^a
Acetic	2.96±0.07 ^c	4.50±0.19 ^b	5.70±0.45 ^a	3.97±0.92	4.67±0.67	4.50±0.64
Propionic	0.33±0.09	0.53±0.01	0.70±0.04	0.50±0.09	0.57±0.05	0.53±0.19
Butyric	0.73±0.02	0.96±0.01	1.13±0.09	0.83±0.06	1.07±0.07	0.93±0.01
Caecal microflora (log₁₀)						
Coliform	9.10±0.11	8.99±0.01	8.66±0.24	8.99±0.1	8.76±0.27	8.99±0.01
Clostridium	8.72±0.01	8.72±0.03	8.69±0.01	8.67±0.02	8.75±0.02	8.73±0.01
Lactobacillus	1.99±0.99 ^c	4.26±1.07 ^b	6.40±0.40 ^a	4.30±1.08	2.20±1.10	6.15±0.08
Total bacterial count	10.02±2.44 ^b	10.39±2.66 ^b	12.34±2.95 ^a	10.60±1.96	10.90±1.18	11.24±2.22
Faecal microflora (log₁₀)						
Coliform	7.97±0.99	8.93±0.03	8.96±0.80	8.96±0.80	8.96±0.60	7.93±0.99
Clostridium	8.65±0.02	8.60±0.08	8.62±0.04	8.68±0.01	8.59±1.08	8.59±0.04
Lactobacillus	2.20±0.10 ^b	3.99±0.11 ^a	3.60±0.14 ^a	5.53±1.71 ^a	2.16±0.82 ^b	2.10±0.105 ^b
Total bacterial count	10.09±1.10	10.11±0.98	10.79±1.09	10.29±0.74	10.21±0.78	10.48±1.25

^{a,b,c} Means on the same row with different superscripts are different significantly ($P<0.05$)

Table 5 : Interaction effect of varying dietary fibre and digestible energy on caecal fermentation, caecal and faecal microflora of rabbits

Fibre level	Low			Optimum			High		
Digestible energy level	Low	Optimum	High	Low	Optimum	High	Low	Optimum	High
Caecal VFA									
Total (mmol/100ml)	4.20±0.67 ^d	4.60±0.29 ^c	4.80±0.91 ^f	5.00±0.62 ^c	6.80±0.16 ^b	7.20±0.71 ^b	8.20±0.93 ^a	8.40±0.74 ^a	7.90±0.90 ^a
Acetic (mmol/100ml)	2.70±0.83 ^c	3.20±0.14 ^c	3.00±0.99 ^c	3.50±0.66 ^c	4.90±0.67 ^b	5.10±0.73 ^b	5.70±0.24 ^a	5.90±0.88 ^a	5.50±0.41 ^a
Propionic (mmol/100ml)	0.30±0.05	0.40±0.09	0.40±0.07	0.50±0.08	0.50±0.05	0.60±0.07	0.70±0.04	0.80±0.03	0.60±0.05
Butyric (mmol/100ml)	0.70±0.01	0.70±0.09	0.80±0.06	0.70±0.09	1.20±0.02	1.00±0.09	1.10±0.29	1.30±0.61	1.00±0.03
Caecal microflora									
Colliform (log ₁₀)	8.99±0.09	9.30±0.12	8.99±0.07	8.99±0.05	8.99±0.02	8.99±0.01	8.99±0.11	8.00±0.10	8.99±0.07
Clostridium (log ₁₀)	8.70±0.12	8.77±0.10	8.70±0.19	8.61±0.11	8.78±0.07	8.77±0.22	8.69±0.91	8.69±0.19	8.70±0.10
Lactobacillus (log ₁₀)	0±0.00 ^c	0±0.00 ^c	5.99±0.12 ^b	6.30±0.17 ^b	0±0.00 ^c	6.48±0.15 ^a	6.60±0.19 ^a	6.60±0.39 ^a	5.99±0.22 ^b
TBC (log ₁₀)	10.04±1.44 ^c	10.02±0.96 ^c	10.00±0.77 ^c	10.09±0.94 ^c	10.01±1.22 ^c	11.02±0.84	11.67±0.95 ^{ab}	12.65±1.92 ^{ab}	12.70±1.88 ^a
Faecal microflora									
Colliform (log ₁₀)	8.96±0.93	8.96±0.77	5.98±0.89	8.96±0.79	8.97±0.66	8.96±0.65	8.97±1.02	8.96±0.97	8.96±0.88
Clostridium (log ₁₀)	8.71±1.09	8.61±0.99	8.61±0.90	8.63±0.88	8.46±0.29	8.70±0.44	8.70±0.69	8.70±0.90	8.70±0.99
Lactobacillus (log ₁₀)	6.61±0.85 ^a	0±0.00 ^d	0±0.00 ^d	5.66±0.90 ^b	0±0.00 ^d	6.30±0.45 ^a	4.33±0.29 ^c	6.48±0.22 ^a	0±0.00 ^d
TBC (log ₁₀)	10.00±0.94	10.12±1.09	10.02±0.77	10.10±0.90	10.00±0.60	10.22±0.89	10.67±0.90	10.50±0.77	11.20±1.01

a,b,c,d Means on the same row with different superscripts are different significantly (P<0.05)

V. CONCLUSION

High fibre diets (containing low or optimum DE) in rabbit nutrition improved growth performance, nutrient digestibility, increased caecal fermentation and favoured high caecal lactobacillus and total bacterial count. Rabbits fed diets containing high fibre + low DE showed the highest weight gain, improved feed to gain ratio and caecal fermentation.

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Leaching of Bifenthrin in two Different Types of Soil under Laboratory Condition

By Sachin Kumari, Ramprakash & Beena Kumari

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Abstract - Leaching studies of bifenthrin in two different soils were carried out under laboratory conditions during October- November 2011. Commercial formulation of bifenthrin available as Talstar 10 % EC were applied at two treatment levels i.e. 25 and 50 μg as single and double dose, respectively. More than 90 per cent of bifenthrin were retained in 5 cm soil section in sandy loam and clay loam soil which shows the immobility of this pyrethroid insecticide in soil. No residues were detected in the leachate fractions of the insecticide.

Keywords : *bifenthrin. residues. persistence. soil.*

GJSFR-C Classification : *FOR Code: 300102*



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Leaching of Bifenthrin in two Different Types of Soil under Laboratory Condition

Sachin Kumari ^α, Ramprakash ^σ & Beena Kumari ^ρ

Abstract - Leaching studies of bifenthrin in two different soils were carried out under laboratory conditions during October-November 2011. Commercial formulation of bifenthrin available as Talstar 10 % EC were applied at two treatment levels i.e. 25 and 50 µg as single and double dose, respectively. More than 90 per cent of bifenthrin were retained in 5 cm soil section in sandy loam and clay loam soil which shows the immobility of this pyrethroid insecticide in soil. No residues were detected in the leachate fractions of the insecticide

Keywords : bifenthrin. residues. persistence. soil.

I. INTRODUCTION

Over use of pesticide is the contamination of soil and water sources including the aquatic system. Several scientists reported effect of pesticides in soil (Frampton et al. 2006; Coupe et al. 2005; Willian and Mueller 1994; Balasubramanya and Patil 1980). Bifenthrin (2-Methyl-3-phenyl phenyl methyl (1*S*, 3*S*)-3-(*Z*)-2-chloro-3, 3, 3-trifluoroprop-1-enyl 2, 2-dimethylcyclopropane-1-carboxylate; Fig. 1) is a third generation pyrethroid. It is used in agriculture, public health and forestry. Pesticides may have potential to bind to soil, the extent of which depends greatly on the nature of the chemical used. Therefore it has been observed that the physico-chemical nature of the soil is important for the persistence, metabolism and binding of pesticides in soil. They include sorption to soil constituents, movement of the soil in runoff, movement into the air by volatilization, movement downward in the soil by leaching, and movement upward in the soil through the soil by leaching, and movement upward in the soil through capillary forces. The main processes potentially affecting the ultimate fate of pesticides in soil are retention by soil materials (involving adsorption/desorption processes), transformation processes (biological and chemical degradation), and transport (through soil, atmosphere, surface water, or ground water) (Saltzman and Yaron 1986; van der Hoff and van Zoonen 1999).

The present study was undertaken to determine the leaching of bifenthrin in two different soil at different doses under laboratory condition.

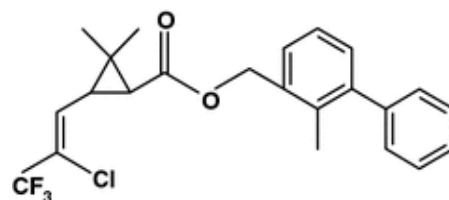


Figure 1 : Chemical structure of bifenthrin

II. MATERIALS AND METHODS

Leaching studies were carried out in sandy loam and clay loam soil. Soil were collected from Haryana. The physico- chemical properties of the soil are presented in Table 1 and 2.

For laboratory experiment, plexi glass columns (90 cm x 5 cm i.d) fitted with perforated plexi glass sieve covered with filter paper (Whatmann No.1) at the bottom were used. Each column was filled with soil up to 60 cm height to a uniform bulk density of 1.35 (sandy loam) and 1.40 (clay loam) g cm⁻³ ± 0.1. Weighed amount of different soils 128 g (sandy loam) and 137 g (clay loam) was poured in the different columns each time with the help of a funnel (1 cm i.d) tapped gently from a fixed height and pressed uniformly by a wooden roller. The soil in each column was covered with a filter paper which was topped by a glass wool swab. Soil column so filled were installed vertically on wooden stand with their bottom resting on the fixed perforated sieve, so as to facilitate collection of effluents during leaching.

Leaching studies were carried out in triplicates. Soil (5 g) was fortified with 25 µg (single dose) and 50 µg (double dose) solution of bifenthrin. Added this fortified soil at the top of the column. Cotton swabs prewashed with acetone dried and were placed on top of soil to avoid disturbance of soil. One column packed with soil was kept as control to which no pesticide was added.

Each time 50 mm lot of leaching water (98 ml) was added with the help of a pipette, with the care that no spattering of soil took place. The leaching with the next 50 mm lot of distilled water was done after 24 hrs. After the disappearance of the standing water in the column, leaching was repeated till each leaching cycle of 200, 400 and 600 mm depth was completed. During leaching, the columns were covered with perforated polythene bags to minimize the loss of leaching water due to evaporation. During leaching 2-3 drops of toluene solution was added to each column to check microbial growth in it.

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Leachate oozing out of all the column were collected and processed for the presence of bifenthrin. Total three fraction of leachates were collected. Collect 8 ml (sandy loam) and 5 ml (clay loam) of each fraction. After completion of leaching, intact soil cores were taken out of the plexi glass column. Leachate samples were processed as per method of Kumari et al. (2008).

Leachate sample was taken in 250ml separatory funnel, to which 5g sodium chloride was added.

The solution was partitioned with 15% dichloromethane (DCM) in hexane thrice (30, 20, 10 ml) by shaking vigorously for 1 minute every time. The organic layers were collected and passed through 2-3 cm pad of anhydrous sodium sulphate. The organic layer was evaporated to near dryness on rotary evaporator followed by gas manifold evaporator. The residues were redissolved in n- hexane to make final volume to 2 ml for GC analysis.

Extraction and clean-up of soil sample was carried out as per method of Kumari et al. (2008). Ground, sieved and dry representative (15 g) of soil mixed with charcoal and florisil (0.3 g each) and 10 g of anhydrous sodium sulphate. The mixture was packed compactly in a glass column (60 cm × 22 mm) in between two layers of anhydrous sodium sulphate. Residues were eluted with 100 mL of hexane:acetone (9:1 v/v) at flow rate of 2–3 mL/min. The eluate was concentrated on flash evaporator and made the final volume to 2 mL for GC analysis. Estimation of micro quantities of bifenthrin residues in leachate as well as in soil samples were carried out by GC-ECD system equipped with capillary column.

III. RESULTS AND DISCUSSION

a) Sandy Loam Soil

The data on leaching potential of bifenthrin in sandy loam soil are presented in Table 3 and Fig.2. Total amount of bifenthrin recovered out of 25 and 50 µg were 92 and 95.00 per cent from single and double dose, respectively. Beyond the plough depth (15 cm) of the soil, the amount of bifenthrin could not be detected in any of the doses. None of the leachate fractions from both the treatments showed the presence of bifenthrin residues.

b) Clay Loam Soil

The data on leaching potential of bifenthrin in clay loam soil are presented in Table 4 and Fig.3. Total amount of bifenthrin recovered out of 25 and 50 µg were 96.40 and 98.50 per cent from single and double dose, respectively. Beyond 10 cm depth of soil, the amount of bifenthrin could not be detected in any of the doses. None of the leachate fractions from both the treatments showed the presence of bifenthrin residues.

Similar results were observed by Chauhan et al. (2011) for leaching behaviour of bifenthrin and lambda-

cyhalothrin in sandy loam soil. More than 90 % of bifenthrin as well as lambda-cyhalothrin were retained in 5 cm soil section, which shows their immobility in soil. Both the insecticides restricted up to ploughed soil (15 cm depth) in both the doses.

Manoj and Gajbhiye (2007) reported low mobility of bifenthrin in soil. Its residues remained with in top 15 cm and more than 99 percent of residues were recovered from top 0-10 cm layer. Tariq *et al*, (2006) reported highest concentrations of lambda-cyhalothrin were found in the top 0–10 cm layer.

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Table 1 : Physico-chemical characteristics of sandy loam soil

Soil Property				
Texture	pH	EC _e (dSm ⁻¹)	O.C. (%)	P ₂ O ₅ (kg ha ⁻¹)
Sandy Loam	7.6	2.0	0.32	33

Table 2 : Physico-chemical characteristics of clay loam soil

Soil Property						
Texture	pH	EC _e (dSm ⁻¹)	O.C. (%)	N (kg ha ⁻¹)	P (kg ha ⁻¹)	K (kg ha ⁻¹)
Clayey loam	8.9	0.4	0.36	180	18	140

Table 3 : Leaching behaviour of bifenthrin in sandy loam soil

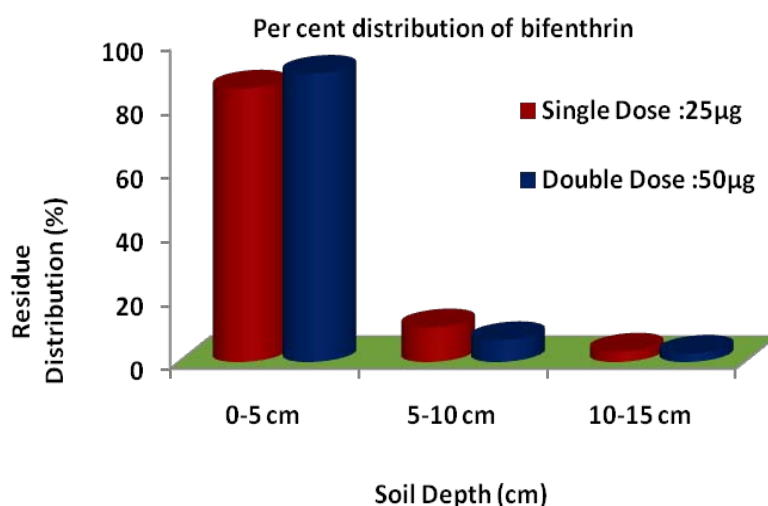
Soil Column Depth (cm)	Residues (μg)*			
	Single dose (25 μg) ±SD	% Retention	Double dose (50 μg) ±SD	% Retention
0-5	19.75±1.1.9	85.86	43.00±3.05	90.52
5-10	2.50±2.30	10.86	3.25±1.64	6.84
10-15	0.75±3.03	3.26	1.25±2.04	2.63

*Average residues of three replicates
Leachate contained no residues

Table 4 : Leaching behaviour of bifenthrin in clay loam soil

Soil Column Depth (cm)	Residues (μg)*			
	Single dose (25 μg) ±SD	% Retention	Double dose (50 μg) ±SD	% Retention
0-5	22.00±2.52	91.28	45.55±3.07	92.48
5-10	2.10±3.41	8.71	3.70±1.11	7.51

*Average residues of three replicates
Leachate contained no residues

**Figure 2 :** Distribution of bifenthrin at different soil depths for two doses in sandy loam soil

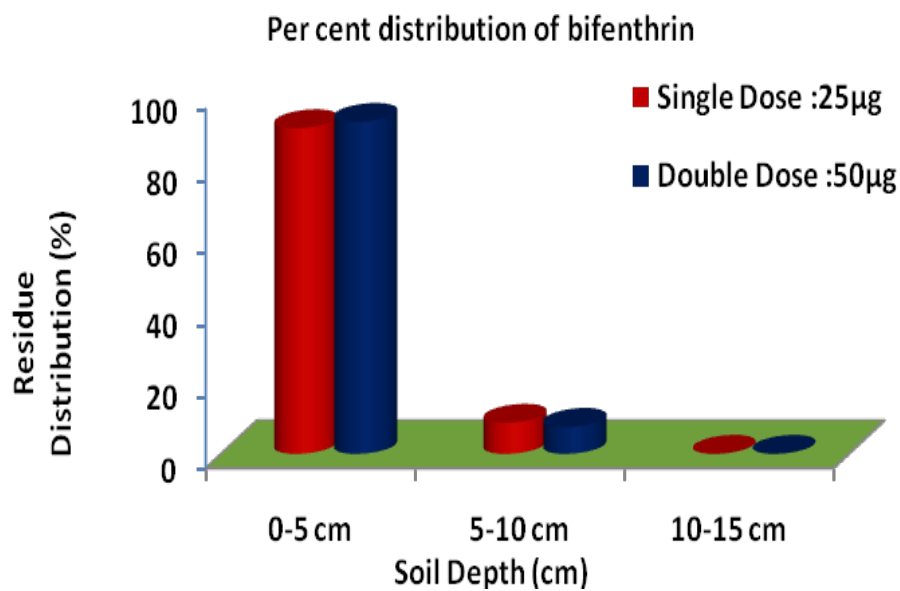


Figure 3 : Distribution of bifenthrin at different soil depths for two doses in clay loam soil



Evaluation of Shoot Fly Resistance through SSR Markers in Sorghum [Sorghum Bicolor (L.) Moench]

By M. K. Choudhary, B. R. Ranwah & V.Saharan

University of Agriculture and Technology

Abstract - The present investigation was carried out with three shoot fly resistant, one having recovery resistance and four susceptible genotypes and their twenty eight crosses. Out of 21 SSR primers used 6 were amplified and gave 12 bands and 327 clear fragments. Out of 12 bands 5 were polymorphic and level of average polymorphism was 41.67 per cent. The most informative band was of Xtxp 88 at 250 bp. The correlation between early vigour, glossiness and trichome density was significant positive. The average value of glossiness ($p=0.00$) and trichome density ($p=0.02$) was significantly low in the genotypes having Xtxp88 250 bp band. Correlation of marker Xtxp88 (250 bp) with glossiness (-0.58) and trichome density (-0.38) was significant and negative. In the stepwise regression analysis only one band Xtxp88 250 bp was entered and explained 33.20 and 14.70 percent variability of glossiness and trichome density, respectively, indicating that the band Xtxp88 250 bp was weakly associated with susceptibility to shoot fly.

Keywords : sorghum, shoot fly, ssr etc.

GJSFR-C Classification : FOR Code: 820404



Strictly as per the compliance and regulations of :



Evaluation of Shoot Fly Resistance through SSR Markers in Sorghum [*Sorghum Bicolor* (L.) Moench]

M. K. Choudhary ^α, B. R. Ranwah ^σ & V.Saharan ^ρ

Abstract - The present investigation was carried out with three shoot fly resistant, one having recovery resistance and four susceptible genotypes and their twenty eight crosses. Out of 21 SSR primers used 6 were amplified and gave 12 bands and 327 clear fragments. Out of 12 bands 5 were polymorphic and level of average polymorphism was 41.67 per cent. The most informative band was of Xtp88 at 250 bp. The correlation between early vigour, glossiness and trichome density was significant positive. The average value of glossiness ($p=0.00$) and trichome density ($p=0.02$) was significantly low in the genotypes having Xtp88 250 bp band. Correlation of marker Xtp88 (250 bp) with glossiness (-0.58) and trichome density (-0.38) was significant and negative. In the stepwise regression analysis only one band Xtp88 250 bp was entered and explained 33.20 and 14.70 percent variability of glossiness and trichome density, respectively, indicating that the band Xtp88 250 bp was weakly associated with susceptibility to shoot fly. There was no specific relationship between Jaccards' similarity coefficient and resistance and susceptibility of parents and crosses. This indicated that most of the polymorphic bands obtained were not related to resistance and susceptibility except Xtp88 250 bp.

Keywords : *sorghum, shoot fly, SSR etc*

I. INTRODUCTION

Sorghum bicolor (L.) Moench is one of the most important crops in the world because of its adaptation to a wide range of ecological conditions, suitability for low input cultivation and diverse uses [6]. It is grown on about 7.93 M ha in India, with an annual grain production of 7.78 Mt (Ministry of Agriculture, 2007-08). During its cultivation, the crop is exposed to several stresses, starting from the seedling stage to harvest, and biotic stresses have the maximum impact on crop growth [4,5]. More than 150 species of insect pests damage the sorghum, of which sorghum shoot fly, *Atherigona soccata* (Rondani), is the most important pest in Africa, Asia and Mediterranean Europe [18] during early stage of crop growth and establishment. Shoot flies of the genus *Atherigona* are also known to cause 'dead hearts' in a number of tropical grass species [16] and wheat [17]. In India, the losses due to shoot fly damage have been estimated to reach as high as 90 percent of grain, and 45 percent of

fodder yield [12]. That leads annual economic losses US\$200 million (ICRISAT, 1992).

Given the economic impact of shoot fly, the improvement of genetic resistance to this pest is one of the major goals in sorghum breeding programs in India. In this context, a better understanding of the inheritance of resistance and identification of genomic regions/QTL that influence resistance can help the breeders to develop more efficient and effective breeding and selection schemes through marker-assisted selection (MAS). Molecular markers have been used for rapid, detailed, and directed genetic manipulation of crop plants, and to identify and characterize quantitative trait loci (QTLs) associated with plant height and days to maturity [15], plant domestication [14], resistance to diseases [10], resistance to insects [1], and tolerance to drought [22]. The advent of PCR based molecular marker techniques has facilitated the analysis of sorghum genome [24]. A high level of genetic variation has been detected among the sorghum accessions, which was high for bicolor, and guinea races and low for kafir race [8]. The SSR primers and linkage map locations have been published for sorghum [2, 3, 13, 19, 20 & 21]. Accordingly present study was undertaken to identify the SSR marker associated with QTL responsible for shoot fly resistance.

II. MATERIALS AND METHODS

In present investigation three shoot fly resistant *viz*, IS 2312, IS 2205 and SUENT 11, one having recovery resistance *viz*, CSV 17 and four susceptible *viz*, 27B, AKMS 14 B, DJ 6514 and CSV 23 genotypes were crossed in diallele fashion during *kharif* 2010 at instructional farm, RCA, Udaipur and during summer 2011 at sorghum research station, Warangal (AP) to obtained the F_1 seed. The parents and F_1 were sown during *kharif* 2011 to score the value of early vigour (1- Plants having minimum vigour, 2- Plants having medium vigour, 3- Plants having maximum vigour), glossiness (1- No glossy (dark green, dull broad and dropping leaves), 2- Medium glossy (medium green and shining Leaves), 3- Highly glossy (light green, shining, narrow and erect leaves) and trichome density (No./mm²) and to obtain the leaves for DNA extraction. These traits were identified for shoot fly resistance by [9]. The genomic

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DNA was isolated from soft leaf tissue using the CTAB method described by [7] and treated with RNase to eliminate RNA. Concentration and quality of DNA was estimated by measuring the optical density at 260 nm and 280 nm using a nano spectrophotometer and the integrity of the purified DNA was verified by visualization of DNA on 0.8 percent Agarose gel with DNA standard uncut lambda DNA.

The PCR amplification reactions for SSR markers were carried out with a total volume of 25 μ l containing 2 μ l of 30 ng of genomic DNA, 1.5 μ l of each forward and reverse primer, 19 μ l of ready to use 1X PCR buffer and 1 μ l of 1 unit *Taq* polymerase. Amplification was performed for 45 cycles (1 min at 94°C, 1 min at 57°C and 1 min at 72°C) followed by a final extension of 10 min at 72°C. Amplified products were separated by 1.2 percent agarose gel electrophoresis.

PCR based SSR analysis was carried out for 28 crosses and eight parents using 21 SSR primers to identify the linked polymorphic markers for component traits of shoot fly resistance. To study the relationship between polymorphic bands and traits independent student t test, correlation and stepwise regression analysis was carried out using SAS 9.2. Similarity coefficient was calculated using following formulae based on Jaccards coefficient [11].

$$S_{ij} = \frac{2 X_{ij}}{X_{ij} + Y_i + Y_j}$$

$$D_{ij} = 1 - S_{ij}$$

Where, S_{ij} = Similarity coefficient between i^{th} and j^{th} genotype.

D_{ij} = Distance coefficient between i^{th} and j^{th} genotype.

X_{ij} = Number of common bands present in both i^{th} and j^{th} genotypes.

Y_i = Number of bands present in i^{th} genotype but absent in j^{th} genotype.

Y_j = Number of bands present in j^{th} genotype but absent in i^{th} genotype.

III. RESULTS AND DISCUSSION

Out of 21 SSR primers 6 were amplified and produced 12 bands and 327 clearly amplified fragments. Five bands were polymorphic lead 41.67 per cent polymorphism. The most informative primers was *Xtxp88* having 64 clear and reproducible amplicons from one monomorphic and one polymorphic bands. The minimum number of amplified amplicons was observed in primer *Xtxp69* and *Xtxp75* i.e. 36 amplicons from one monomorphic band in each.

All the three shoot fly resistance component traits were positively correlated with each other. The correlation of early vigour with glossiness and trichome density was 0.46 and 0.35 and glossiness with trichome density was 0.71 (table 1). These results confirm the findings of [2]. In the genotypes where *Xtxp88* 250 bp band was present, value of glossiness ($p=0.00$) and trichome density ($p=0.02$) was significantly low (table 2). Marker *Xtxp88* (250 bp) showed significant and negative correlation with glossiness (-0.58) and trichome density (-0.38) (table 1) [2,23]. In the stepwise regression analysis only one band *Xtxp88* 250 bp was entered and explained 33.20 and 14.70 percent variability of glossiness and trichome density, respectively, indicating that the band *Xtxp88* 250 bp was associated with susceptibility to shoot fly, whereas [23] reported positive correlation with shoot fly resistance components. But, it explained very low variability.

Table 1 : Correlations between primers and shoot fly resistance component traits

	<i>Xtxp37</i> 300bp	<i>Xtxp88</i> 250bp	<i>Xtxp248</i> 200bp	<i>Xtxp248</i> 250bp	Early Vigour	Glossiness	Trichome Density
<i>Xtxp32</i> 250bp	-0.17	-0.04	0.23	0.07	0.32	0.18	0.25
<i>Xtxp37</i> 300bp		0.06	-0.36*	-0.24	-0.22	0.04	0.19
<i>Xtxp88</i> 250bp			0.16	0.20	-0.21	-0.58**	-0.38**
<i>Xtxp248</i> 200bp				0.44**	0.16	-0.29	-0.28
<i>Xtxp248</i> 250bp					0.17	0.02	-0.13
Early Vigour						0.46**	0.35*
Glossiness							0.71**

*, ** Significant at the 0.05 and 0.01 level, respectively.

Table 2 : Mean comparison of polymorphic bands for shoot fly resistance component traits

S. No.	Band	Characters	Bands		Probability
			Absent	Present	
1.	<i>Xtxp32</i> 250bp	Early vigour	2.33 $\pm$ 0.12	2.78 $\pm$ 0.15	0.06
		Glossiness	1.85 $\pm$ 0.13	2.11 $\pm$ 0.20	0.31
		Trichome density	2.11 $\pm$ 0.51	3.56 $\pm$ 0.58	0.14

2.	<i>Xtxp37</i> 300bp	Early vigour	2.48±0.11	2.00±0.00	0.19
		Glossiness	1.91±0.11	2.00±0.58	0.82
		Trichome density	2.33±0.42	4.00±2.08	0.28
3.	<i>Xtxp88</i> 250bp	Early vigour	2.67±0.17	2.37±0.12	0.21
		Glossiness	2.56±0.18	1.70±0.10	0.00
		Trichome density	4.11±0.94	1.93±0.42	0.02
4.	<i>Xtxp248</i> 200bp	Early vigour	2.33±0.19	2.52±0.11	0.36
		Glossiness	2.13±0.17	1.76±0.14	0.09
		Trichome density	3.13±0.77	2.00±0.44	0.18
5.	<i>Xtxp248</i> 250bp	Early vigour	2.36±0.14	2.57±0.14	0.32
		Glossiness	1.91±0.15	1.93±0.17	0.93
		Trichome density	2.73±0.60	2.07±0.52	0.45

The similarity coefficient using all the bands was ranged from 0.58 to 1.00, whereas from polymorphic bands, the value was ranged from 0.00 to 1.00. The trend of the similarity was similar in both the ways. However, similarity was increased after inclusion of monomorphic bands. The correlation between them was 0.80. Looking to this trend the similarity based on all the bands were used.

The maximum similarity coefficient 0.88 was observed between resistant parents IS 2312 & IS 2205 and SUEENT 11 followed by 0.78 between IS 2205 & SUEENT 11. Between resistant and susceptible parents maximum similarity coefficient 1.00 was observed between IS 2312 & CSV 17 and minimum similarity coefficient 0.70 was observed between IS 2205 & CSV 23 and SUEENT 11 & DJ 6514. The maximum similarity coefficient 1.00 was observed between susceptible parents 27 B & AKMS 14B and minimum similarity coefficient 0.78 was observed between CSV 17 & DJ 6514 and CSV 23. This indicated no relationship between these bands and resistance.

The maximum similarity coefficient 0.88 was observed between resistant parents and resistant x resistant F_1 's IS 2205 & IS 2205 X IS 2312 and minimum similarity 0.58 was observed between IS 2205 & IS 2205 X SUEENT 11. For resistant parent and resistant x susceptible F_1 's maximum similarity coefficient 0.89 was observed between IS 2205 & IS 2205 X 27 B and SUEENT 11 & SUEENT 11 X CSV 17 and minimum similarity 0.64 was observed between IS 2205 & IS 2205 X CSV 23 and IS 2205 X CSV 17. The maximum similarity coefficient 0.80 was between resistant parent and susceptible x susceptible crosses SUEENT 11 & 27 B X AKMS 14 B, 27 B X CSV 17, 27 B X CSV 23 and AKMS 14 B X DJ 6514 while minimum similarity coefficient 0.58 was between IS 2205 & 27 B X DJ 6514. The maximum similarity coefficient 0.89 was observed between susceptible parents and susceptible x susceptible F_1 's AKMS 14 B & AKMS 14 B X CSV 17 and AKMS 14 B X CSV 23 and minimum similarity 0.73 was observed between 27 B & 27 B X DJ 6514. For susceptible parent and susceptible x resistant F_1 's maximum similarity coefficient 1.00 was observed between 27 B & 27 B X IS 2312 and minimum similarity 0.64 was observed between DJ 6514 & DJ

6514 X SUEENT 11. The maximum similarity coefficient 1.00 was observed between susceptible parent CSV 17 and resistant x resistant cross IS 2205 X IS 2312 while minimum similarity coefficient 0.64 was between DJ 6514, CSV 23 & IS 2312 X SUEENT 11.

The average similarity was maximum between susceptible parents and crosses involved at least one susceptible parent followed by resistant parent and crosses involved both resistant parents, susceptible parents and crosses without susceptible parents, resistant parents and crosses without one resistant parent and resistant parents and crosses without resistant parents. The difference between the similarity coefficients was very low as well as there was no specific trend. This indicated that most of the polymorphic bands obtained were not related to resistance and susceptibility except *Xtxp88* 250 bp. This band was present in all the susceptible parents (except CSV 17) viz; 27 B, AKMS 14 B, DJ 6514 and CSV 23 and their crosses except IS 2205 (R) X DJ 6514 (S) and DJ 6514 (S) X SUEENT 11 (R). This band was absent in CSV 17 though it was not having trichomes but having medium early vigour and glossiness that caused recovery resistance. Its cross with SUEENT 11 was also not having this band. These results indicated that this marker was dominant and present in all the crosses where susceptible parents were involved.

In all we can concluded that resistance was dominant over susceptibility and band *Xtxp88* was associated with susceptibility though it can explained only 33.20 and 14.70 percent variability of glossiness and trichome density, respectively. Therefore, to identify QTL responsible for resistance some other markers may be used.

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One must be persistent and creative in using keywords. An effective keyword search requires a strategy and planning a list of possible keywords and phrases to try.

Search engines for most searches, use Boolean searching, which is somewhat different from Internet searches. The Boolean search uses "operators," words (and, or, not, and near) that enable you to expand or narrow your affords. Tips for research paper while preparing research paper are very helpful guideline of research paper.

Choice of key words is first tool of tips to write research paper. Research paper writing is an art. A few tips for deciding as strategically as possible about keyword search:



- One should start brainstorming lists of possible keywords before even begin searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in research paper?" Then consider synonyms for the important words.
- It may take the discovery of only one relevant paper to let steer in the right keyword direction because in most databases, the keywords under which a research paper is abstracted are listed with the paper.
- One should avoid outdated words.

Keywords are the key that opens a door to research work sources. Keyword searching is an art in which researcher's skills are bound to improve with experience and time.

Numerical Methods: Numerical methods used should be clear and, where appropriate, supported by references.

Acknowledgements: Please make these as concise as possible.

References

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<i>Result</i>	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
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<i>References</i>	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

INDEX

A

Acclimatized · 9
Anaérobique · 1
Appropriate · 7

B

Balasubramanya · 22

C

Cartography · 3
Chromatography · 9, 23
Comprehensive · 19

D

Dichloromethane · 23
Disappearance · 22

E

Enteropathogenic · 12

F

Fabricated · 9

G

Geographical · 4

L

Lactobacillus · 7, 11, 15, 17
Lignocellulose · 12, 18

M

Microflora · 7, 8, 9, 10, 11, 12, 15, 17, 18, 19, 20
Monogastriques · 19

N

Nicodemus · 7, 12, 18, 19

O

Organiques · 1, 5
Orthophosphoric · 9

Q

Quantitative · 26

R

Recommended · 7, 9, 12
Renouvelable · 1

S

Slaughtered · 9
Statistique · 5



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