Online ISSN : 2249-4626
Print ISSN : 0975-5896
DOI : 10.17406/GJSFR

Global Journal

of Science Frontier Research: D

Agriculture & Veterinary

Effects of Sources of Protein

Conditions of Pasture Breeding

Highlights

Study on Behavioral Reactions

Analysis for Saturated Hydrocarbons

Discovering Thoughts, Inventing Future

VOLUME 18 ISSUE 1 VERSION 1.0

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Global Journal of Science Frontier Research: D
Agriculture & Veterinary

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Agriculture & Veterinary

Volume 18 Issue 1 (Ver. 1.0)

Open Association of Research Society

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Contents of the Issue

Global Journal of Science Frontier Research: D
Agriculture and Veterinary
Volume 18 Issue 1 Version 1.0 Year 2018
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Study on Behavioral Reactions of Female Calves Under Conditions of Pasture Breeding

By Nikolay Markov & Tatyana Bozhanska
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Abstract- The study was conducted in the late spring and early summer of 2017, in a foothill area of the Balkan Mountains, at an altitude of 386 m. We observed the ethological reactions of female calves of 'Bulgarian Rhodope Cattle' and 'Montbeliarde' breeds in the conditions of an artificial pasture. Two technological groups of animals were observed (six female calves intended for breeding), at the same age (180 days) and live weight of calves (107 kg on average for 'Bulgarian Rhodope Cattle' and 129 kg of 'Montbeliarde' breeds) by the method of analogs. Female calves of 'Bulgarian Rhodope Cattle' used 17.7% (436 min) of their time within twenty-four hours for grazing and animals of 'Montbeliarde' – 18.6% (453.24 min). For a 24-hour period, the animals of 'Bulgarian Rhodope Cattle' spent in a standing (187.5 min) and lying (523.3 min) position by 8.1 and 32.0 min more time in comparison with 'Montbeliarde' breed.

The terrain, abiotic factors, and the available grassland had an impact on the quality and quantity of grazing, hence the ethological reactions of the calves.

Keywords: *'bulgarian rhodope cattle', 'montbeliarde', behavior, pasture, reactions.*

GJSFR-D Classification: *FOR Code: 070305*

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Study on Behavioral Reactions of Female Calves under Conditions of Pasture Breeding

Nikolay Markov [α] & Tatyana Bozhanska [σ]

Abstract- The study was conducted in the late spring and early summer of 2017, in a foothill area of the Balkan Mountains, at an altitude of 386 m. We observed the ethological reactions of female calves of 'Bulgarian Rhodope Cattle' and 'Montbeliarde' breeds in the conditions of an artificial pasture. Two technological groups of animals were observed (six female calves intended for breeding), at the same age (180 days) and live weight of calves (107 kg on average for 'Bulgarian Rhodope Cattle' and 129 kg of 'Montbeliarde' breeds) by the method of analogs. Female calves of 'Bulgarian Rhodope Cattle' used 17.7% (436 min) of their time within twenty-four hours for grazing and animals of 'Montbeliarde' – 18.6% (453.24 min). For a 24-hour period, the animals of 'Bulgarian Rhodope Cattle' spent in a standing (187.5 min) and lying (523.3 min) position by 8.1 and 32.0 min more time in comparison with 'Montbeliarde' breed.

The terrain, abiotic factors, and the available grassland had an impact on the quality and quantity of grazing, hence the ethological reactions of the calves.

Keywords: 'bulgarian rhodope cattle', 'montbeliarde', behavior, pasture, reactions.

I. Introduction

There are differences among breeds and individuals within a species (Gonyou and Lou, 2000), and the environment is a main factor determining its behavior and development. The behavioral reactions of female calves allow prognosticating the temperament of herds (groups) as an indicator of their adaptation and co-existence over a definite period. A lot of studies in Bulgaria and abroad (Motyska et al., 1995; Krastev et al., 1999; 2002; Kudrin et al., 2008; Varlyakov et al., 2011; Stoycheva, 2014) noted the relationship between the ethological reactions of cattle and the various systems characterizing the breeding regime.

According to Medvedski (1983), the breeding of calves is a planned and guided process with a defined purpose and focus. Every young organism is very plastic and can be used to form the individuals in the desired direction. Zimmerman (2000), Grandin (2001), Yonikovski et al. (2008), and Kudrin et al. (2016) assumed that by a variety of factors such as breed, sex, breeding technology, live weight produced the behavior of cattle and the same is a relation between productivity and environmental performance

There are two types of grasslands: natural and cultivated (Malay, 2012). Cultivated pastures have been sown with grass species of high productivity and nutritional value for breeding and feeding the livestock. Kirilov et al. (2013) reported that according to the proposed technology, the average annual yield of artificial grasslands was 7500 kg/ha of hay, 9000 kg/ha of haylage (from the hay harvests) and 22500 kg/ha of green matter (of the pasture areas).

The number of studies, related to the behavior of female calves under pasture technology, is relatively small.

The purpose of the present study is to observe the ethological reactions of female calves of 'Bulgarian Rhodope Cattle' and 'Montbeliarde' breeds during the spring and summer period under the conditions of artificial pasture at the foothill of the mountain.

II. Materials and Methods

We conducted the study in the late spring and early summer (May, June, and July) in 2017, at a foothill area of the Balkan Mountains, at an altitude of 386 m. Two technological groups of animals were observed of 'Bulgarian Rhodope Cattle' and 'Montbeliarde' breeds. We followed the norm for their number (six female calves intended for breeding), at the same age (180 days) and live weight (107 kg on average for 'Bulgarian Rhodope Cattle' and 129 kg of 'Montbeliarde') by the method of analogs. The average daily growth during the grazing period was 294 ± 2.45 g for calves of 'Bulgarian Rhodope Cattle' and 387 ± 3.71 g for calves of 'Montbeliarde' breed. We identified the breeds with a different spray color and individual numbers (from 1 to 6).

Until May, the animals were bred in a covered cattle-shed (free), fed with a day ration mixture of coarse and concentrated fodder. Fourteen days before the grazing period, the ration was reduced by 25% to restrict the behavioral and physiological stress of the animals in the first days of the free pasture farming.

The artificial pasture is situated on flat terrain (Bukovets locality), typical for the conditions of the Central Balkan Mountains and isolated with an electric fence. All calves started grazing in the morning at 6.30 a.m., which lasted for 14 hours (until 8.30 p.m.) in an area of 1.5 ha. Calves slept and had a rest in natural conditions on the pasture under a group of tall trees. The watering was carried out in a trough connected to the plumbing system.

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The study on ethological reactions of calves was conducted by continuous observations (24 hours per day) according to the group timing technique in 10 minutes by zootechnical methods. We have observed six main behaviors: grazing, resting (standing, lying down), rumination, movement, water intake, and defecation. We reflected the results in daily records.

We indicated meteorological data given from regulated observations in a meteorological observation post, located on the territory of the pasture. Measurements are three times: daily readings of t˚C (dry and wet thermometer), humidity (thermohydrograph apparatus), wind (kata thermometer), cloudiness and precipitation.

Grass samples from the experimental pasture were analyzed in the analytical laboratory of RIMSA using a classic Weende method for establishing nutrients and chemical composition. *In vitro* enzyme dry matter digestibility (DMD) was determined by a two-phase pepsin-cellulose method of Aufrere (1982).

We calculated the relative feed value – RVF by dry matter digestibility and consumption in comparison with RVF for alfalfa (Jeranyama & Alvaro, 2004; Ward & de Ondarza, 2008).

III. Results and Discussion

a) The influence of the environment on the behavior of female calves of 'Bulgarian Rhodope Cattle' and 'Montbeliarde' breeds

The abiotic indicators characterizing mainly the climate conditions in the experimental area – the average air temperature ranging from 14.4 °C (01-10.05) to 22.9 °C (21-30.06) are typical for the region (Table 1). The lowest minimum air temperature (9.9 °C) was in the period of the third ten days of May and the highest maximum (31.1 ° C) in the third ten days of June.

The average relative humidity of the atmospheric air was highest during the first ten days of June (83.0%). In the period of 01-10.05, the lowest average values (75.0%) of the indicator were when the average air temperatures were the lowest.

The third ten days of June were relatively warm, sunny, with the least clouds, wind, and rainfall. We observed the highest cloudiness at the end of May and the highest precipitation (70.3 mm) in the first ten days of July.

Table 1: Abiotic ecological characteristics of the environment

Period	Temperature, ˚C			Humidity, %			Cloudiness	Wind, m/s	Precipitation, mm
	Min	Max	Average	Min	Max	Average			
01-10.05.17	8.3	20.4	14.4	58.0	92.0	75.0	5.8	1.5	55.4
11-20.05.17	14.9	22.4	18.7	65.0	88.0	76.5	5.5	1.8	8.0
21-30.05.17	9.9	20.4	15.2	71.0	92.0	79.0	6.4	1.5	69.7
01-10.06.17	13.7	24.5	19.1	72.0	94.0	83.0	5.6	1.5	30.1
11-20.06.17	12.1	24.4	18.3	71.0	92.0	81.5	4.8	2.0	51.7
21-30.06.17	14.6	31.1	22.9	64.0	75.0	69.5	1.5	1.4	1.3
01-10.07.17	13.7	29.0	21.4	66.0	94.0	80.0	2.4	1.8	70.3
11-20.07.17	14.6	27.4	21.0	71.0	94.0	82.5	4.4	1.4	62.0
21-30.07.17	13.7	28.9	21.3	71.0	91.0	81.0	3.2	1.5	54.3

Given the values of abiotic environmental indicators in the area of experimental pasture, the ethological reactions of the female calves are of interest.

The data reported in Table 2 show that calves of 'Bulgarian Rhodope Cattle' use 17.7% (436 min) of the twenty-four hours for grazing and those of 'Montbeliarde' breed spend 18.6% (453.24 min). The hours between 6.30-10.30 a.m. and 2.30-8.30 p.m. are the most active grazing period for both groups of animals. The results obtained are similar to those found by Krastev et al. (2001), Yonikovsky et al., (2008) and Kudrin et al., (2008), in pasture breeding of calves and heifers.

Table 2: Correlation and trends in the behavior of female calves

Elements of behavior	Breeds			
	Bulgarian Rhodope Cattle (I group) n=6		Montbeliarde (II group) n=6	
	min	%	min	%
Grazing	436.0	17.7	453.2	18.6
Total time spent in rest	710.8	28.9	670.6	27.6
Standing	187.5	7.6	179.4	7.4
Lying	523.3	21.3	491.3	20.2
Rumination	308.7	12.6	318.4	13.1
Movement	279.0	11.4	301.7	12.5
Water intake	8.5	0.3	9.1	0.4
Defecation	5.7	0.2	5.4	0.2
Total	1440.0	100.0	1440.0	100.0

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During the grazing period, the average time spend in lying position for calves of 'Bulgarian Rhodope Cattle' is 523.3 min or 21.3% of the day, and for those of 'Montbeliarde' – 491.26 min or 20.2% of the time within twenty-four hours (Table 2). The animals spend the longest time in lying position from 10:30 p.m. to 1.30 a.m. – 43.71%, a decrease is observed after that to 4.12% till 6.30 a.m. (Fig. 1).

For a 24-hour period, the female calves of 'Bulgarian Rhodope Cattle' spend 187.5 min in a standing position, which is 8.1 min longer than the representatives of 'Montbeliarde' breed. Calves are most active during a definite period in the morning (6.30 a.m. - 10.00 a.m.), before noon (11.30 a.m. to 2.00 p.m.) and evening hours (from 6.30 p.m. to 9.30 p.m.).

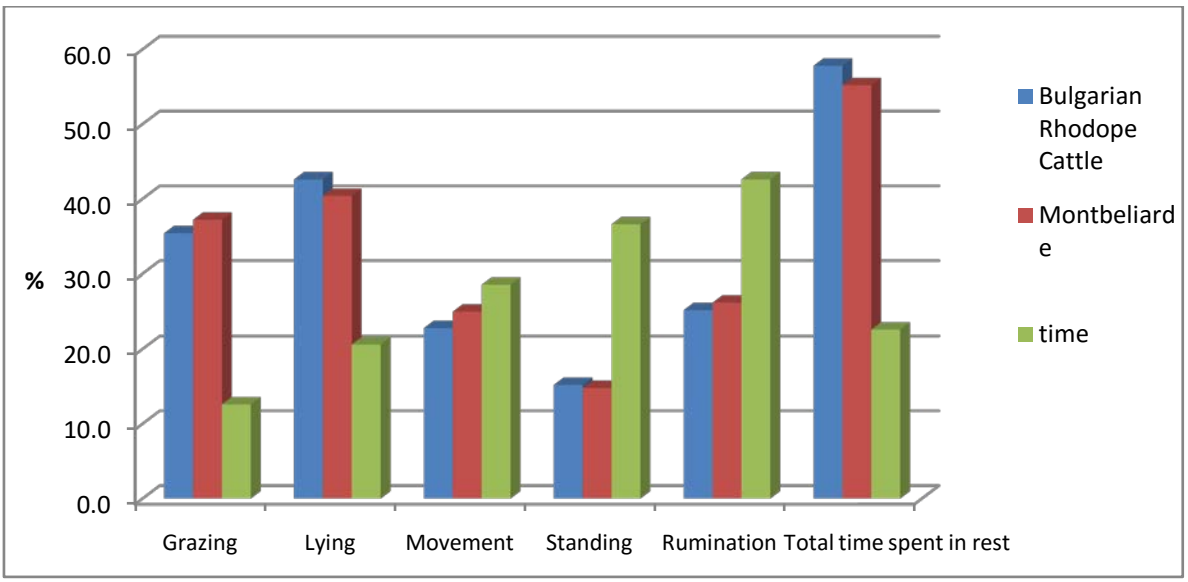

Fig. 1: Ethological reactions in dynamics for 24 hours

During the study, we observed sporadic instances of sexual aggression. Diseases in experimental calves were not allowed.

b) *Productivity and qualitative characteristics of artificial grassland*

Artificial grasslands for grazing (under non-irrigated conditions) form their yields mainly in April, May, and June. In the summer months, because of the lower soil and air humidity, grass growing is weaker. Proper use of the pasture ensures the maximum yield per unit area and a slight change in grass quality.

The average fresh and dry matter yield of the artificial grassland included in the experiment is 17066.8 kg/ha and 3625.3 kg/ha, respectively.

The group of motley (38%), in which 17% are weeds, followed by grasses (36%) and legumes (26%) – Fig. 2, formed the density and productive potential of the pastureland. The plants of *Dactylis glomerata* L. and *Phleum pretense* L. predominate in the botanical composition of the grassland. The share of legumes (*Lotus corniculatus* L. and *Trifolium repens* L.) is considerably more limited in the grass association. The feeding value of the sward in the grassland is high because the grazing does not allow grasses to become coarse hence the quality of plant biomass does not deteriorate.

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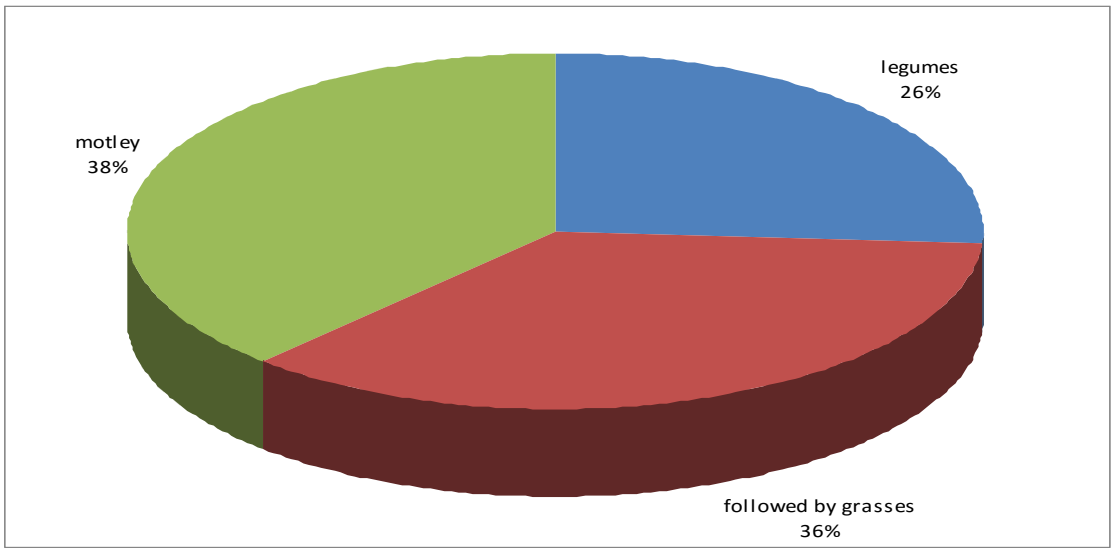

Fig. 2: Botanical composition of artificial grassland (weight, %)

The chemical characteristics of fodder determine to some extent the quantity of feed intake.

Table 3 and 4 show the basic chemical composition and structural fibrous components of cell walls in forage dry matter, where the crude protein (CP) values – 159.4 g kg^{-1} DM, crude fiber (CFR) – 384.7 g kg^{-1} DM, nitrogen-free extractable substances (NFE) – 330.6 g kg^{-1} DM and the fully digestible hemicellulose – 193.2 g kg^{-1} DM are high. They correspond to the high dry matter digestibility (653.5 g kg^{-1} DM).

Table 3: Basic chemical composition of dry matter of artificial grassland (g kg^{-1} DM)

CP	CFr	CF	Mineral substances	NFE	Ca	P
159.4	384.7	24.3	101.1	330.6	25.6	1.83

In biochemical cycles, the amount and ratio of Ca and P elements in the forage mass composition are interrelated and affect the feeding of young cattle. In this instance, the calcium/phosphorus ratio in the dry matter of the analyzed feed is 2.3: 0.2%.

The relative feed value (RFV – 120.2), used for the comparison of biomass quality, compared to alfalfa biomass in the blooming phase shows that grass biomass has a slightly better nutritional value because of the lower content of acid detergent fibers (ADF).

Table 4: Structural fibrous components and *in vitro* dry matter digestibility of an artificial grassland (g kg^{-1} DM)

NDF	ADF	ADL	Hemicellulose	Cellulose	Degree of lignification	DMD	DMI	RVF
505.8	312.6	107.7	193.2	205.0	199.6	653.5	2.37	120.2

The grass composition of the pastures is a factor determining the nutrient composition, feed consumption and productivity of animals (Komarek et al., 2007; Thomas et al., 2010).

The total energy nutrition value of dry biomass is 18.41 MJ/kg DM (GE) and the energy required to meet the physiological needs of the animal organism – 7.75 MJ/kg DM. Feed units of growth (0.64) in kg of dry matter allow the optimum regrowth and development of female animals (Table 5).

Table 5: Energy nutrition value of dry matter of artificial grassland

GE	EE	FUM	FUG
18.41	7.75	0.71	0.64

IV. Conclusions

The main ethological reactions of female calves of 'Bulgarian Rhodope Cattle', intended for breeding, with a live weight of 217 kg, are distributed as follows: grazing – 17,7% (436 min), time spent in a lying position – 21.3% (523.3 min), time spent in a standing position – 7.6% (187.5), time spent in movement 17.13% (279 min), time spent in water intake – 0.3% (8.5 min), and time for defecation – 0.2% (5.7 min).

The main ethological reactions of female calves of 'Montbeliarde' intended for breeding, a live weight of 294 kg were: grazing – 18.6% (453.24 min), lying

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position – 20.2% (491.26 min), a standing position – 7.4% (179.43), movement – 12.5% (301.7 min), time spent in water intake – 0.4% (9.1 min) and time for defecation – 0.2% (5.4 min).

Periods of increased activity were morning, afternoon and evening hours.

The terrain, the abiotic factors, and the available grassland had an impact on the quality and quantity of grazing, hence the ethological reactions of the calves.

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Global Journal of Science Frontier Research: D
Agriculture and Veterinary
Volume 18 Issue 1 Version 1.0 Year 2018
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Effects of Sources of Protein on Productive Performance and Immune Response in Commercial Broiler

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Abstract- The study was conducted to investigate the effect of different protein supplemented feed (animal protein, plant protein and combination of animal protein and plant protein) on the growth performance of broiler and the immune response of the birds against Newcastle Disease (ND). A total of 99 broiler birds were divided into 3 groups, each group consist of 33 individuals. Rations supplemented with animal protein, plant protein and combination of animal protein and plant protein were formulated for 3 treatment groups. Newcastle Disease vaccine was administered according to the vaccination schedule. Growth performance was measured using Feed Conversion Ratio (FCR) and immune response against ND using Haemaglutination Inhibition (HI) test. There was significant difference in growth performance due to variation in protein source of the supplied ration FCR was found 2.06, 1.64 and 1.68 for group A, B and C respectively.

Keywords: *ration, protein source, growth performance, immune response.*

GJSFR-D Classification: *FOR Code: 309999*

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Effects of Sources of Protein on Productive Performance and Immune Response in Commercial Broiler

Sabuj Kanti Nath [α], Jotan Kar [σ], Rajib Chakrabarty [ρ], Arup Sen [ω], Saurav Kumar Ghosh [¥], Md Bayzid [§], & Mohammad Mahbub Hasan [χ]

Abstract- The study was conducted to investigate the effect of different protein supplemented feed (animal protein, plant protein and combination of animal protein and plant protein) on the growth performance of broiler and the immune response of the birds against Newcastle Disease (ND). A total of 99 broiler birds were divided into 3 groups, each group consist of 33 individuals. Rations supplemented with animal protein, plant protein and combination of animal protein and plant protein were formulated for 3 treatment groups. Newcastle Disease vaccine was administered according to the vaccination schedule. Growth performance was measured using Feed Conversion Ratio (FCR) and immune response against ND using Haemaglutination Inhibition (HI) test. There was significant difference in growth performance due to variation in protein source of the supplied ration FCR was found 2.06, 1.64 and 1.68 for group A, B and C respectively. Antibody titre of the birds against ND vaccine virus was highest at day 7 is 7.10 and at day 28 is 6.90 in group-A birds supplemented with animal protein. The finding shows that immune response in broilers against ND depends on the protein source of the ration and animal protein is the best to have strong immunity.

Keywords: ration, protein source, growth performance, immune response.

I. Introduction

Chicken contributes 51% of the total meat production in Bangladesh (BBS, 2009). Per capita annual consumption of meat in this country is 5.9 kg which is very low and only 7.98% of the universal standard *(Sabrin et al., 2012)*. The annual per capita egg consumption is only 23 although it should be 100 from a nutritional point of view. The predominant poultry breed in Bangladesh is local, kept in scavenging system. Disease challenge is one of the many factors that will have an effect on the nutrient requirements of poultry. Insufficient nutrient consumption will reduce the effectiveness of the bird's defense mechanisms. Therefore, poultry must be supplied enough dietary nutrients and energy to allow the bird to express desired growth and feed efficiency. Poultry have been produced commercially since the early 1900's, and research has been conducted for years to improve production efficiency. Poultry nutrition experts employed by the industry have access to a great amount of information allowing them to optimize the particular production parameters most important to the producers (such as breast meat yield, feed conversion, weight gain). A good example of some of this information is that supplied by the National Research Council for Poultry (NRC). The protein and essential amino acids (EAA) requirements for broilers proposed by NRC (1994) are unable to accommodate the terms of production for modern strains of birds. In order to catch up the additional growth, levels of different commercially available proteins are generally increased (Corzo et al., 2002). Among these, particularly soybean meal is now being supplemented extensively enabling increasing energy production on the body (Corzo et al., 2002). Lysine requirements of broilers are higher in low protein diets for maximum weight gain and feed efficiency (Labadan et al., 2001). Even at normal crude protein (CP) level, high lysine content has been reported to increase the growth rate in broilers (Saima et al, 2010). Increasing soybean meal above NRC (1994) recommendations has been reported to improve weight gain; feed efficiency and breast meat yield (Si et al., 2004) and This protein source reduce the deposition of extra fat in the carcass. As widely described, increasing dietary proteins generally results in improved feed intake, feed conversion, and body weight gain (Sterling et al., 2006). Kidd et al., (2004) reported that essential amino acid (lysine) produced after break down of dietary proteins in poultry diet in concentration recommended by the NRC (1994) support proper immune system functions in healthy chicks. Improvements in immunity, as affected

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by dietary proteins in animals include improved thymic weight and function, enhanced lymphocyte mitogenesis, improved immunity against tumors and enhanced wound healing (Faluyi et al, 2015).

Among infectious diseases of backyard poultry, Newcastle disease (ND) and fowl cholera (FC) are highly endemic in Bangladesh. The prevalence rate of Newcastle disease in poultry is 28.9% (Adene, 2004). The major tools that can be used to provoke immunity in birds for both the prevention and control of the spread of the disease are vaccination, good nutrition and immunomodulation. Information as to the effect of different types of protein supplementation in different levels on growth and immunological responses to Newcastle disease in broiler chickens are rare. Although vaccination is being practiced to control ND in Bangladesh but outbreaks of NDV infection has been reported by several investigators (Sarkar *et. al.,* 2012).

Malnutrition and infection are major obstacles to survival, health, growth and reproduction of animals and humans worldwide. Recent studies indicate that dietary amino acids deficiency, which reduces concentrations of most amino acids in plasma and destroys the immune system, remains a significant nutritional problem in developing countries and also occurs in populations of developed nations. Thus, there is growing interest in the role of amino acids in the immune function of mammals, birds, fish and other species (Grimble, 2006). However, only in the past 15 years the underlying cellular and molecular mechanisms began to unfold (Newsholme *et al.,* 2003). Dietary supplementation with methionine has beneficial effects on the immune system under various catabolic conditions. For example, increasing total methionine levels from 0.35 to 1.2% in the diet for chickens infected with the Newcastle Disease Virus (NDV) markedly enhanced the following key aspects of the immune response: plasma levels of IgG (Tsiagbe *et al.,* 1987), leucocyte migration and antibody titre (Swain and Johri, 2000). The energy for adaptation comes from the three energy-yielding nutrients: carbohydrates, lipids and proteins. These nutrients are only available from feed and from nutrient reserves in the animal body. Lack of energy and protein hardly damages the humoral immunity. Choosing the proper level of energy that will optimize growth, carcass quality and feed efficiency, while still allowing for profitable production, is a major concern to any commercial operation. It has been consistently demonstrated that if essential nutrients are maintained in relation to dietary energy, an increased growth rate and improved feed efficiency are observed as a result of increasing the level of dietary energy (Bartov, 1992 and Leeson *et al.,* 1996). Therefore, the major objective of this study was to provide an insight into the specific roles of different protein sources in diets on the immune function and performance of broilers.

II. Materials and Methods

a) Study area

The study was conducted at the Department of Animal Science and Nutrition in Chittagong Veterinary and Animal Sciences University (CVASU), Chittagong.

b) Duration of study

The study was carried out from April to May, 2016.

c) Sampling strategy

A total of 99 day old Cob-500 broiler chicks were obtained from commercial hatchery (MM Agha Hatchery Ltd.) and reared using common practice for broiler chicks and fed on starter broiler diets special emphasis on different sources of protein (animal, plant, mixed animal & plant protein).

d) Study design and management

A total number of 99 day old cob-500 strain chicks were collected with average weight of approximately 40g and randomly divided into three (3) groups A, B and C. Each group consisted of 33 numbers of chicks. Then chicks were reared upto 28 days and first group was reared with ration supplemented with animal protein while the second and third groups were fed the plant protein and mixed animal & plant protein diet, respectively. All groups were kept at the similar conditions of room temperature and under normal periods of light/dark. Feed and water supplementation were *ad libitum* throughout the experimental period.

e) Vaccination of the experimental broiler

All the birds from each group were chosen and each bird was vaccinated against Newcastle disease with NDV clone 30 (Nobilis ND Clone 30; Intervet) following standard ND vaccination schedule (At 5 days and 12th days of age of bird).

f) Live body weights and body weight gain

Birds were weighed (g) individually at 7, 14, 21 and 28 days of age. Chicks were weighed in the morning before offering the feeds. Body weight gains were calculated by subtracting body weight at the end of each period from the initial body weight at the same period using individual record for each bird.

g) Measurement of feed intake and protein intake

In each experiment, feed intake was recorded weekly according to the replicate feeding system followed in the present work. Each group was provided daily with enough pre-weighed amount of its corresponding diet. The remainder and scattered feeds as well as the consumed feed was weekly calculated for each replicate and thereafter, the average weekly feed intake per bird was calculated by division of group consumed by their chick number whereas, protein

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intake was calculated by multiply dietary CP% of the feed by feed intake.

h) Calculation of feed conversion ratio (FCR)

Feed conversion ratio was calculated in the form of units of feed intake required to produce one unit of live body weight gain.

i) Cumulative feed consumption ratio

Cumulative feed consumption ratio was calculated at 7, 14, 21 and 28 days of age from each group of chicks. It was done by total feed consumed by birds of each groups divided by total numbers of birds in each groups.

j) Mortality rate

Mortality rate for whole experiment period was presented as a number of dead birds in each treatment at the end of each experiment.

k) Immunological test

Blood samples were collected from wing vein using an insulin syringe at two times 4 and 21 days of post-vaccination. Blood was allowed to clot then centrifuged immediately to separate serum. After collection of serum Hemagglutination inhibition (HI) test of serum samples was done according to the method of King and Seal, (1998) to determine the immune response (antibody titer) of the chickens derived from vaccination against Newcastle Disease virus.

l) The procedure of HI test is given below

To obtain a preparation of virus with known HA titer or determine its HA titer we have to prepare two-fold dilutions of patient/test serum to be tested e.g. from 1:4 to 1:1024. Add a fixed amount of virus to every well of a 96-well plate, equivalent to 4 HA units (varies according to virus), except for the serum control wells. The plate is then allowed to stand at room temperature for 60 minutes (time varies according to specific requirements). Add red blood cells (RBC) and incubate at 4oC for 30 minutes. Then Read the wells. The highest dilution of serum (Ab) that prevents hemagglutination is called the HI titer of the serum. A smooth or jagged shield of cells or an irregular button indicates agglutination. Observation of movement of the button of red cells when the plate is tilted may help to clarify the end point. (https://microbeonline.com)

m) Ration formulation

We used animal protein, plant protein and combination of animal protein and plant protein supplement for broiler starter and grower.

Animal protein supplemented ration for broiler starter

Ingredients	Amount	ME	CP	CF	Ca	P
Maize	55	1819.95	5.06	1.32	0.0385	0.22
Auto Rice Polish	10	293.7	1.19	1.24	0.035	0.12
Full fat soya	6.8	239.292	2.584	0.34	0.017	0.0408
Fish meal	7.5	207.75	4.35	0.3	0.5775	0.2925
Meat and bone meal	7.5	158.325	4.035	0.1725	0.8475	0.40425
Vegitable oil	1.4	125.3			0.091	
Protein Conc.	8	232	4.8	0.24	0.52	0.2
Molasses	0.5	12	0.014		0.00755	0.0033
Lime stone	1				0.358	0.0002
Vit. & Min. premix	0.25					
Common salt	0.25					
DCP	0.5				0.1215	0.091
D-L Methionine	0.14					
Enzyme	0.05					
Emulsifier	0.04					
L-Lysine	0.05					
Antioxidant	0.012					
Coccidiostat	0.02					
Toxin binder	1					
TOTAL	100.01	3088.32	22.03	3.61	2.61	1.37

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Animal protein supplemented ration for broiler grower

Ingredients	Amount	ME	CP	CF	Ca	P
Maize	58	1919.22	5.336	1.392	0.0406	0.232
Auto Rice Polish	8	234.96	0.952	0.992	0.028	0.096
Full fat soya	6.8	239.292	2.584	0.34	0.017	0.0408
Fish meal	7	193.9	4.06	0.28	0.539	0.273
Meat and bone meal	7	147.77	3.766	0.161	0.791	0.3773
Vegitable oil	1.4	125.3			0.091	
Protein Conc.	8	232	4.8	0.24	0.52	0.2
Molasses	0.5	12	0.014		0.00755	0.0033
Lime stone	1				0.358	0.0002
Vit. & Min. premix	0.25					
Common salt	0.25					
DCP	0.5				0.1215	0.091
D-L Methionine	0.14					
Enzyme	0.05					
Emulsifier	0.04					
L-Lysine	0.05					
Antioxidant	0.012					
Coccidiostat	0.02					
Toxin binder	1					
TOTAL	100.01	3104	21.51	3.41	2.51	1.31

Plant protein supplemented ration for broiler starter

Ingredients	Amount	ME	CP	CF	Ca	P
Maize	54	1786.86	4.968	1.296	0.0378	0.216
Auto Rice Polish	4.34	127.4658	0.51646	0.53816	0.01519	0.05208
Full fat soya	2.5	87.975	0.95	0.125	0.00625	0.015
Soyabean meal	34	761.6	15.3	2.04	0.1088	0.2278
Vegitable oil	1.4	125.3			0.091	
Molasses	0.5	12	0.014		0.00755	0.0033
Lime stone	1				0.358	0.0002
Vit. & Min. premix	0.25					
Common salt	0.25					
DCP	0.5				0.1215	0.091
D-L Methionine	0.14					
Enzyme	0.05					
Emulsifier	0.04					
L-Lysine	0.05					
Antioxidant	0.012					
Coccidiostat	0.02					
Toxin binder	1					
TOTAL	100.05	2901.2	21.75	3.99	0.75	0.61

Plant protein supplemented ration for broiler grower

Ingredients	Amount	ME	CP	CF	Ca	P
Maize	56	1853.04	5.152	1.344	0.0392	0.224
Auto Rice Polish	2.34	68.7258	0.27846	0.29016	0.00819	0.02808
Full fat soya	1.5	52.785	0.57	0.075	0.00375	0.009
Soyabean meal	34	761.6	15.3	2.04	0.1088	0.2278
Vegitable oil	3	268.5			0.195	
Molasses	0.5	12	0.014		0.00755	0.0033
Lime stone	1				0.358	0.0002
Vit. & Min. premix	0.25					
Common salt	0.25					
DCP	0.5				0.1215	0.091
D-L Methionine	0.14					
Enzyme	0.05					

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Emulsifier	0.04					
L-Lysine	0.05					
Antioxidant	0.012					
Coccidiostat	0.02					
Toxin binder	1					
TOTAL	100.65	3017	21.31	3.75	0.84	0.58

Broiler starter ration supplemented with both animal and plant protein

Ingredients	Amount	ME	CP	CF	Ca	P
Maize	54	1786.86	4.968	1.296	0.0378	0.216
Auto Rice Polish	4.34	127.4658	0.51646	0.53816	0.01519	0.05208
Full fat soya	2.5	87.975	0.95	0.125	0.00625	0.015
Soyabean meal	30	672	13.5	1.8	0.096	0.201
Meat and bone meal	3	63.33	1.614	0.069	0.339	0.1617
Vegitable oil	1.4	125.3			0.091	
Protein Conc.	1	29	0.6	0.03	0.065	0.025
Molasses	0.5	12	0.014		0.00755	0.0033
Lime stone	1				0.358	0.0002
Vit. & Min. premix	0.25					
Common salt	0.25					
DCP	0.5				0.1215	0.091
D-L Methionine	0.14					
Enzyme	0.05					
Emulsifier	0.04					
L-Lysine	0.05					
Antioxidant	0.012					
Coccidiostat	0.02					
Toxin binder	1					
TOTAL	100.05	2903.93	22.16	3.86	1.14	0.77

Broiler grower ration supplemented with both animal and plant protein

Ingredients	Amount	ME	CP	CF	Ca	P
Maize	58	1919.22	5.336	1.392	0.0406	0.232
Auto Rice Polish	5.34	156.8358	0.63546	0.66216	0.01869	0.06408
Full fat soya	2.5		0.95	0.125	0.00625	0.015
Soyabean meal	24	537.6	10.8	1.44	0.0768	0.1608
Meat and bone meal	2.5	52.775	1.345	0.0575	0.2825	0.13475
Vegitable oil	3	268.5			0.195	
Protein Conc.	1	29	0.6	0.03	0.065	0.025
Molasses	0.5	12	0.014		0.00755	0.0033
Lime stone	1				0.358	0.0002
Vit. & Min. premix	0.25					
Common salt	0.25					
DCP	0.5				0.1215	0.091
D-L Methionine	0.14					
Enzyme	0.05					
Emulsifier	0.04					
L-Lysine	0.05					
Antioxidant	0.012					
Coccidiostat	0.02					
Toxin binder	1					
TOTAL	100.15	2976	19.68	3.71	1.17	0.73

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n) Statistical analysis

All required data were recorded and finally analyzed using software. All data were inputted to the Microsoft Office Excel-2007 and transferred to the software STATA/IC-11 for analysis. Descriptive statistics was done by using the STATA software and expressed as percentage of different variables.

III. Results

a) Effect of different protein on growth performance of broiler

Data of the effect of animal protein, plant protein and mixed animal & plant protein supplementation on growth of broiler chicks from 7 to 28 days of age are shown in Table-1 and Table-2. We found in our study that when we use plant protein then there is significant effect in body weight gain in group B as compared with group A group C. In group A the average body weight gain is 675 g when the protein source is animal protein. In group B the average body weight gain is about 1027 g when we used plant protein source and it was the highest body weight gain among 3 groups. When protein source is composed of both animal protein and plant protein then group showed that average body weight gain is 937 g.

b) Feed intake

Data for the effect of different protein supplementation on feed intake of broiler chicks from 7 to 28 days of age are presented in Table-3. In our study we found that the average feed intake weekly was significantly height is group B is about 57.6 g when protein source is plant protein. In group A and group B the average feed intake weekly were 46.70 g and 53.89 g respectively where animal protein .

c) Feed conversion ratio (FCR)

Data for the influence of different protein supplementation on FCR from 7 to 28 days are shown in Table-4. We found in our study that feed conversion ratio is only 1.24 in group B where in group A and group C it were 1.32 and 1.25 respectively. So we can say that FCR is lesser in group B in comparison with group A and group C.

d) Mortality rates

Data for the influence of different protein supplemented diet on broilers mortality are shown in Table-5. We found mortality rate in group C where mixture protein source are used is only 9%. But group A showed the highest mortality rate is 18% when protein source is animal protein and group B showed 12% mortality rate when protein source is plant protein.

e) Effects of different protein supplementation on antibody titers to Newcastle disease virus of broiler chicks

Data for the effects of different protein supplementation on antibody titers to ND virus are given in Table-6. In our study we found highest titres for ND at 4th days and 21th days in group A were 7.1 and 6.9 respectively. It showed the titres for ND at 4th days and 21th days in group B were 5.6 and 5.4 respectively. In group C at 4th and 21th days the titres of ND were 6.3 and 6.6 respectively.

IV. Discussions

It was found that, weekly body weights and average daily weight gain Table-1 were significantly increased in the plant protein consumed group-B as compared to the group-A and group-C during overall experimental period (Table -1 and Table- 2). Plants provide the major portion of protein requirements by animals. However, due to their deficiency in one or more amino acids, plant proteins are usually fortified with synthetic amino acids or another protein source such as processed oilseed meal or animal protein concentrates. Instead of animal protein feeds in poultry nutrition, plant protein feeds are used with the supplementation of synthetic amino acids (Cmiljani *et al.,* 2005). Increasing the efficiency of protein and amino acid utilization is crucial for the reduction of feed costs and maximization of meat production with an absolute minimum intake of amino acids. Synthetic amino acids have been found to facilitate the formulation of diets with an ideal amino acid profile (Han and Lee, 2000). These findings are in agreement with several reports demonstrating that probiotic supplemented to the birds improved the body weight gains of the broiler chickens (Benites *et al.,* 2008).

Weekly feed consumption and average daily feed intake were significantly decreased in the group-A where it was treated with animal protein and highest feed intake found in the plant protein treated group-B and then it was in the group-C (Table -3) Feeding with plant and animal protein did not affect feed intake of broiler chicks from day 7 to 28. These results are similar to those reported by Pinchasav *et al.* (1990), Bunchasak *et al*. (1997), Yonemochi *et al*. (2003), and Yamazaki *et al*. (2006) who found that feed intake of broiler chicks was not significantly affected by low CP amino acid supplemented diets. On the other hand, Attia *et al*. (2001) and Ghazalah *et al*. (2006) observed that feed intake was higher of broiler chicks fed low CP diet than that of those fed high CP diet may be due to the higher requirement of nutrients. Results indicated that, the FCR values were significantly decreased in the group B compared to the group-A and group C. It was positive things that, plant protein supplementation to the feed in group B consumed less amount feed to gain higher body weight where group-A and group C consumed more amount feed but body weight gain was lower than the group-B (Table- 4).

Results indicated that number of dead birds ranged from 3-6 birds/treatment. The postmortem

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investigations indicated that mortality was not related to dietary treatments. Similar results were reported by Attia *et al*. (2001) and Aletor *et al*. (2001) (Table-5). There is increasing interest in evaluating non-medical alternatives for antimicrobials and antiviruses in terms of their ability to improve disease resistance, and enhance overall animal health and production in poultry. Therefore, in the present study, attempts were made to evaluate the use of animal protein, plant protein and mixed animal & plant protein to the broiler feeds and investigate the influence of such feed supplements on immune response. Serum antibody titers against Newcastle disease virus based on HI test in chicken fed basal diet supplemented with different protein was significantly higher in group-A than those of chickens in the group-B and C on days 4 and 21 post vaccinations (Table-6). These findings are in agreement with several studies. Rowghani *et al.* (2007) reported that broiler chickens fed a diet supplemented with probiotics had a significant increase in the Newcastle antibody titers than the control group. They also reported that the antibody titers against ND in broilers fed with diets supplemented with probiotics containing *B. subtilis* was significantly higher at 10 days post-immunization compared to the control birds.

Acknowledgements

We would like to express our all sorts of praises and thanks to Almighty, Who teaches the use of the pen to man, who bestowed us with the potential and the ability for making a humble contribution / addition to the existing knowledge. It's our honor to express the heartiest gratitude and deep sense of obligation to our respectable and considerate supervisor Dr. Goutam Buddha Das, Professor of the department of Animal Science and Nutrition, Faculty of Veterinary Medicine of Chittagong Veterinary and Animal Sciences University for his keen help for the successful accomplishment of the present study. We especially thanks to DR. Avijit Dutta, Lecturer, Department of Microbiology and Veterinary Public Health and DR. Mohammad Bayazid Bostami, MS student in Surgery, Department of Medicine And Surgery, Chittagong Veterinary and Animal Sciences University for their constant help during research work.

Table-1: Effects of different protein supplementation on live weight gain of broiler

Parameters	Experimental groups			
	A (X±SE)	B (X±SE)	C (X±SE)	(P-value)
Initial body weight (g)	40.1 ± 2.33^{a}	40.5 ± 2.27^{a}	40.5 ± 2.27^{a}	0.90
BWG 1st week	124.1 ± 0.87^{a}	147.0 ± 1.15^{b}	152.0 ± 2.00^{c}	0.00
BWG 2nd week (g)	238.2 ± 1.40^{a}	310.0 ± 1.56^{c}	307.0 ± 1.76^{b}	0.00
BWG 3rd week (g)	507.0 ± 1.41^{a}	712.0 ± 1.76^{c}	618.0 ± 0.81^{b}	0.00
BWG 4th week (g)	675.0 ± 1.15^{a}	1027.0 ± 1.82^{c}	937.0 ± 1.76^{b}	0.00

BWG=Body weight gain, A= Animal protein supplementation, B= Plant protein supplementation, C= Mixed animal and plant protein supplementation

Table-2: Effects of different protein supplementation on weight gain of broiler

Body weight gain (gm)	Experimental groups			
	A (X±SE)	B (X±SE)	C (X±SE)	(P-value)
At 1st week	84 ± 0.76^{a}	106.50 ± 0.85^{b}	111.50 ± 0.77^{c}	0.00
At 2nd week	114.10 ± 0.48^{a}	163.00 ± 0.73^{c}	155.00 ± 1.01^{b}	0.00
At 3rd week	268.80 ± 0.73^{a}	402.00 ± 0.63^{c}	311.0 ± 0.61^{b}	0.00
At 4th week	168.00 ± 0.91^{a}	315.00 ± 0.68^{b}	319.00 ± 0.56^{c}	0.00
Average daily weight gain	22.68 ± 0.03^{a}	35.23 ± 0.09^{c}	32.01 ± 0.07^{b}	0.00

A= Animal protein supplementation, B= Plant protein supplementation, C= Mixed animal and plant protein supplementation

Table-3: Amount of feed consumed by birds in experimental groups

Parameter	Experimental groups			
	A (X±SE)	B (X±SE)	C (X±SE)	(P-value)
Feed intake(FI) At 1st week (g)	110 ± 3.59^{a}	129.2 ± 3.70^{b}	130.7 ± 1.94^{b}	0.00
FI At 2nd week(g)	289 ± 6.29^{a}	357 ± 5.77^{b}	441 ± 6.34^{c}	0.00
FI At 3rd week(g)	746 ± 3.89^{a}	983 ± 8.58^{b}	878 ± 4.90^{a}	0.00
FI At 4th week(g)	1155 ± 4.01^{a}	1613 ± 6.29^{c}	1509 ± 4.05^{b}	0.00
Average daily feed intake	46.70 ± 0.35^{a}	57.64 ± 0.35^{c}	53.89 ± 0.26^{b}	0.00

A= Animal protein supplementation, B= Plant protein supplementation, C= Mixed animal & plant protein supplementation

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Table-4: Feed conversion ratio in each group

Feed conversion ratio (g feed/g)	Experimental groups			
	A (X±SE)	B (X±SE)	C (X±SE)	(P-value)
At 1st week	0.88±0.06[b]	0.87±0.04[a]	0.86±0.03[a]	0.00
At 2nd week	1.21±0.04[b]	1.15±0.03[a]	1.11±0.06[c]	0.00
At 3rd week	1.47±0.02[c]	1.38±0.02[b]	1.42±0.02[a]	0.00
At 4th week	1.73±0.03[c]	1.57±0.02[b]	1.61±0.01[a]	0.00
Average FCR	1.32±.0.04[c]	1.24±0.03[a]	1.25±0.03[b]	0.00

A= Animal protein supplementation, B= Plant protein supplementation, C= Mixed animal and plant protein supplementation

Table-5: Number of dead birds in each group

Experimental groups	Number of dead birds	Mortality rates (%)
Group-A	6	18%
Group-B	4	12%
Group-C	3	9%

A= Animal protein supplementation, B= Plant protein supplementation, C= Mixed animal and plant protein supplementation

Table-6: Antibody titers against Newcastle Disease (ND) virus in each group

ND titre at different days	Antibody titers against ND virus vaccine in different experimental groups			
	A (X±SE)	B (X±SE)	C (X±SE)	(P-value)
4th day	7.1±0.13[c]	5.6±0.24[a]	6.3±0.25[b]	0.001
21th day	6.9±0.07[b]	5.4±0.20[a]	6.6±0.25[b]	0.00

A= Animal protein supplementation, B= Plant protein supplementation, C= Mixed animal and plant protein supplementation

V. Conclusion

He label of protein could be reduced of a certain limit in broiler rations along with the supplememtation of lysine plays an important role on feed consumption and weight gain, without the affects of feed convertion ratio (FCR).

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Global Journal of Science Frontier Research: D
Agriculture and Veterinary
Volume 18 Issue 1 Version 1.0 Year 2018
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Groundnut Response to Boron and Molybdenum

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Abstract- As micronutrients required by the plants in a small amount but it perform different function in different plants. Micronutrients play an important role in the phsyio-morphological characteristics of many crops especially in leguminous crops. The aim of this review was about to study the role of micronutrients like boron and molybdenum in growth and yield parameters of groundnut or peanut plant as well as in nutrients concentration and uptake by the plant. Boron and molybdenum has the ability to improve yield and yield parameters of peanut plant. Several results showed that root nodulation, nodules numbers per plant and nitrogen fixation in groundnut plant were significantly improved while applying boron and molybdenum fertilizers. Molybdenum involve in nitrogenaze an enzyme which is responsible for biological nitrogen fixation which further improve nitrogen content of the soil. Furthermore, increasing level of boron and molybdenum showed significant increased in the concentration of micronutrients (B and Mo) in both plant and soil.

Keywords: *boron, molybdenum, nodulation, nitrogen fixation, nitogenase and foliar.*

GJSFR-D Classification: *FOR Code: 070199*

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Groundnut Response to Boron and Molybdenum

Jamal Nasar [α], Gao Qiang [σ] & Ashfaq Alam [ρ]

Abstract- As micronutrients required by the plants in a small amount but it perform different function in different plants. Micronutrients play an important role in the phsyio-morphological characteristics of many crops especially in leguminous crops. The aim of this review was about to study the role of micronutrients like boron and molybdenum in growth and yield parameters of groundnut or peanut plant as well as in nutrients concentration and uptake by the plant. Boron and molybdenum has the ability to improve yield and yield parameters of peanut plant. Several results showed that root nodulation, nodules numbers per plant and nitrogen fixation in groundnut plant were significantly improved while applying boron and molybdenum fertilizers. Molybdenum involve in nitrogenaze an enzyme which is responsible for biological nitrogen fixation which further improve nitrogen content of the soil. Furthermore, increasing level of boron and molybdenum showed significant increased in the concentration of micronutrients (B and Mo) in both plant and soil. As several researcher found earlier that application of boron and molybdenum significantly affect nutrients uptake by the plants. Boron and molybdenum availability of soil depends upon on soil type and soil pH, some soil type have boron and molybdenum deficiency but these deficiency can be recovered by the application of these nutrients to the plant which can be helpful in the uptake of nutrients from the soil and the soil status also improved. Micronutrients can be applied to the plant in two ways soil application and foliar application but the foliar method is the most efficient way of micronutrients application.

Keywords: boron, molybdenum, nodulation, nitrogen fixation, nitogenase and foliar.

I. Introduction

Groundnut (*Arachis hypogaea* L.) is a legume crop of family Fabaceae (or Leguminosae) mostly grown for its edible seeds and oil production in the world. It is also known as peanut or gobber. It is native to South America (Brazil), best grown in tropical and sub tropical region of the world at a latitudes 400 N to 400 S. The groundnut seed is called kernel which is used in confectionary nut flour production, protein and peanut milk (Woodroof, 1966). Seed oil content of groundnut is 46-52 %, while carbohydrates and protein percentage is 18 and 30% respectively. Pure form of groundnut seed oil is mainly use as cooking oil, as a salad oil and margarine while raw oil is use in soap manufacturing. Like other legumes groundnut has the capacity to fix atmospheric nitrogen through symbiotic nitrogen fixing bacteria in root nodules which mean peanut plant required less N containing fertilizers, it also improve N content in soil which make this plant valuable in crop rotation. Total area under groundnut production were 26.4 million ha worldwide with and annual production of 37.1 million metric tonne having an average productivity of 1.4 t/ha (Kalamkar *et al.*, 2006).

China, India, Indonesia and USA are the major groundnut producing courtiers in the world the two third of groundnut production comes from these countries.

As micronutrient required by the plant in a small amount but it play a vital role in the growth yield and production. Soil is the major source of all the plant required nutrients but they are not available in proper amount therefore plant need some essential nutrients to be applied in two methods soil and foliar. Soil application is best for macronutrients while micronutrients act best when applied as foliar spray.

Boron is one of the key micronutrient required by the plant for their growth and development. It has known that peanut need for boron is a bit higher than other legumes crops. Boron has the ability to increase photosynthetic and enzymatic activity in peanut plant. It also involve in protein and nucleic acid metabolism. Boron maintains structural integrity of the plant and protects plasma membrane from external damage (Ismail and Volkar, 1997). It also helpful in sugar transport, division and elongation of cell, involve in transport of auxin and metabolism in roots and improve ATPase activity (Gupta, 2007).

Boron deficiency causes pollen grain germination. Pollen tube growth and viability of pollen grains is also effected due to boron deficiency (Dugger, 1973). Boron is the only element which is available in soil solution and plant can easily take up from the soil as a non-ionized molecule at a suitable pH range (Oertli and Grgurevic, 1975).

Molybdenum involves in nitrogenase an enzyme which is responsible for the nitrogen fixation process by bacteria symbiotically with legumes crops. It also plays a key role in nitrogen metabolism, protein synthesis and sulphur metabolism. Molybdenum is required in pollen formation so Mo deficient plant will cause effect in their fruits and pollen grains formation. It is also important for the absorption and translocation of iron in the plants (Subba Rao and Adinarayan, 1995).

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II. Boron and Molybdenum Status of Soil

The amount of water soluble boron is higher in neutral and basic soil than in acidic soil (Yang. 1960). The high pH and organic matter soil was enriched in molybdenum content, as the pH and organic matter increasing it showed slightly increase in molybdenum level of the soil (Kavimandan *et al*., 1964). Boron availability is higher in fine texture soil while lower in coarse texture soil (Gupta., 1968). The deep black colored soil has the high molybdenum content than the other colored soil (Rai *et al*., 1972). Boron are much more deficient in light texture soil than any other soil type (Annamalai., 2014). Bororn availability depends upon soil pH and texture class of the soil as the pH of such soil is increases the availability of boron is decreases. Sandy to loam soil with a pH 5.5 to 6.5 required 0.30 mg kg^{-1} boron while soil with a pH 7.0 required 0.5 mg kg^{-1} boron required for optimum growth and production of plant (Loue. 1986).

III. Effect of Boron on Growth and Yield of Groundnut

The application of boron at 15 kg ha^{-1} significantly increased the growth and yield of groundnut special improvement was found in pods per plant (Asokan and Raj., 1974). Pod yield of groundnut showed 13.25% increased due to the application of 5 kg Borax ha^{-1} Shinde and Kale (1985) in Maharashtra India. The highest pod yield production was observed by Golakiya and Patel (1986) during a pot culture experiment when boron and calcium carbonate was applied at the rate of 10 % C and 2 mg kg^{-1} B respectively. The combined application of S @ 60 kg, B @ 0.92 kg and FYM @ 5.5 t ha^{-1} significantly enhanced pod yield (22.5 q ha^{-1}), oil content (47.61 %) and protein content (27.24 %) in groundnut plant Suruvase *et al*. (1986). The significant increased in pod yield of groundnut from 0.80 to 0.95 t ha^{-1} compared to control treatment in shallow Vertisols with low available B was due to the application of boron at 5 kg borax ha^{-1} at sowing and two foliar sprays of 0.1% (Jadhao *et al*., 1989). Plants treated with boron fertilization @ 7.5 kg borate ha^{-1} produced maximum plant height, number of branches per plant, 100 seed weight and yield in groundnut than control (Luo *et al*., 1990). The application of zinc and boron directly in soil or as a foliar spray enhanced significantly yield and yield components in groundnut plant, pod yield, 100 kernel weight, shelling percentage, oil and protein content in seed was increased due to zinc and boron fertilization Ramamoorthy and Sudarshan (1992). Boron applied @ 0.275 mg kg^{-1} soil and 135 mg kg^{-1} in plant tissue was best for optimum growth of groundnut (Cv. JL-24) (Kadag *et al*., 1994). The increased in yield of dry pod, harvest index and total N uptake of groundnut plant was obtained because boron application at the rate of 0.5 kg ha^{-1} Mahajan *et al*. (1994). Boron applied at the 100, 200 and 300 ppm alone or in combination with rhizobium inoculation significantly increased the growth and yield parameters of groundnut (Nasef, 2006). Peanut produced higher flowering and yield arttribute due to boron fertilization 1.0 kg ha^{-1} as a soil and 0.1% as a foliar spray (Sing *et al.,* 2008). When boron applied at the rate 5 kg ha^{-1} it gave maximum plant height and number of branches per plant in peanut plant Vishwakarma *et al.* (2008).

IV. Effect of Molybdenum on Growth and Yield of Groundnut Plant

The increase in nitrogen uptake and protein content in groundnut plant was found consequently when plants treated with molybdenum Chatterjee *et al*. (1985). Molybdenum plays a significant role in nodulation and N content of nodules in groundnut plant Kene *et al*. (1988). Different growth and yield parameters like plant height, number of pods per plant, 100 kernel weight, seed oil content and pod yield of groundnut plant were significantly improved when seed of groundnut were soaked in 150 gm ha^{-1} of molybdenum along with soil application of $CaCo_3$ and NPK Lumpungu and Muteba (1985). Groundnut plant in calcarious soil showed significant increase in nodules number, pods yield and 100 kernel weights when fertilized with Zn and M micronutrients Joshi *et al*. (1987). The oil content of groundnut plant was increased when sulphur and molybdenum @ 120 kg S ha^{-1} + 1.2 kg Mo ha^{-1} Singh and Abidi (1989). Molybdenum has a key role in nitrogenase an enzyme which is responsible for nodulation and nitrogen fixation, the improvement in nitogenase activity in groundnut plant was observed when seed of the plant was treated with 100 g ha^{-1} of Mo along with soil application 16 kg P ha^{-1} Hafner *et al*. (1992). Molybdenum applied at the rate of 0.2 and 0.4 kg ha^{-1} to groundnut plant increased dry pod weight significantly Aghatise and Tayo (1994). Application of molybdenum significantly improved the growth and yield parameters in groundnut, it was noticed that molybdenum applied along with nitrogen fertilizer improved the growth and yield parameters of groundnut, nodulation and nitrogen fixation was also significantly enhanced Nadia, 2012).

V. Combined Effect of Boron and Molybdenum on Yield and Growth of Groundnut Plant

Micronutrients has the ability to improve different growth and yield parameters in different plants like Fe, Mn, Zn,Cu, B and Mo are the micronutrients which significantly increased plant height, chlorophyll

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content, pod and fodder yield in groundnut plant when applied in combination Singh *et al.* (1990). Foliar application of Zn, B and Mo either alone or in combination consequently enhanced the vegetative growth of groundnut plant (Das, 1992). Combined application of boron and molybdenum at the rate of 1 kg Mo and 2 kg B ha^{-1} produced maximum pod yield, nodules number and seed oil content over control in groundnut plant Noor *et al*. (1997). The groundnut pod yield, pod number, pod weight, shelling percentage was significantly increased when plant were fertilized with combined application of Mn @ 5 kg, Zn @ 4 kg, Cu @ 1.0 kg, B @ 0.6 kg and Mo @ 0.1 kg ha^{-1} Sarkar *et al.* (1998). Nodulation and nitrogease activity in groundnut plant was increased when plant were treated with Zn @ 25 kg, B 10 kg and Mo 1.0 kg ha^{-1} alone or in combination but pod yield, dry matter and leaf area was enhanced with combined application Tripathy *et al*. (1999). Boron and molybdenum both in combination significantly improved chlorophyll content in leaves, photosynthetic activity of the leaves, dry matter accumulation, and flowering, yield and reproductive organs of groundnut plant were also enhanced Duyingqiong *et al*. (2002). When the seeds of groundnut treated with micronutrients fertilizers like Mo, B and Zn 8, 6 and 8 g kg^{-1} produced maximum pod yield than control Bagewadi *et al*. (2003). Shankhe *et al*. (2004) found that foliar application of boron (0.5 % borax) + soil application of molybdenum 1 kg ha^{-1} significantly enhanced the production of groundnut as well as the availability of B and Mo and their uptake was also found improved.

VI. Effect of B and Mo Application on Nutrients Concentration and Uptake by Groundnut Plant

Boron and molybdenum play a significant role in the concentration and uptake of nutrients by groundnut. several investigations were found on the effect of B and Mo application on the concentration and uptake by crop. Groundnut plant fertilized with NPK, Mg and B showed significant increased in the uptake of N, P, K and Mg by kernel Longnathan and Krishnamoorthy (1977). When sulphur @ 60 mg kg^{-1} and molybdenum @ 2 mg kg^{-1} applied to groundnut in pot experiment an increment was noticed in nitrogen uptake by seed and haulm (Narasi Reddy and Sreenivasa Rao, 1985). The increase in the concentration and uptake of nutrients was observed in groundnut plant when boron as borax at the rate of 0, 1.5 and 2.5 kg ha^{-1} was supplied to the plant Sinha *et al*. (1991). As the level of boron application increased to the groundnut plant it showed significant increased in the concentration of B and uptake of nitrogen by groundnut plant Jiang *et al*. (1994). The uptake and concentrations of nutrients increased with increasing level of boron level when applied to groundnut plant (Nasef, 2006). Groundnut plant when treated with molybdenum application significantly improved the uptake and concentration of macro and micronutrients (Nadia, 2006).

VII. Effect of B and MO Application the Available Nutrient Status of the Soil

Application of boron and molybdenum not even affect the plant but also found significant effect on the available nutrient status of the soil by many researchers. As the boron level increased from 0.32 to 1.60 ppm it significantly increased the hot water extractable boron in the soil from 0.90 to 2.04 ppm under groundnut crop, it was noticed that boron as borax was more superior in increasing hot water extractable boron in the soil Ashokan and Raj (1974). Shinde and Kale (1985) observed that when boron applied as borax at the rate of 5 kg ha^{-1} to the groundnut plant it significantly increased the boron concentration in the soil at harvest. The availability of N, P, Ca and micronutrients like B and Mo in the soil was enhanced when plant were treated with the foliar application of boron and molybdenum Shankhe *et al*. (2004). Application of boron at the rate of 1 and 2 kg ha^{-1} to the groundnut plant significantly increased the boron availability in the soil Singh *et al*. (2005). The application of boron improved the level of boron in the low available boron sandy loam soil Powel and Waldemar (2006). When the boron (5 kg ha^{-1}) treated groundnut plant were harvested at full maturity, a significant increase in the hot water extractable boron in the soil was observed Nadaf (2007).

VIII. Conclusion

It has been observed by several researchers that applying micronutrients like boron and molybdenum to the plants has beneficial effect on the growth yield and production of groundnut. Boron and molybdenum also involve in the nitrogen fixation of many plants due to the application of these nutrients the BNF of the plants improved which further improve the N content of the soil. So from the review it is concluded that boron and molybdenum are must required micronutrients for the better growth, yield, nitrogen fixation and other physio-morphological parameters of groundnut plant. Further researches are suggested to work out on the effect of boron and molybdenum on leguminous crops.

Acknowledgement

Author are thankful to all Lab mattes for helping and supporting in the review, special thank to prof: Dr. Gao Qiang for guiding while writing this review.

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Global Journal of Science Frontier Research: D
Agriculture and Veterinary
Volume 18 Issue 1 Version 1.0 Year 2018
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

GC-MS Analysis for Saturated Hydrocarbons From Jasmine (*Jasminum sambac L.*) Leaves Damaged by Jasmine Leaf Webworm, *Nusinoe geometralis* Guenee

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Abstract- The hexane extracts of *Jasminum sambac* L. leaves damaged by jasmine leaf webworm, *Nausinoe geometralis* (Guenee) and also healthy jasmine laeves were subjected to Gas Chromatography-Mass spectrometry (GC-MS) to determine the saturated hydrocarbons. The results revealed that both the healthy and damaged leaves had hydrocarbon compounds numbering 21 and 27 respectively. The variation in the hydrocarbon profile of healthy and damaged leaves might be related to the quality of semiochemicals these plants emit, which is important for the attraction of natural enemies in jasmine ecosystem so as to reduce further infestation by budworm. In the healthy jasmine leaves, the hydrocarbons, hentriacontane and tetracosane were detected at 25.89 mins exhibiting the largest peak area of 24610830 mm^2 octacosane, tetracosane, eicosane, nonacosane and heptacosane followed the order and emitted in enormous quantities.

Keywords: *semiochemicals, synamones, saturated hydrocarbons, nausinoe geometralis, GC-MS, jasmine.*

GJSFR-D Classification: *FOR Code: 079999*

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GC-MS Analysis for Saturated Hydrocarbons from Jasmine (*Jasminum Sambac L.*) Leaves Damaged by Jasmine Leaf Webworm, *Nusinoe Geometralis* Guenee

I. Merlin Kamala [α] & J. S. Kennedy [σ]

Abstract- The hexane extracts of *Jasminum sambac* L. leaves damaged by jasmine leaf webworm, *Nausinoe geometralis* (Guenee) and also healthy jasmine laeves were subjected to Gas Chromatography-Mass spectrometry (GC-MS) to determine the saturated hydrocarbons. The results revealed that both the healthy and damaged leaves had hydrocarbon compounds numbering 21 and 27 respectively. The variation in the hydrocarbon profile of healthy and damaged leaves might be related to the quality of semiochemicals these plants emit, which is important for the attraction of natural enemies in jasmine ecosystem so as to reduce further infestation by budworm. In the healthy jasmine leaves, the hydrocarbons, hentriacontane and tetracosane were detected at 25.89 mins exhibiting the largest peak area of 24610830 mm^2 octacosane, tetracosane, eicosane, nonacosane and heptacosane followed the order and emitted in enormous quantities. The natural enemy attractant, methyl salicylate and allyl isovalerate was also detected in healthy jasmine leaves. With regard to jasmine leaf web worm infested leaves, allyl isothiocyanate, allyl isovalerate, divinyl sulfide, oxalic acid potential candidates for attracting natural enemies were detected in along with an array of saturated hydrocarbons. Bis (2-ethylhexyl) phthalate, a six carbon compound was detected in maximum quantity detected with the largest peak area of 144295751 mm^2 at 38.984 mts in web worm damaged leaves.The quality and quanity of these semiochemicals emitted by the leaves might be the reason for attraction of natural enemies in the jasmine ecosystem there by further reducing the infestation of leaf webworm, as well as other pests. This feature can be exploited to enhance the efficacy of natural enemies in integrated management of jasmine pests.

Keywords: semiochemicals, synamones, saturated hydrocarbons, nausinoe geometralis, GC-MS, jasmine.

I. Introduction

Jasmine (*Jasminum sambac* L.) known in persian as yasmin *ie*. 'Gift of God' is one of the oldest fragrant flowers of India. It is traditionally as well as commercially cultivated for its sweet scented flowers. Globally, jasmine is celebrated in many countries as their national flower and utilized as decoration for ceremonies and rituals as an important part of cultural heritage. Flowers and un opened buds are used for making garlands, bouquets, in religious and ceremonial functions, perfumed hair oils, attars, soaps, wine and drinks (Thakur *et al.,* 2014). It is used for production of jasmine concrete, which is the base in cosmetic and perfumery industries and hence the phrase "no perfume without jasmine". The dried flowers are used for making the famous 'jasmine tea', scented with aroma from jasmine blossoms, which is a popular drink in South East Asian countries. An infusion of jasmine tea is beneficial in treating fever, relieving stress and anxiety. The flowers and other parts of the plant like leaf, stem, bark and root are also used for medicinal purposes (Bose and Yadav, 1989).

Flower or essential oil, jasmine find a place in useful medicines as an aphrodisiac, sedative, antiseptic, antidepressant, antispasmodic, and analgesic relieving pains and relaxing the nervous system (Ranadas *et al.,* 1985; Kanniamal and Divya, 2016). As the demand for high grade perfumes has greatly increased in recent times, there is tremendous scope for the production of concretes and oils from jasmine flowers. Also, the need for the mesmerizing jasmine flowers for diverse necessities like religious ceremonies, official and home decorations, weddings, funerals etc. is ever rising. The countries growing jasmine on a commercial scale are France, India, Italy, Moracco, Algeria, North Africa, Spain, Egypt and Israel. The area and production of total flowers in India were increasing impressively over the years. The world production of jasmine concrete is around 20 tonnes per annum, out of which India is producing and exporting about 2 tonnes (Ray *et al.,* 2014). The largest area under jasmine cultivation is in Tamil Nadu and Karnataka from where it is distributed to metropolitan cities (Nimisha and Razia, 2014). Since recent past, this commercial jasmine is affected by a number of pests like jasmine budworm (*Hendecasis duplifascialis* Hampson), the galleryworm (*Elasmopalpus jasminophagus* Hampson), the leaf webworm (*Nausinea geometralis* Guenee), the leaf roller (*Glyphodes unionalis* Hubner), the hawk moth (*Achreontia styx* Westwood), the blossom midge (*Contarinia maculipennis* Felt) and the two spotted mite

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(*Tetranychus urticae* Koch) posing serious threat to jasmine cultivation.

The existing recommendation of synthetic chemicals is only a short term solution, as the pest population increases after few months, later disproportionately requiring repeated application with high dosages, which finally became hazardous and uneconomical, leading to the endangerment of ecosystem by reducing the diversity of natural enemies. In addition, direct toxicity to human beings, animals and environment is of serious concern. It is pertinent that a change in the insect pest management strategy may form a meaningful solution to avoid the ill-effects caused by the synthetic chemical insecticides especially as environmental contaminants. Therefore, in search of safer alternatives, attention has been focused on exploration of semiochemical mediated approaches through host plant defense mechanisms. An approach of using semiochemicals in pest management is to exploit ways to chemically augment, conserve or enhance the efficacy of natural enemies in a crop ecosystem. Use of these biochemicals especially, the synomones released by host plants is of significance in biological control. Hydrocarbons present in host plants were found to act as synomones for natural enemies in different crop ecosystems. In particular, synomones play a major role by guiding the natural enemies to the potential host or prey on the plant (Hilker and Meiners, 2006). Such clues may be utilized to stimulate foraging and host selection behavior of entomophages thereby increasing their effectiveness for IPM (Ahmad *et al.,* 2004). Therefore in the present study, the hexane leaf extracts of jasmine damaged by leaf webworm and healthy leaves were analyzed through GC-MS to determine the saturated hydrocarbon profiles in them.

II. Materials and Methods

Jasmine plants were raised in an area of 20 cents at the Botanical garden premises, Tamil Nadu Agricultural University, Coimbatore during January-May 2016. All the recommended agronomic practices were followed. Healthy and leaf webworm damaged leaves were collected from the field during the period of heavy infestation by leaf webworm.

The saturated hydrocarbons were extracted from the jasmine leaves damaged by leaf webworm and healthy leaves using HPLC grade hexane as follows. The healthy and leaf webworm defoliated leaves of jasmine were plucked carefully and used for extraction.

Ten gram of leaves was immersed overnight in 100 ml of HPLC grade hexane. The filtrate was then passed through silica gel (60-120 mesh) column. The hexane solvent was allowed to evaporate and the left over residue was collected by rinsing the container with a small quantity of HPLC grade hexane (Merck) and stored in separate vials for GC-MS Analysis. Gas chromatography combined with mass spectroscopy is a preferable methodology for routine analysis of compounds.

Hexane based leaf extracts were analysed on GC-MS (Agilent Technologies 7890B GC System with 5977B MSD) mass selective detector (70eV) equipped with a 10:1 split injector. The gas chromatography is equipped with 30m fused Oven temperature programming: 60°C (1 min hold) to 100°C at 5°C/min rate (1 min hold), then to 220°C at 10° C/min rate (5 min hold) and then to 240°C at 50°C/min rate (8 min hold). Injector temperature was set at 275°C. One microlitre of the extract was injected using auto sampler into the gas chromatography-mass spectroscopy (GC-MS) System for analysis injections was done in split 10:1 mode. Agilent data analysis software was used for the analysis of compounds in the extracts. Injected sample was separated into various constituents with different retention time which were detected by mass spectrophotometer. The compounds of interest were identified using standard NIST mass spectral (NIST MS 2) library. The chromatogram, a plot of intensity against retention time was recorded by the software attached to it. From the graph, the compounds were identified by comparing the data with the existing software libraries.

III. Results and Discussion

Induction of plant defense in response to herbivore involves the emission of volatile compounds called synamones that act as attractants for natural enemies of herbivores. Synamones produced by plants are reported to be very significant in eliciting host-seeking response in many natural enemies. Synamones attract predators and parasitoids, which elucidate the tritrophic interaction in a crop ecosystem (Ferry *et al.,* 2004).

Gas Chromatography mass - spectroscopy analysis of synamone extracts of healthy leaves showed the presence of 21 hydrocarbons *viz.,* cyclohexanol, 2-butanol, 2-methyl butanoic anhydride, allyl isovalerate, azetidine 1, 2 dimethyl, cyclo hexane, phenyl ethyl alcohol, methyl salicylate, heneicosane, nona decane 9 methyl, eicosane, octacosane, tetracosane, hentriacontane, eicosane, nona decane 9-methyl, pentacosane, octadecane, heptacosane, hexacosane and nonacosane (Fig 1).

But the leaf web worm damaged leaves showed the presence of 27 hydrocarbons (Table 1) *viz.,* allyl isothiocyanate, divinyl sulfide, dodecane 5-methyl, 2-amino ethanol, allyl isovalerate, azetidine, oxalic acid, dodecane, 2-penetene, dodecyl octyl ether, acetophenone, cyclohexane, cycloheptanol, 4-amino-5-(4-acetyl phenylazo) benzo furazan, iso butyl angelate, cyclo hexa siloxane,trifluoro octoxy hexadecane, butyl angelate, 2-butenoic acid, cyclohepta siloxane, benzene, benzene butanoic acid, diethylmalonic acid,

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cyclotetradecane, decyl trifluoroacetate, tetracosane, bis (2-ethylhexyl) phthalate, di - n- octyl phthalate and pthalic acid, di (2-propyl pentyl ester) (Fig 2).

In the healthy jasmine leaves, the hydrocarbons, hentriacontane and tetracosane (Fig 3, 4) were detected at 25.89 mins exhibiting the largest peak area of 24610830 mm^2 followed by eicosane displaying a peak area of 15511968 mm^2 in 31.462 mins. The compounds, octacosane, tetracosane and eicosane (Fig 5,6) were detected at 25.713 mins, exhibiting the third largest peak area of 13642707 mm^2. Hydrocarbons eicosane, nonacosane and heptacosane (Fig 7) were detected at 33.519 mins displaying a peak area of 9932421 mm^2.

Apart from several saturated hydrocarbons, natural enemy attractants, methyl salicylate was detected in healthy jasmine leaves at 11.92 in a peak area of 981341 mm^2 .Volatile methyl esters are common constituents of plant volatiles with important function in plant defense. Methyl salicylate, (Fig 8) a herbivore-induced volatile has been shown to attract natural enemies and affect herbivore behavior. But methyl salicylate is present in healthy jasmine leaves itself in a meager quantity.

Methyl salicylate lures examined for its effectiveness against organic soybean aphids, *Aphis glycines* Matsumura showed reduced population of aphids, with significantly greater number of syrphid flies (Diptera : Syrphidae) and green lace wings (Neuroptera: Chrysopidae) (Mallinger *et al.,* 2011). The results are in line with Du *et al.,* (1998) ; Kesseler and Baldwin, (2001) reporting that a number of herbivory induced plant volatiles have been characterized for their individual contrition to indirect defense in behavioural set ups including methyl salicylate.

Allyl isovalerate(Fig 9), a fragrant compound is also detected, in healthy jasmine leaves at 5.465 mins in a peak area of 6178643 mm^2, which findings of Zada *et al.* (2003) who found lavandulyl isovalerate in headspace volatiles of vine mealybug, *Planococcus ficus*.

With regard to jasmine leaf web worm infested leaves, allyl isothiocyanate, allyl isovalerate, divinyl sulfide, naphthalene, oxalic acid potential candidates for attracting natural enemies were detected in along with an array of saturated hydrocarbons.

The potential natural enemy attractant allyl iso-thiocyanate (Fig 10) was detected at RT of 4.307 mins at an area of 533535 mm^2 in leaf webworm webbed leaves, which is 1.073 per cent of the total compounds present. Allyl iso-thio cyanate, a naturally occurring organo-sulfur compound in mustard, radish, horseradish, is responsible for their pungent taste.

Allyl isothiocyanate serves the plant as a defense against herbivores; since it is harmful to the plant itself, it is stored in the harmless form of the glucosinolate. When the plant is damaged, the enzyme myrosinase is released and acts on a glucosinolate known as sinigrin to give allyl isothiocyanate.

Synthetic allyl isothiocyanate is used as an insecticide, bacteriocide and nematocide, and is used in certain cases for crop protection (Romanowsk and Klenk, 2005). Zabża, 1989; Titayavan and Altieri, 1990, reported that the parasitoid *Diaeretiella rapae* M'Intoshis was attracted to allyl isothiocyanate emitted by cabbage plants damaged by cabbage aphids (*Brevicoryne brassicae* L.) and increased aphid parasitism from 8.5% to 22.5%. Thus, allyl isothiocyanate release due to budworm attack in jasmine plant might attract lot of natural enemies that check the pest naturally due to tri trophic interactions. Though, allyl iso thiocyanate peak area is only 1.073 per cent of the total compounds, which is less compared to most hydrocarbons released, which means, the compound is elicited only in meager quantity, it has already proved its efficiency in attracting natural enemies in various crop ecosystems.

Moreover, divinyl sulfide (Fig 11) was detected at 4.688 mins in a peak area of 260362 mm^2. Though its emission is in meager quantity it might also play its own role in natural enemy attraction as corroborated by Ferry *et al*. (2009) who found higher cabbage fly *Delia radicum* egg predation in broccoli *Brassica oleracea* plots with dimethyl disulphide lures (2.1 eggs predated/patch of eggs).

Bis (2-ethylhexyl) phthalate, di – n- octyl phthalate, pthalic acid, di (2-propyl pentyl ester), six carbon compounds were the compounds present in maximum quantity detected with the largest peak area of 144295751 mm^2 at 38.984 mts. In general, green leaf volatiles are six carbon compounds which are very quickly produced and/or emitted upon herbivory which play an important role in plant defenses and as bis (2-ethylhexyl) phthalate (Fig 12) is also six carbon compound, produced due to leaf herbivory in jasmine ecosystem, there are chances for its potential role in natural enemy attraction. Liu *et al,* (2007) reported the presence of volatile, bis (2-ethylhexyl) phthalate in honeydew from both *B. tabaci* on cabbage and *T. vaporariorum* on cucumber and its role as kairomone in host-searching of parasitoids.

Moreover oxalic acid (Fig 13) was emitted in enormous amount in a peak area of 42578330 mm^2 at 5.962 mins. Oxalic acid and oxalates are produced and present in plants in different amounts (Korth, 2006; Nakata, 2012; Franceschi and Nakata, 2005). They provide biochemical as well as mechanical defense against insect pests and animals (Prasad and Shivay, 2017). Foliar application of acetylsalicylic and oxalic acids has the potential to encourage aphid parasitisation, parasitoid *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae) (Karatolos and Hatcher, 2008).

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Napthalene, (Fig 14) an aromatic hydrocarbon was detected in bud worm infested leaves at 17.493 mins with peak area of 233218 $mm^{2.}$ which was reported to be a semiochemical attracting natural enemies of stemborer in maize ecosystem (Peshwin and Pimental, 2014).

Among the other hydrocarbons, tetracosane and triacontane were detected in 29.045 mins in a peak area of 11570984 $mm^{2.}$ 17.846% of the total compounds which implies their emission in maximum quantity.

The GC-MS studies on the volatile profile emitted from leaf folder damaged leaves in the susceptible rice variety TN1 showed more of the presence of docosane, which could be responsible in attraction of *Trichomma cnaphalocroccis* Uchida and *Cotesia angustibasis* Gahan (Rathika and Nalini, 2011). Seenivasagan and Paul (2011) analyzed the extracts of cruciferous host plants of diamond back moth and revealed the presence of saturated hydrocarbons. Cauliflower leaf extract contain 12 hydrocarbons with carbon number ranging from C10-C30 in which C29 (nonacosane) was detected in highest quantity. In cauliflower extracts exclusively C10 (decane) and C12 (dodecane) hydrocarbons were identified which were not detected in other host plant extracts. The hydrocarbon C14 (tetradecane) was detected only in cauliflower and broccoli extracts, whereas C16 hexadecane was detected only in cabbage, cauliflower and broccoli extracts. C18 (octadecane) and C20 (eisocane) were detected in cabbage and cauliflower extracts. C22 (docosane) and C25 (pentacosane) were detected only in cauliflower, while C26 (hexacane) was found only on knoll-knol leaf extracts. Similarly hexane extracts of ten different varieties of tomato (*Lycopersicon esculentum* Mill.) obtained in the vegetative and flowering phase of growth contained tricosane (C23), heneicosane (C 21), pentacosane (C25) and hexacosane (C26) during the vegetative period and heneicosane (C21) and hexacosane (C26) during the flowering period (Paul *et al.,* 2008).

Comparing the peak areas of common hydrocarbon compounds *viz.,* allyl isovalerate and tetracosane, present in healthy and damaged leaves, allyl isovalerate was found to be 1.043 percent more present in leaf webworm damaged leaves, but the hydrocarbon tetracosane was 0.848 per cent more pronounced in healthy jasmine leaves.

Behaviour of a natural enemy can be manipulated potentially by enhancing their foraging ability in an ecosystem. The interface, where tritrophic interactions take place in natural condition is often the cuticle of a plant. The saturated hydrocarbons present in the epicuticular wax layer of plants have been shown to influence the foraging success of natural enemies. Therefore the hydrocarbon compounds found in the extracts of damaged leafwebworm damaged leaves have to be explored for the attraction of natural enemies to parasitize and/or to predate the herbivore or reduce the further infestation by the herbivores for efficient pest management.

Acknowledgement

The financial assistance provided by UGC, fellowship to pursue Ph.D. in Agricultural Entomology at Tamil Nadu Agricultural University, Coimbatore is gratefully acknowledged for the senior author.

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Table 1: Saturated hydrocarbon profile of the healthy and leaf webworm, *Nausinoe geometralis* damaged leaves of jasmine, *Jasminum sambac* L.

Healthy leaves			Leaf webworm damaged leaves		
RT(min)	Area(mm^2)	Name of the compound	RT(min)	Area(mm^2)	Name of the compound
4.305	3527444	Cyclohexanol	4.308	533535	Allyl isothiocyanate
4.688	3319684	2-Butanol	4.688	260362	Divinyl sulfide
5.030	10691889	2-Methyl butanoic anhydride	4.908	2214865	Dodecane 5-methyl
5.465	6178643	Allyl isovalerate	5.030	24241992	2-Amino ethanol
5.669	3556699	Azetidine 1,2 dimethyl	5.465	6480251	Allyl isovalerate
5.962	8563751	Cyclo hexane	5.670	4018056	Azetidine
7.787	1894648	Cyclo hexane (2 methyl propyl-)	5.962	42578330	Oxalic Acid
9.671	1782054	Phenyl Ethyl Alcohol	6.144	260869	Dodecane
11.924	981341	Methyl salicylate	6.545	4971755	2-Penetene
24.098	2270549	Heneicosane	7.136	125362	Dodecyl octyl ether
	2270549	Nona decane 9 methyl	8.421	65691	Acetophenone
25.713	13642707	Eicosane	11.52	83629	Cyclohexane
25.713	13642707	Octacosane	12.22	200022	Cycloheptanol
25.713	13642707	Tetracosane	12.70	91452	4-Amino-5-(4-acetyl phenylazo) benzo furazan
25.894	24610830	Hentriacontane	14.221	185482	Iso butyl angelate
25.894	24610830	Tetracosane	14.562	289982	Cyclo hexa siloxane
27.546	3828465	Eicosane	17.062	85400	Trifluoro octoxy hexadecane
27.546	3828465	Nona decane 9-methyl	17.123	464305	Butyl angelate
27.546	3828465	Hentriacontane	17.123	464305	2-Butenoic Acid
30.855	3809329	Eicosane	17.493	233218	Napthalene
30.855	3809329	Pentacosane	17.155	464305	Cyclohepta siloxane
30.855	3809329	Octadecane	17.704	145509	Benzene

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31.462	15511968	Eicosane	17.817	126852	Benzene butanoic acid
33.132	4254520	Heptacosane	18.511	124684	Diethylmalonic acid
33.132	4254520	Hexacosane	19.556	140095	Cyclotetradecane
33.519	9932421	Eicosane	19.556	140095	Decyl trifluoroacetate
33.519	9932421	Nonacosane	29.045	11570984	Tetracosane
33.519	9932421	Heptacosane	29.045	11570984	Triacontane
			31.984	144295751	Bis (2-ethylhexyl) phthalate
			31.984	144295751	Di – n- octyl phthalate
			31.984	144295751	Pthalic Acid Acid, di (2-propyl pentyl ester)

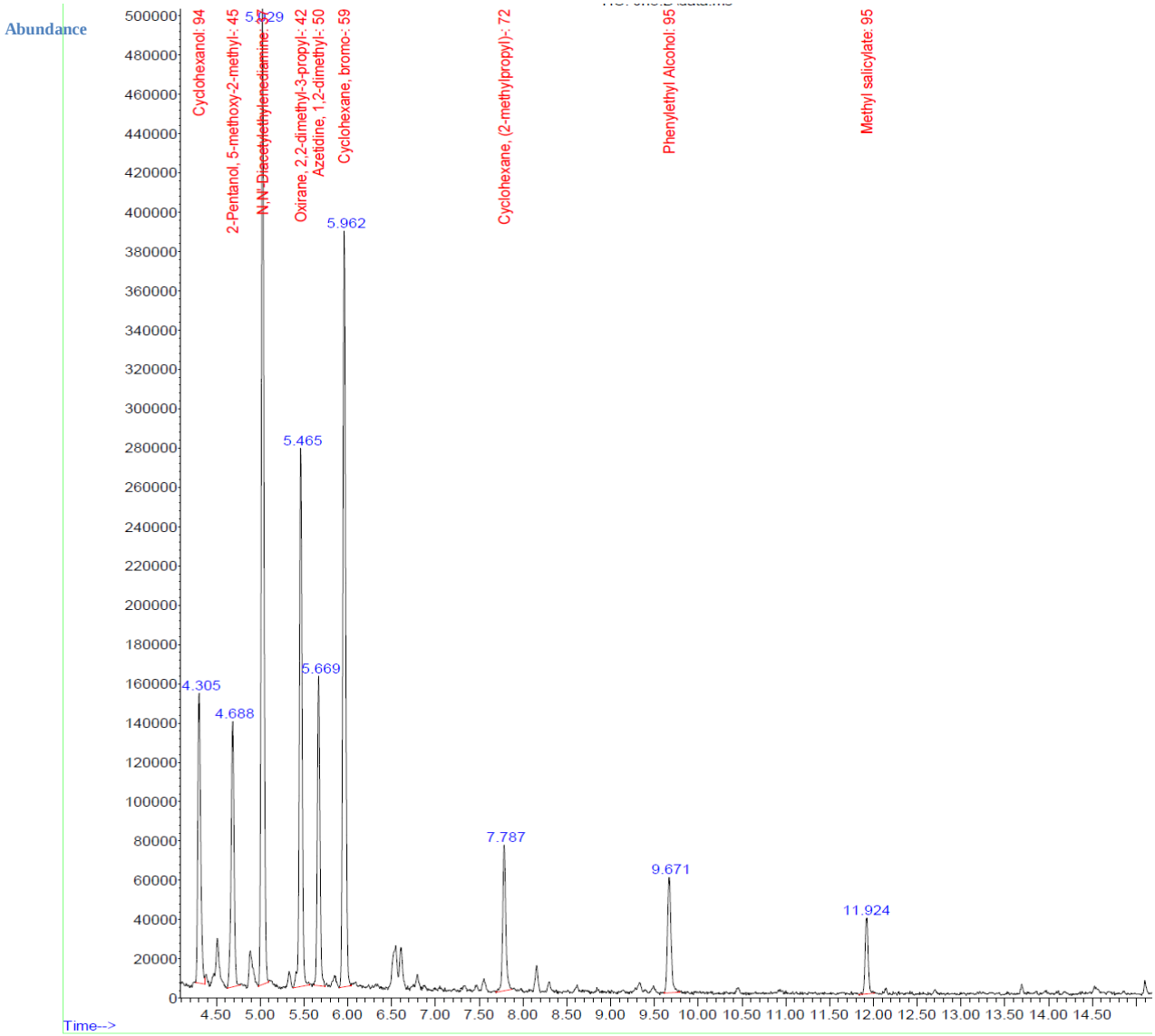

Fig. 1a: Chromatographic profiles of saturated hydrocarbons from healthy jasmine (*Jasminum sambac* L.) leaves

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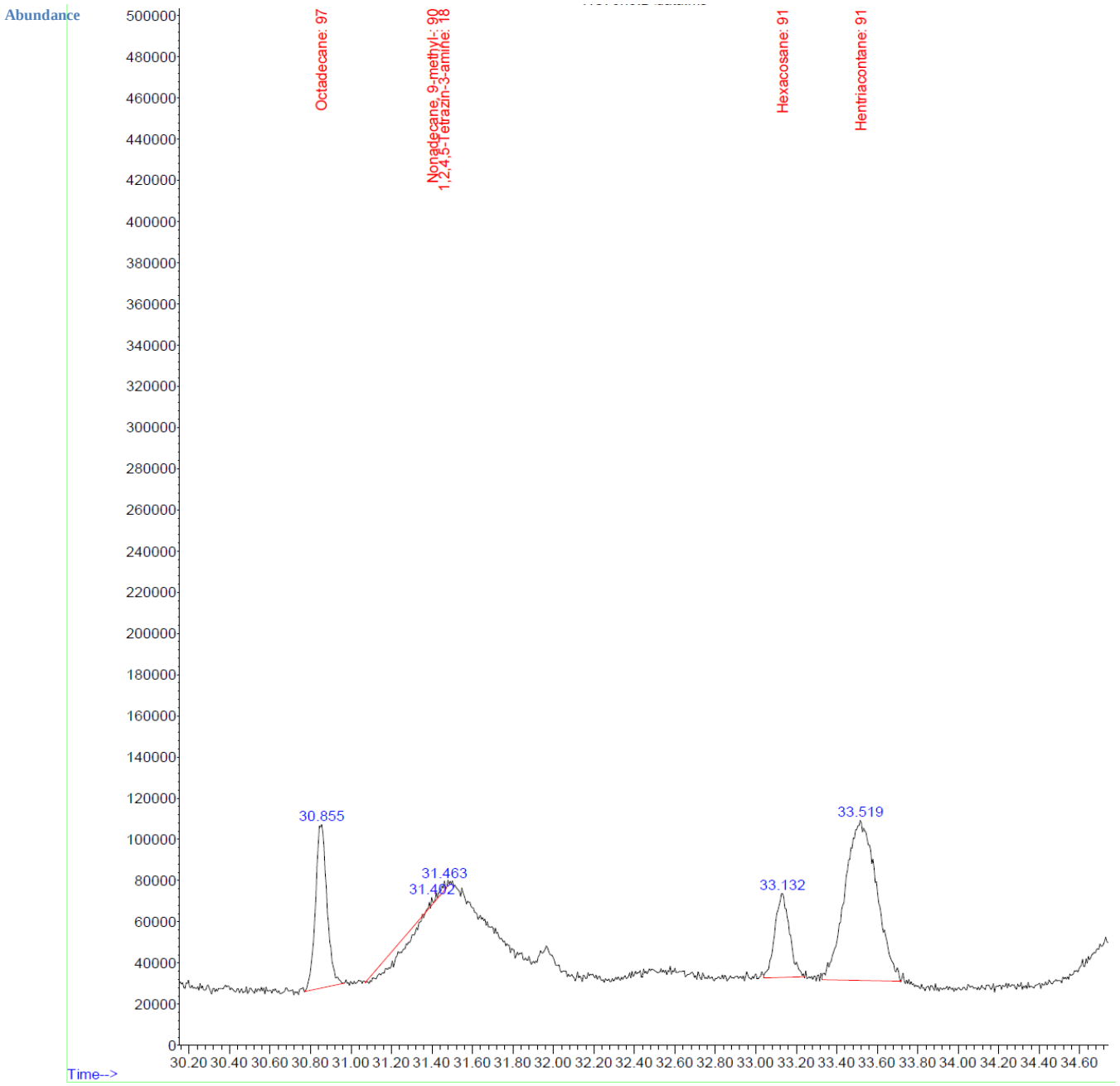

Fig. 1b: Chromatographic profiles of saturated hydrocarbons from healthy jasmine (*Jasminum sambac* L.) leaves

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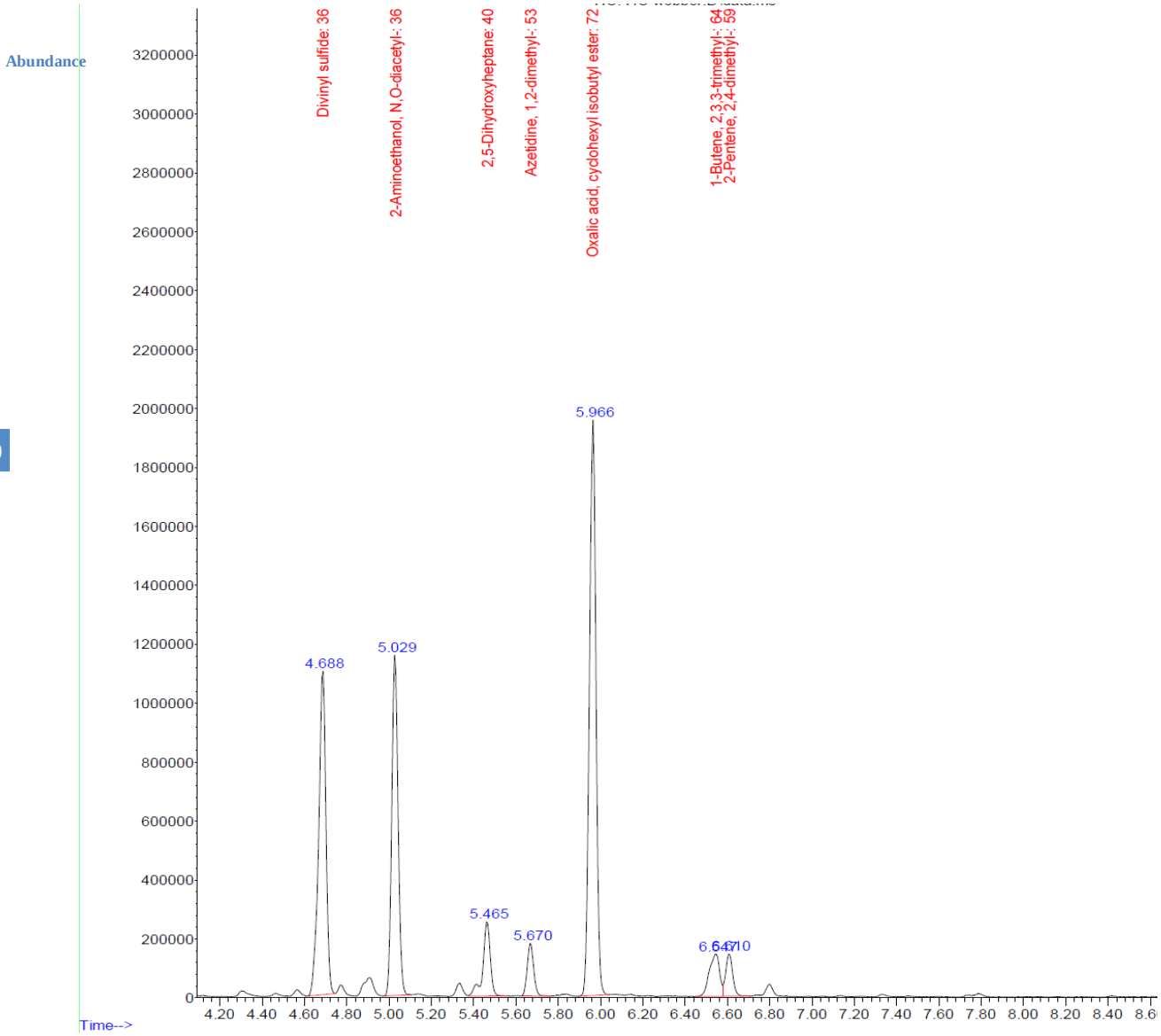

Fig. 2a: Chromatographic profiles of saturated hydrocarbons from leaf webworm, *Nausinoe geometralis* infested jasmine leaves

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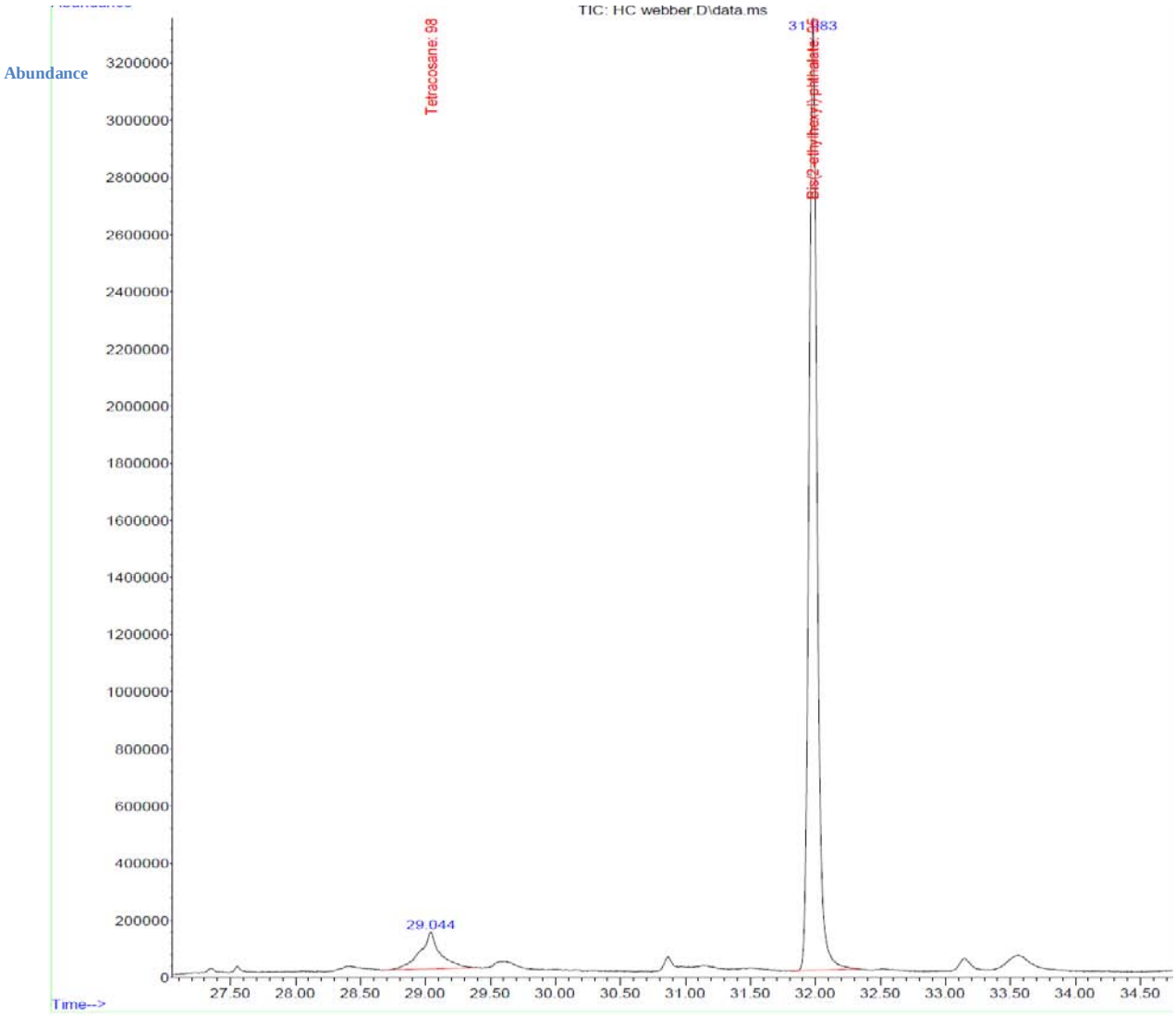

Fig. 2b: Chromatographic profiles of saturated hydrocarbons from leaf webworm, *Nausinoe geometralis* infested jasmine leaves

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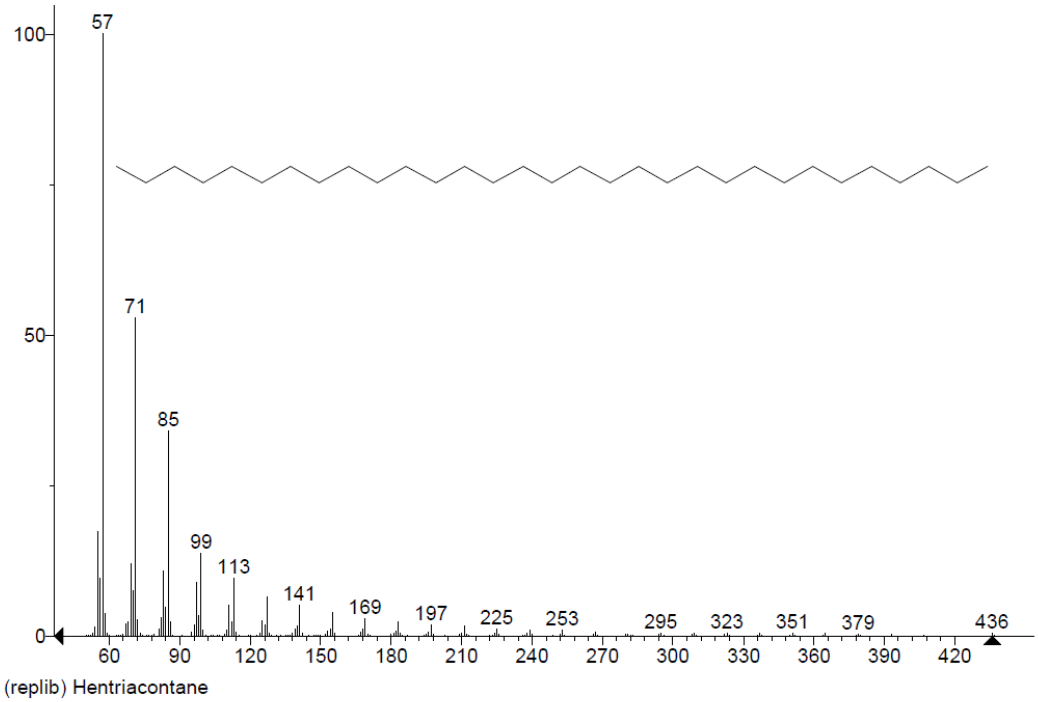

Fig. 3: Mass spectrum and structure of hentriacontane

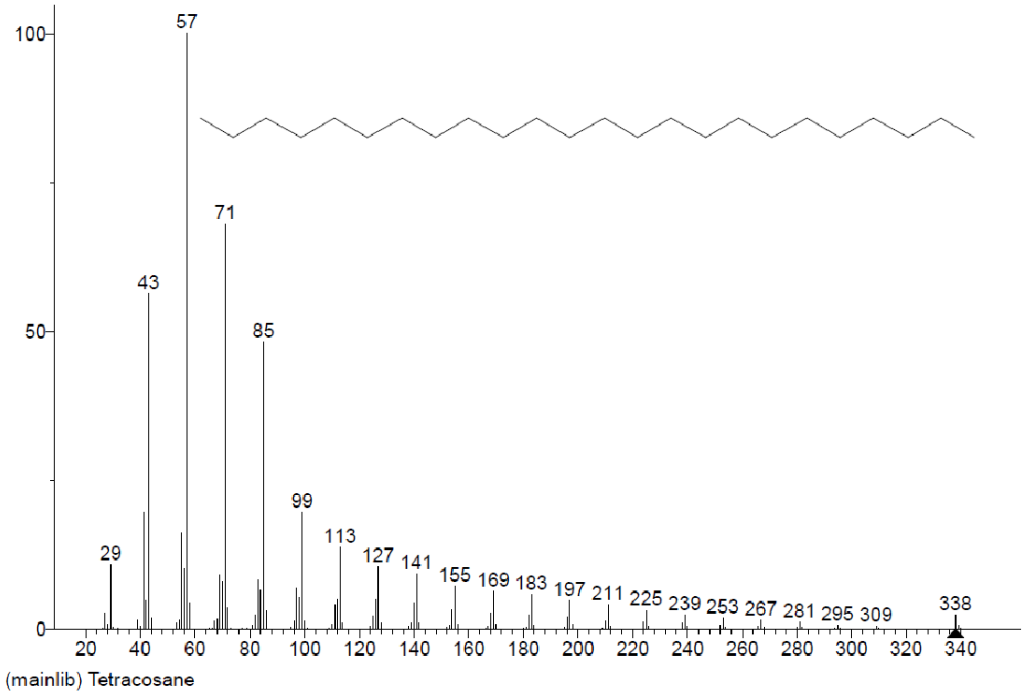

Fig. 4: Mass spectrum and structure of tetracosane

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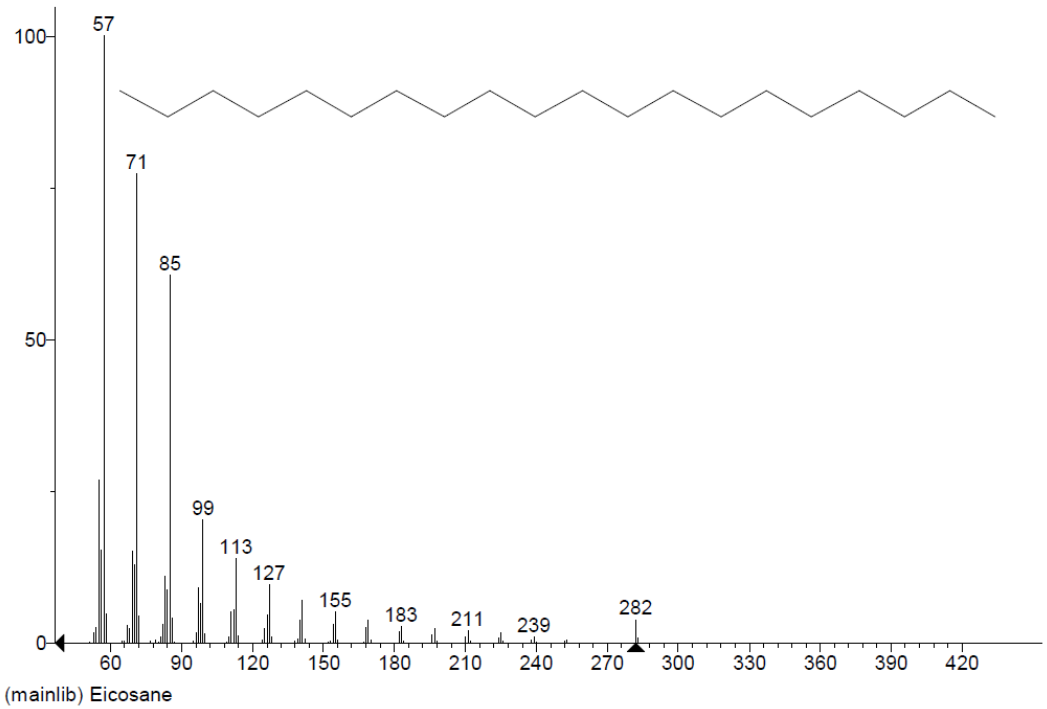

Fig. 5: Mass spectrum and structure of eicosane

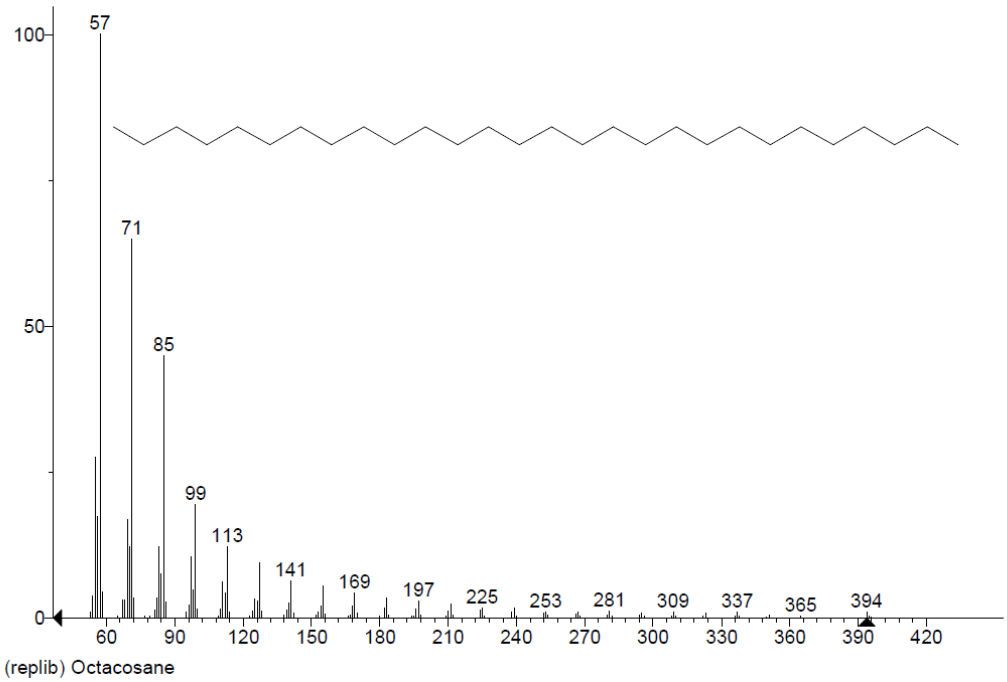

Fig. 6: Mass spectrum and structure of octacosane

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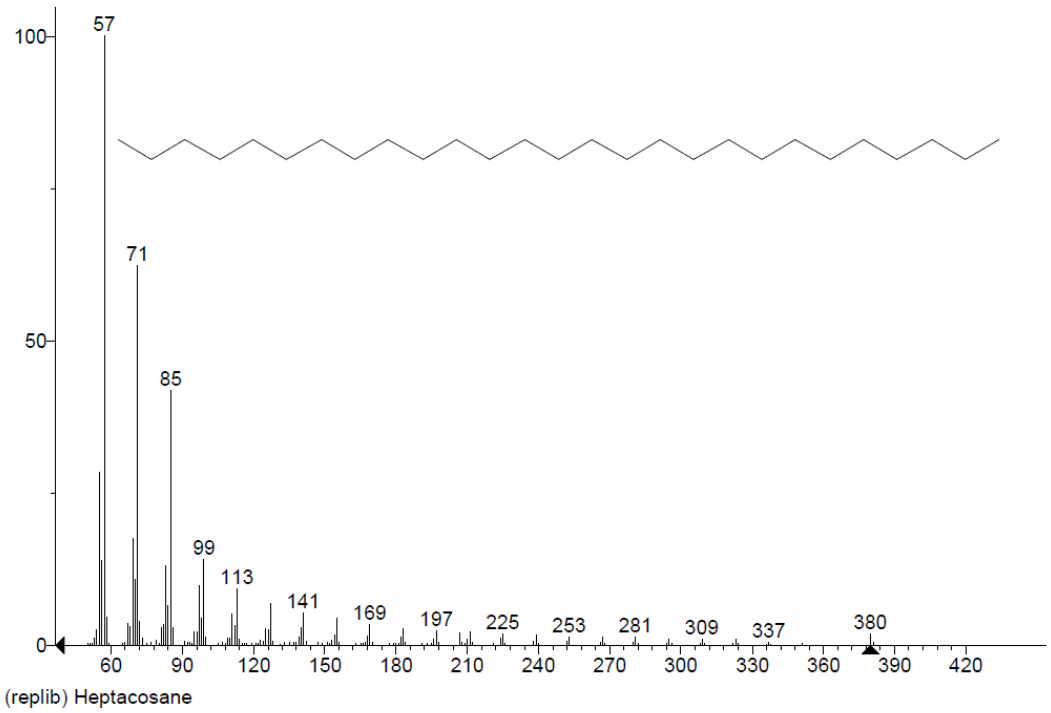

Fig. 7: Mass spectrum and structure of heptacosane

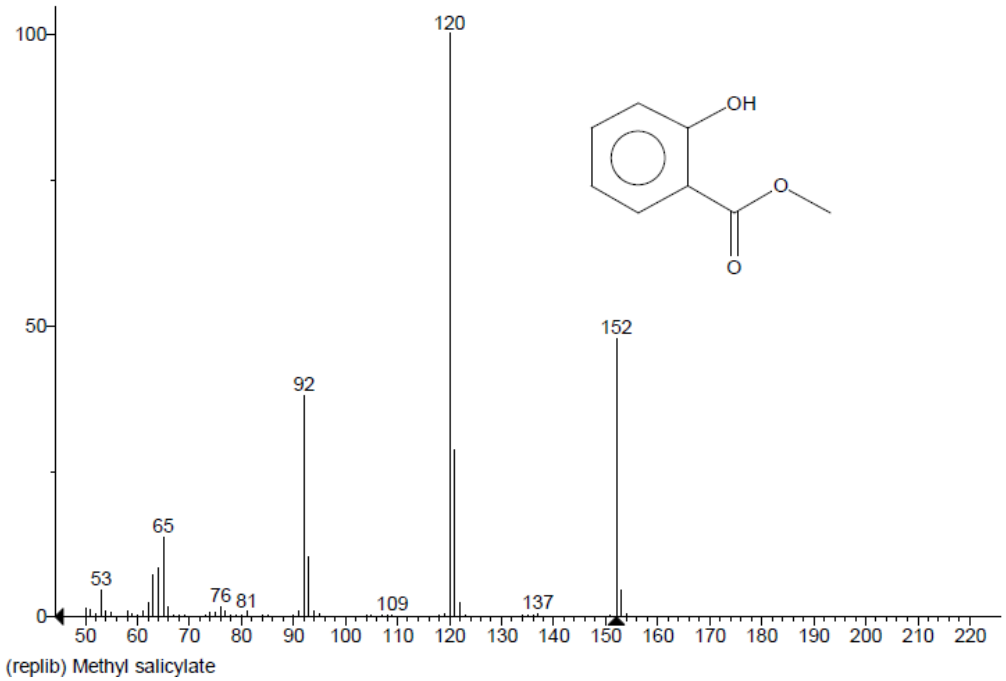

Fig. 8: Mass spectrum and structure of methyl salicylate

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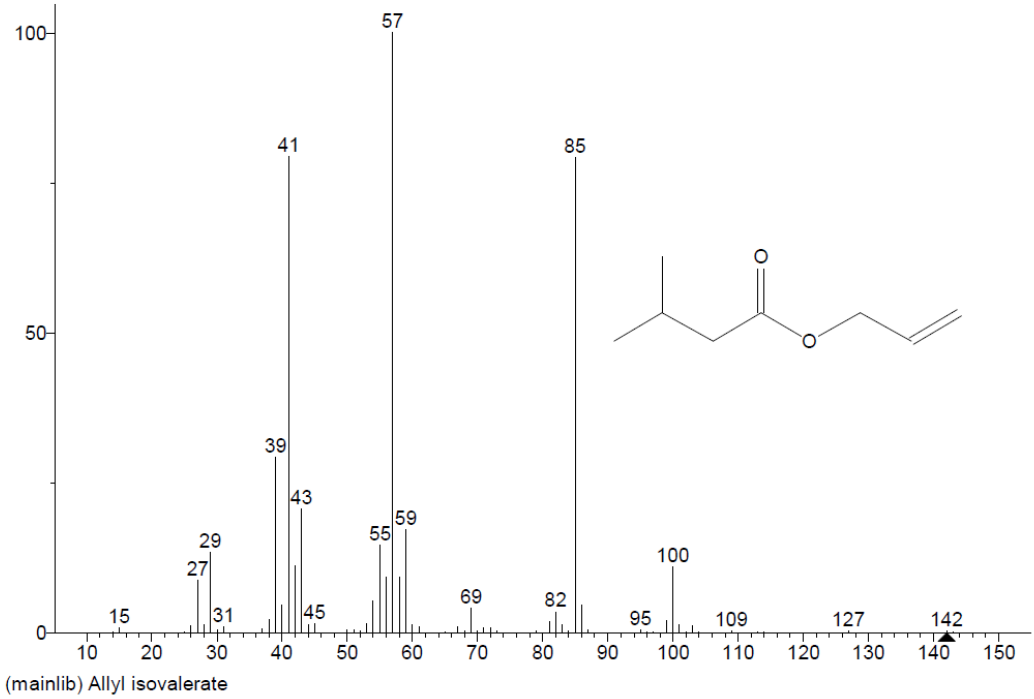

Fig. 9: Mass spectrum and structure of allyl isovalerate

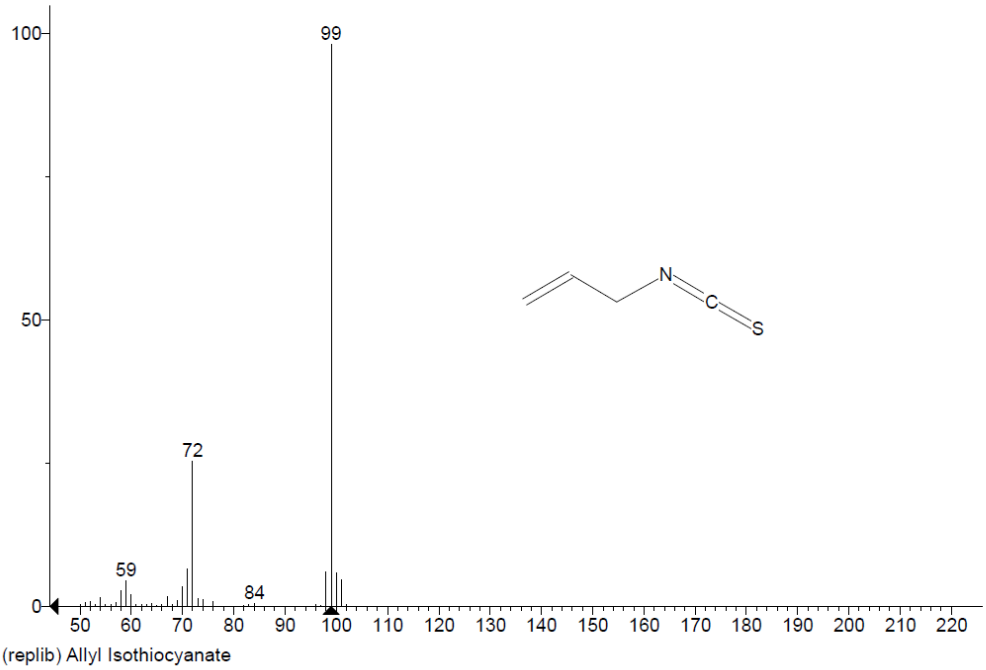

Fig. 10: Mass spectrum and structure of allyl isothiocyanate

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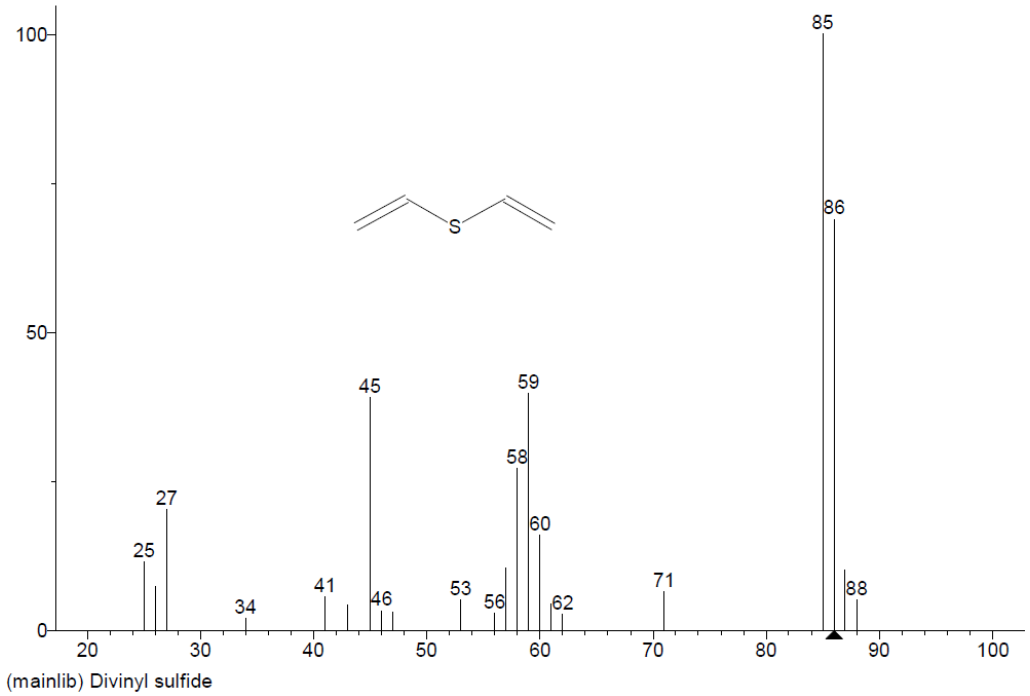

Fig. 11: Mass spectrum and structure of divinyl sulfide

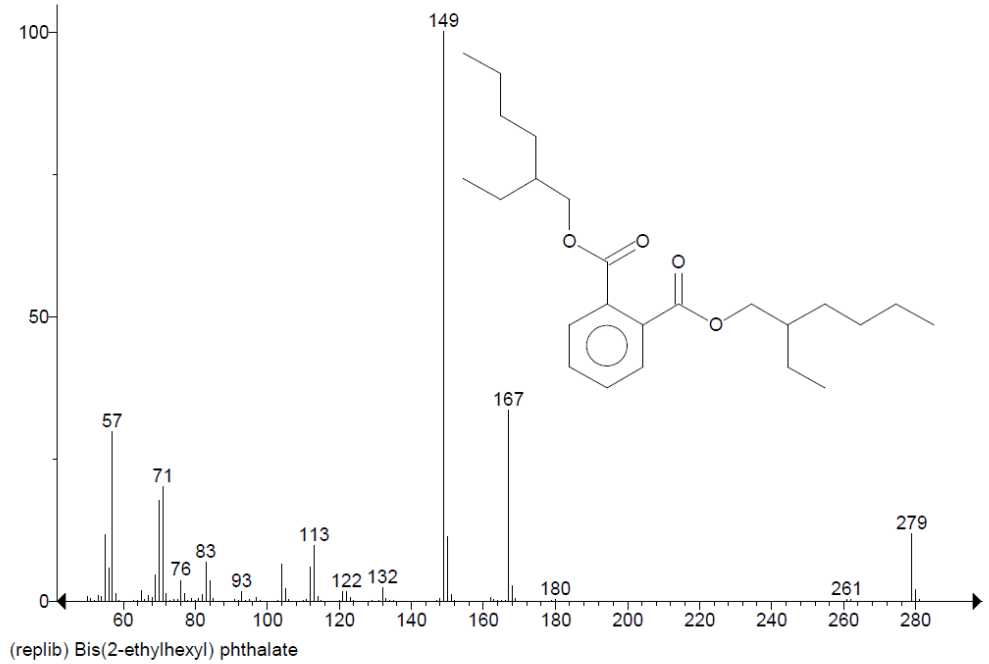

Fig. 12: Mass spectrum and structure of bis (2-ethylhexyl) phthalate

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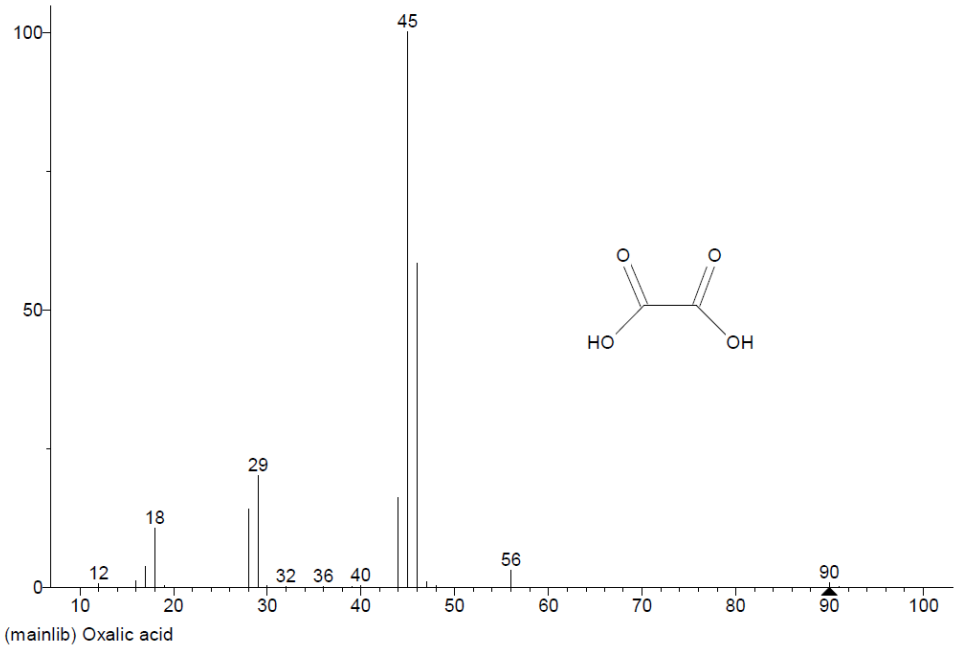

Fig. 13: Mass spectrum and structure of oxalic acid

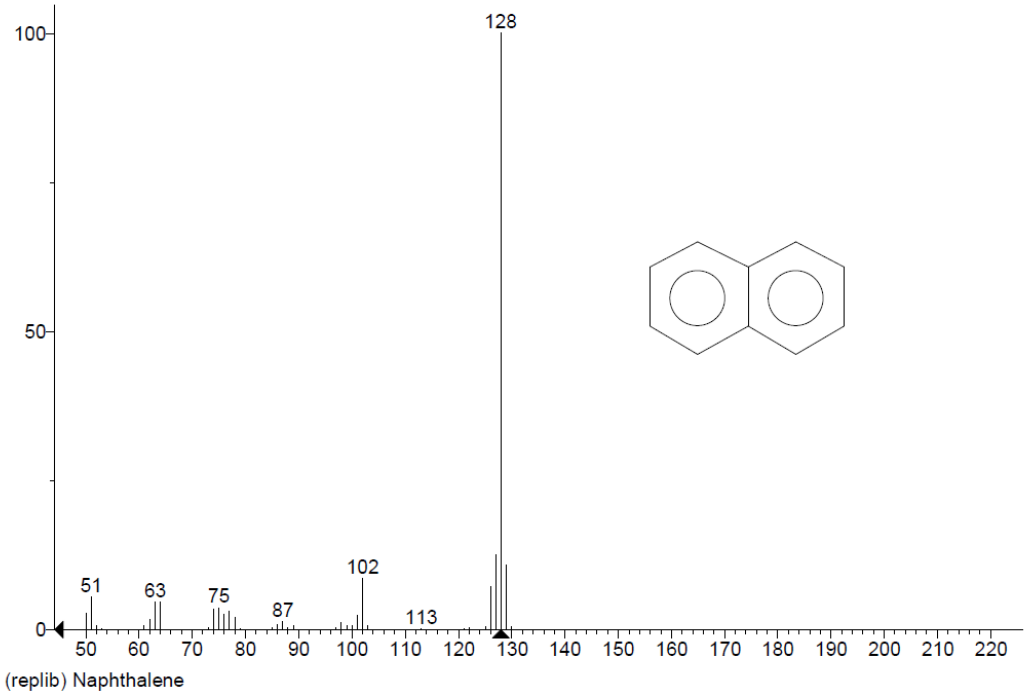

Fig. 14: Mass spectrum and structure of napthalene

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Global Journal of Science Frontier Research: D
Agriculture and Veterinary
Volume 18 Issue 1 Version 1.0 Year 2018
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Investigation of the Energy Flow and its Effects on Global Warming Potential (GWP) of Rainfed Wheat Farms in the Golestan Province (Aqal City)

By Mohammad Taghi Feyzbakhsh & Alireza Alazmani
Research Institute of Mountain Stockbreeding and Agriculture

Abstract- Recently evaluation of input, output and global warming potential (GWP) have been an extension in sciences of agricultural. For this study, 95 farmers were selected for rained Wheat in the semi-salty farm in the north of Golestan Province (Aqal City).The data including (machines, seeds, fertilizers, fuel, and pesticides) were collected by questioner. Then fuel, input and output energy, energy evaluation indexes and global warming potential (kg CO_2/ha^{-1}) were calculated. Results showed that the most direct input energy from fuel in rained Wheat was 38.8 percent. Also, the highest indirect input energy in rainfed Wheat was 31.3 that related to fertilizers. The ratio of output to input energy in rainfed Wheat was calculate 5.01. The amount of GWP was 943.5 (kg CO_2/ha^{-1}) in rainfed Wheat. The highest GWP was related to nitrogen fertilizer and fuel consumption. For Wheat, the consumption of fuel and fertilizer constitute the high percent of energy consumption and greenhouse gas emissions. So that, the use of devices that reduce fuel consumption is recommended, also need for research and investigation on crop rotation and nitrogen fixation plants were revealed.

Keywords: *specific energy, indirect energy, fuel, field operations.*

GJSFR-D Classification: *FOR Code: 070199*

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Investigation of the Energy Flow and its Effects on Global Warming Potential (GWP) of Rainfed Wheat Farms in the Golestan Province (Aqal City)

Mohammad Taghi Feyzbakhsh [α] & Alireza Alazmani [σ]

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Keywords: specific energy, indirect energy, fuel, field operations.

I. Introduction

Because of the many factors, Wheat is grown all around the world. The main Factor is varieties available, which can under many climatic and soil conditions. According to Ministry of Jihad e Agriculture in 2016, Wheat is the most important crop in Iran and its 63.1 percent shares of the total Cultivating area. Also according to the latest statistics, 209 thousand hectares were sown rainfed Wheat in the Golestan province (Ministry of Jihad e Agriculture, 2016). Energy productivity is one of the most factors for sustainable agriculture. The use of fossil fuels and chemical fertilizers were threatening the environment (Kocheki, 1994). Comparison of energy consumption of crops is one way of selecting different in each region. (Feyzbakhsh and Soltani, 2015). Recently evaluation of input, output and global warming potential (GWP) have been an extension in sciences of agricultural. There are many studies on energy usage pattern for crops (Feyzbakhsh and Soltani, 2015; Strapatsa et al., 2006; Darlington,1997; Nasirian et al., 2006; Shahin et al., 2008; Ghorbani et al., 2011).

The crucial role of energy in the development of economic sectors such as industry, transport, and agriculture has led researchers to study management in energy consumption (Strapatsa et al., 2006).

Most of the energy used to produce agricultural products is due to the use of inputs such as machinery, fossil fuels, fertilizers, and pesticides, which inappropriate use of these while lowering energy efficiency in production, also cause health and human health problems. At the moment, due to the energy crisis in the world, it is necessary to study more about energy consumption and find solutions for its optimal use (Darlington,1997).

Energy has defined the ability to do the work. Given the growing need for in today's world, high prices and limited energy resources, as well as the effects of unusual and excessive use of in greenhouse gas emissions, as well as accelerating the global warming process, today the energy category in all economic infrastructures, including the industry, Services and agriculture have become one of the most topics for researchers and scientists (Abdollahpour et al., 2009). Human has turned to using available and affordable fossil fuels to meet his energy needs. This kind of resource accounts for 95% of the world's energy consumption. The rise in fossil fuel consumption contributes to air pollution and global changes in the climate of the planet. Based on the global agricultural scale, it consumes about 5 percent of the total energy of fossil fuels. Industrial crop energy is divided in direct and indirect (Nasirian et al., 2006).

Shahin et al. (2008) in their research in Ardabil provinces indicated that Wheat production consumed a total of 38.36 GJ ha^{-1} of which fertilizer energy consumption was 38.45% followed by diesel and machinery energy. And Output–input energy ratio and energy productivity were found to be 3.13 and 0.16 kg of Wheat MJ^{-1}, respectively. Also, large farms were more successful in energy use and energy ratio.

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Esengun et al. (2007) reported that energy use in agriculture had been developer in response to increasing populations, a limited supply of arable land and desire for an increasing standard of living. In all societies, these factors have encouraged an increase in energy inputs to maximize yields, minimize labor-intensive practices, or both.

Tipi et al. (2009) reported that Wheat production consumed a total of 20,653.54 MJ ha-1 energy depending mainly on fossil fuels. Also, they showed that the energy input of diesel (45.15%) has share in the total energy consumption followed by fertilizers (34.21%), mainly nitrogen (31.77%).

Valadiani et al. (2005) evaluated energy consumption in rainfed Wheat of East Azarbaijan and showed that the highest energy consumption in these fields was related to nitrogen fertilizer, machinery ,and diesel fuel respectively. They that the lowest energy consumption belongs to human labor and herbicide.

There are much research regarding energy (input and output rates) in different crops. Low input systems compared to high input systems have greater energy use efficiency and lower emissions of greenhouse gasses (Dalgaard et al., 2001).

Beheshti Tabar et al. (2010) reported that with higher yields and improved agricultural practices in the Wheat irrigated systems, the unit of land used per unit of output, reduced by 32% in 2006 compared to 1990.

Ghorbani et al. (2011) reported that total energy input used in irrigated Wheat production was about 45367.63 MJ ha^{-1}, which is five times more than that of dryland fields (9354.2 MJ ha^{-1}) in North Khorasan, Iran. They stated that the factor resulting in excessive energy use in irrigated production was application chemical fertilizers. Also, they reported that the amount of energy used in different agricultural practices such as machinery, irrigation, electricity, and diesel in irrigated production system was higher than those of dryland systems.

This study was conducted to determine the GWP and its energy evolution and global warming potential (GWP) in Wheat production in northern lands of Gorgan to identify suitable strategies for avoiding energy losses and reducing environmental impacts of resources.

II. Materials and Methods

This research was did in the semi-salty farm in the north of Gorgan- Golestan province (Aqal City). This is locating in the North of Iran near the Caspian Sea. Overall, Golestan has a moderate and humid climate known as "the moderate Caspian climate." The factors lead to such are the Alborz mountain range, the direction of the mountains, vicinity to the Caspian sea, especial vegetation surface, local winds, and altitude and weather fronts. As a result of the above factors, three different climates exist in the region: plain moderate, mountainous, and semi-arid. The weather in this area is moderately cool in the spring and warm and humid in the summer (Mokhtarpour 2011). This province extends from 36°_44' N to 38°_05' N, and from 51°_53' E to 56°_14' E. The Golestan province area covers approximately 2115 Km2 (Kazemi Poshtmasari et al. 2012). The data for this study were collecting from 93 Wheat farms by using a face-to face questionnaire in the production year 2015/2016. Farms were randomly choose from the villages in the area. The data including (machines, seeds, fertilizers, fuel, and pesticides) were collected by questioner.

After this stage, data were calculated by Excel software in three parts: Fuel consumption, Energy consumption and Global Warming Potential (kg CO_2/ha^{-1}).

Based on the energy equivalents of the inputs and output (Tables 2.3.4 and 5), the energy ratio (energy use efficiency), energy productivity specific energy and the Net energy were calculated (Feyzbakhsh and Soltani, 2015).

Energy use efficiency = Energy Output (MJ ha^{-1}) / Energy Input (MJ ha^{-1}) (1)

Energy productivity = Grain output (kg ha^{-1}) / Energy Input (MJ ha^{-1}) (2)

Specific energy = Energy input (MJ ha^{-1}) / Grain output (kg ha^{-1}) (3)

Net energy = Energy Output (MJ ha^{-1}) - Energy Input (MJ ha^{-1}) (4)

The global warming potential was calculate as follows:

1. Estimated energy consumption of each input and crop operation.
2. Calculate the energy consumption for each and operation from various sources such as electricity, gas oil, natural gas, and oil about each of these energies.
3. Calculation of the amount of three greenhouse gas CO_2, N_2O, and CH_4 produced by using the product of the amount of energy consumed and the coefficients of production of each gas.

Given the different power of N_2O and CH_4 in global warming potential, the total greenhouse gas emissions were calculation as equivalent to Co_2.

III. Results and Disruptions

Variable inputs for Wheat are give in Table (3). Fuel consumption as one of the energy inputs for land preparation, cultivation and transportation is use. The results of this study showed that fuel consumption is high in Wheat cropping. The reason for the high fuel consumption in these fields can be due to the use of

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non-efficient machines and equipment. In general, the amount and percentage of energy consumed in different operations in agriculture and crops and different countries are different. Unclear antecedent is due to the different climatic, ecological and agricultural conditions of these countries. The use of fossil fuels in agriculture has begun since about 70 years ago and continues. There is a need to find other fossil fuels other than fossil fuels, even for exporting countries (Kocheki et al., 1999). One of the ways to reduce fuel consumption is using new and suitable agro-industry such as a multi-purpose machine (Combined). This fuel consumption (Rajabi et al., 2010). Adhering to sustainable agricultural systems and adhering to low farming practices such as reduced plowing can be a means of reducing the high fuel consumption in agriculture. It is reported that tillage can reduce up to 55% of fuel consumption without lowering yield (Bonari et al., 1995).

Table (4) shows the input energy of the rainfed Wheat regarding megajoules per hectare. Among different inputs, seed with the average input energy of 3140 MJ/ha^{-1} (24.23%), had the highest share. The amount used depends on the type and manner of use of planting machines. Factors such as tillage and proper land preparation for cultivation and the use of efficient planting machines can in seedling consumption. Also, the herbicide with 7.3% had the lowest input of energy.

In Table 5, energy consumption is divide into two direct and indirect energy consumption. The highest direct input energy is related to fuel. The high share of fuel in the input energy of these fields can be due to the use of low-productivity machinery. Also, the most indirect input energy is related to nitrogen fertilizer with values of 31.3%.

Table 6 shows that the output to input ratio in rainfed Wheat fields is 1.05, which energy efficiency in low, which can be due to increasing energy input in the form of fuel and chemical fertilizers. Input-to-output ratio in Wheat cultivation in Turkey, 2.8 (Canakci et al., 2005), in Ardabil Wheat fields 1.92 (Shahan et al., 2008), the Wheat fields in the Rey, 2.63 (Alipour et al., 2014), and in the Saveh, 0.1-0.6 (Tabatabayefar, 2002) had been reported. It seems that one of the reasons for the relative efficiency of energy use in Wheat fields of Gorgan is to regard crop rotation and use its benefits in the cultivation of this product. Constant cultivation of a plant in one land, in addition to reducing plant yield due to nutrient evacuate, causes weed infestation, pests, and diseases that the invading of these factors inevitably leads to more use of inputs, which, in addition to reducing energy efficiency, causes Increases environmental pollution. In general, using high yielding cultivars, compact farming systems, increasing the use of fertilizers and chemical pesticides, and high levels of agricultural mechanization has led to an increase in energy consumption in modern agriculture (Singh et al., 2004).

In this study net, energy efficiency was estimated at Wheat fields 58701, and the specific energy content was 6.3. Konkhani et al. (2005) reported energy were 5.24 and 3.88 for Wheat and Corn respectively.

Table 7 shows the GWP per kilogram CO_2 per hectare. The greatest GWP is in rainfed Wheat fields, related to fuel with a share of 42.7 percent. The highest amount of greenhouse gas emissions from nitrogen fertilizers was obtain, and the least amount of greenhouse gas emissions was due to potassium fertilizer. This indicates that the sectors with the highest fuel consumption had the highest greenhouse gas emissions and hence the GWP. Also, the GWP was obtained Per unit area, Per unit weight, Per unit energy input and Per unit energy output (kg eq-CO_2 GJ^{-1}) with 943.5, 410.2, 64.6 and 12.8 respectively in rainfed Wheat in the Aqal City. Feyzbakhsh and Soltani (2013) their study on maize in Golestan province reported that the least GWP was obtain from spring planting with 2349 kg eq-CO_2 GJ^{-1}.

IV. Conclusions

In rainfed Wheat, the consumption of fuel and fertilizer constitute the highest percent of energy consumption and greenhouse gas emissions. So that, the use of devices that reduce fuel consumption is recommend, also need for research and investigation on crop rotation and nitrogen fixation plants were reveal. With attention on the results we suggested that with improved management practices, efficient use of fertilizers, efficient use of fertilizers, increased yield and with increasing the yield, improved energy efficiency.

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Table 1: Agricultural operations times for rainfed Wheat in Aqal City

Field operations	Time
Tillage (30 cm)	November
Disk (1)	Erlay December
Disk (2)	Erlay December
Mixing fertilizer with soil	Erlay December
Basal dressing	Erlay December
Planting seeds	Erlay December
Weed control	March
Top dressing	Erlay March
Fungi Disease Control	April
Harvest	Late June

Table 2: Energy equivalent of inputs and outputs in rainfed Wheat in Aqal City

Iinputs / outputs	Unit	Energy equivalent (MJ/unit)	Reference
Wheat Seed	kg	15.7	(Canakci*et al*., 2005; Rathke*et al*., 2007)
Human labor	Hour	1.96	(Akcaoz *et al*., 2009)
N fertilizers	kg	60.6	(Ozkan *et al*., 2004; Akcaoz *et al*., 2009)
P fertilizers	kg	11.1	(Ozkan *et al*., 2004; Akcaoz *et al*., 2009)
K fertilizers	kg	6.7	(Ozkan *et al*., 2004; Akcaoz *et al*., 2009)
Gasoline	L	38	(Hydrocarbon balance sheet of Country, 2008)
Herbicide	kg a.i.b	287	(Tzilivakis *et al*., 2005; Rathke *et al*., 2007)
Insecticide	kg a.i.b	237	(Tzilivakis *et al*., 2005; Rathke *et al*., 2007)
Wheat grain	kg	15.7	(Canakci *et al*., 2005)
Stems and leaves of Wheat	kg	9.25	(Rajabi et al.,2012)
Fungicide	kg a.i.b	99	(Strapasta et al.,2006)

Table 3: Inputs used for Rainfed Wheat in Aqal City

Input	Unit	Rainfed Wheat
Fuel	l ha^{-1}	136
Seed	kg ha^{-1}	200
Fertilizer		
Nitrogen	kg ha^{-1}	150
Phosphorus	kg ha^{-1}	70
Potassium	kg ha^{-1}	50
Herbicide	kg a.i.b	257
Human labor	Hour	18
Agricultural operations		
Plow	Time	1
Fertilizer Distribution	Time	2
Planting with row planter	Time	1
Spraying (pesticides, fungicides)	Time	3
Harvest	Time	1

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Table 4: Energy inputs (MJ^{-1} ha^{-1}) dividing farming group in rainfed Wheat

Field operations	Average	Percent of total
Plow	1379.4	10.3
Disk	1909.4	14.2
Basal dressing	1004.5	7.5
Seed	3140	23.4
Row planter	1083.2	8.1
Herbicides	510.9	3.7
Fungiicides	506.3	3.8
Top dressing	3084.1	23
Harvest	748.2	5.6
Total	13366.5	100

Table 5: Direct and indirect energy inputs for rainfed Wheat in Aqal City

Energy inputs	Average	Percent of total
Direct		
Fuel for field operations	5168	38.7
Human labor	27.4	0.2
Indirect		
N fertilizers	4178.6	31.3
P fertilizers	322	2.4
K fertilizers	160.8	1.2
Seed	3140	23.5
Herbicide	133	1
Fungiicides	128.3	0.9
Machinery	661.2	5
transportation	696.4	5.2
Total	14615.7	100

Table 6: Different energy indices for rainfed Wheat in Aqal City

Indices energy	Rainfed Wheat
Inputs	
Direct input energy (GJ ha^{-1})	5.1
Indirect input energy (GJ ha^{-1})	22.6
outputs	
Stem+leaf output energy (GJ ha^{-1})	39.5
Grain output energy (GJ ha^{-1})	33.8
Ouput/input ratio	5.01
Specific energy (GJ ton^{-1})	6.3
Energy productivity (ton GJ $^{-1}$)	0.157
Net energy (GJ ha^{-1})	58701

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Table 7: GHG emissions (kg e-CO_2 ha^{-1}) for rainfed for rainfed Wheat Aqal City

Operations	Average	Percent of total
Production and transportation		
Nitrogen	304.2	32. 24
Phosphorus	26.4	2.79
Potassium	13.1	1.38
Pesticides	26.9	2.85
Fuel	403.1	42.72
Production, transportation and maintenance equipment and machinery	138.6	14.68
Total GWP	943.5	100

Table 8: GHG emissions in per unit area, per unit weight, per unit energy input and per unit energy output in rainfed Wheat in Aqal City

Per unit area (kg eq-CO_2 ha^{-1})	943.5
Per unit weight (kg eq-CO_2 t^{-1})	410.2
Per unit energy input (kg eq-CO_2 GJ^{-1})	64.6
Per unit energy output (kg eq-CO_2 GJ^{-1})	12.8

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Unless specified in the notification, the Editorial Board's decision on publication of the paper is final and cannot be appealed before making the major change in the manuscript.

Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

Declaration of funding sources

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Preparing your Manuscript

Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11'", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.

Format Structure

It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

All manuscripts submitted to Global Journals should include:

Title

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

Author details

The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

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

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning 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 a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for Writing a Good Quality Science Frontier Research Paper

Techniques for writing a good quality Science Frontier Research paper:

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of science frontier then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.

6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.

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Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

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Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.

Segment draft and final research paper: You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

Written material: You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.

Criterion for Grading a Research Paper (Compilation) by Global Journals

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	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
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

INDEX

Global Journal of Science Frontier Research

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ISSN 9755896

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