ISSN: 2156-8553 Vol.1, No.2, August 2010

Scientific Research

# Agricultural Sciences

ISSN: 2156-8553

9 772156 855001 02

www.scirp.org/journal/as

# Journal Editorial Board

**ISSN Print: 2156-8553  ISSN Online: 2156-8561**
**Hhttp://www.scirp.org/journal/as/**

# TABLE OF CONTENTS

## Volume 1, Number 2, August 2010



# Agricultural Sciences

## Journal Information

### SUBSCRIPTIONS

The *Agricultural Sciences* (Online at Scientific Research Publishing, www.SciRP.org) is published quarterly by Scientific Research Publishing, Inc., USA.

**Subscription rates:**

Print: $40 per copy.

To subscribe, please contact Journals Subscriptions Department, E-mail: sub@scirp.org

### SERVICES

**Advertisements**

Advertisement Sales Department, E-mail: service@scirp.org

**Reprints (minimum quantity 100 copies)**

Reprints Co-ordinator, Scientific Research Publishing, Inc., USA.

E-mail: sub@scirp.org

### COPYRIGHT



### PRODUCTION INFORMATION

For manuscripts that have been accepted for publication, please contact:

E-mail: as@scirp.org

**Vol.1, No.2, 51-55 (2010)**
doi:10.4236/as.2010.12007

Agricultural Sciences

# Effects of treatment methods on the nutritional value of cotton seed cake for laying hens

**David Friday Apata**

Department of Animal Production, University of Ilorin, Ilorin, Nigeria; dfapataunilorin@yahoo.com

Received 17 June 2010; revised 8 July 2010; accepted 21 July 2010.

## ABSTRACT

**The effects of treatment methods on the nutritive value of cotton seed cake (CSC) for laying hens were examined. Olympia laying hens (n = 96) were assigned randomly to a 2 × 3 factorial combination of fermented or unfermented CSC supplemented with enzyme, Vitamin E and ferrous sulphate over a 4-week feeding period. CSC was incorporated at 15% in the diets. Results showed no significant differences ($P > 0.05$) in feed intake, body weight gain and egg weight due to treatments. However, hen-day egg production was significantly reduced ($P < 0.05$) among dietary treatments compared with the control. Layers fed on unfermented CSC + Vitamin E and unfermented CSC + $FeSO_4$ had the lowest ($P < 0.05$) values for packed cell volume and haemoglobin. Treatment differences in red blood cell and white blood cell were not significant ($P > 0.05$). Serum concentration of total protein among dietary treatments was lower ($P < 0.05$) than the control, while albumin and cholesterol values were similar ($P > 0.05$) for all treatments. Generally, activities of alanine and aspartate aminotransferases increased ($P < 0.05$) among CSC dietary treatments compared with the control diet. The interaction between CSC form and additives had no significant effect on any of the parameters. The results of this study indicate that the treatment methods employed for CSC could not significantly improve its nutritive value for egg production at 15% level in the diet of laying hen.**

**Keywords:** Cotton Seed Cake; Haematology; Serum Constituents; Laying Hen

## 1. INTRODUCTION

One major limiting factor in the expansion of egg production enterprise is the high cost of feedstuffs such as soybean meal, groundnut cake and fish meal. This has made egg production expensive, thereby worsening the intake of protein sources by the populace of developing countries. One approach to reduce pressure on conventional protein ingredients is greater utilization of alternative sources of dietary protein alongside other strategies.

Cotton seed cake (CSC) is a by-product of the cotton processing industry. The cake is rich in protein (42%) but it contains gossypol which has been recognized since the turn of the century to be toxic to animals [1,2]. In monogastric animals, gossypol interfered with protein digestion, bind lysine (making it unavailable) and reduced growth rate and productivity [3,4]. There is another problem as the cake contains a high level of fibre, perhaps 23% because undelinted seeds are used in the processing [5]. This high fibre content has limited the extent to which it can be used in the diets of poultry which lack the appropriate enzymes capable of degrading fibre [6,7].

Currently, CSC is used by feed millers in Nigeria at 7.5% inclusion level with ferrous sulphate supplement to inactivate gossypol in balanced layers feed. This chemical is usually not available to local farmers at farm sites. It becomes imperative therefore that simple treatment method be found that will overcome the gossypol and degrade the high fibre in CSC so that its nutritive value could be enhanced. Vitamin E is an antioxidant that prevents the damage of free radicals at the cellular level [8].

This study was designed to investigate the effectiveness of enzyme, vitamin E and ferrous sulphate treatments in improving the nutritive value of CSC for laying hens.

## 2. MATERIALS AND METHODS

Cotton seed cake was obtained from a feed miller in Ilorin. Samples (20 kg) of the cake were placed in a double layer polythene bag, tied up and placed in an empty plastic drum, sealed and fermented for a period of 7 days. Thereafter, the fermented cake was removed and sundried for 3 days. The fermented and a batch of the unfermented cake (20 kg) were used in preparation of the diets described below.

Seven isonitrogenous (18% crude protein) and isoaloric (2623 kcal/kg ME) experimental diets (**Table 1**) were formulated by incorporating fermented or unfermented CSC at 15% and supplemented with enzyme, vitamin E and Ferrous Sulphate ($FeSO_4$) at 0.01, 0.03 and 0.20%, respectively in a 2 × 3 factorial design. Maize soybean meal-based diet was used as control. The enzyme preparation (Allzyme) derived from *Aspergillus niger* contains amylase, beta-glucanase, cellulase, pectinase, phytase, protease and xylanase activities. The level of enzyme supplementation was chosen according to manufacturer's recommendation.

Ninety six hens of the Olympia strain that had been in lay for 10 weeks were purchased from a commercial poultry farm in Ilorin. The birds were individually caged in a poultry pen which was illuminated at night and were randomly assigned to the dietary treatments. The trial lasted for 4 weeks. Feed intake was measured daily and body weight weekly. Records of egg production per cage swere kept daily throughout the experimental period. All eggs laid each day were weighed individually.

**Table 1.** Composition of the experimental diets (%).

| | Unfermented Cotton Seed Cake | | | Fermented Cotton Seed Cake | | | Control |
|---|---|---|---|---|---|---|---|
| Ingredient | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Maize | 50.00 | 50.00 | 50.00 | 50.00 | 50.00 | 50.00 | 50.00 |
| Wheat bran | 12.64 | 12.62 | 12.45 | 13.14 | 13.12 | 12.95 | 15.85 |
| Soybean meal | 12.50 | 12.50 | 12.50 | 12.50 | 12.50 | 12.50 | 27.50 |
| Cotton seed cake | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 0.00 |
| Fish meal | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Blood meal | 3.50 | 3.50 | 3.50 | 3.00 | 3.00 | 3.00 | 0.30 |
| Oyster shell | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 |
| Bone meal | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| [a]Premix | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| DL-Methionine | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| Lysine | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| Salt (Nacl) | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Enzyme | 0.01 | - | - | 0.01 | - | - | - |
| Vitamin E | - | 0.03 | - | - | 0.03 | - | - |
| $FeSO_4$ | - | - | 0.20 | - | - | 0.20 | - |
| **[b]Chemical Composition (%)** | | | | | | | |
| Crude protein | 18.08 | 18.10 | 18.30 | 18.07 | 18.05 | 18.20 | 18.00 |
| Crude fibre | 5.36 | 5.31 | 5.32 | 5.34 | 5.30 | 5.34 | 4.71 |
| Ether extract | 4.01 | 4.01 | 4.01 | 3.92 | 3.92 | 3.92 | 3.89 |
| ME (Kcal/kg) | 2623 | 2623 | 2623 | 2625 | 2625 | 2625 | 2624 |
| Lysine | 1.17 | 1.17 | 1.17 | 1.19 | 1.19 | 1.19 | 1.22 |
| Methionine | 0.52 | 0.52 | 0.52 | 0.53 | 0.53 | 0.53 | 0.49 |

[a]Provided 1.5 mg retinol, 25 mg cholecalciferol, 16 mg α-tocopherol, 1mg menadione, 0.8 mg thiamine, 2.4 mg riboflavin, 14 mg nicotinic acid, 4 mg calcium D-pantothenate, 1.4 mg pyridoxine, 10 mg cyanocobalamin, 0.4 mg folic acid, 0.02 mg biotin, 120 mg choline chloride, 0.62 mg zinc bacitracin, 36 mg avaten, 40 mg Mn, 18 mg Zn, 0.8 mg Cu, 0.09 mg Co, 20 mg Fe and 0.04 mg Se $kg^{-1}$ diet; [b]Determined values except for metabolisable energy (ME), lysine and methionine which were calculated from the published (NRC, 1995) compositions of the ingredients used.

At the end of the experimental period, four hens were randomly selected from each dietary treatment, fasted overnight and blood samples collected from the wing vein. Blood was collected into bottles containing EDTA anticoagulant for haematology and clean dry bottles for serum constituent analysis. Serum samples were separated by centrifugation at 1600 × g for 15 min. and stored at –20℃. The whole blood was analysed for packed cell volume (PCV), haemoglobin (Hb), red blood cell count (RBC) and white blood cell count (WBC) using standard haematological techniques [10]. Serum total protein, albumin, cholesterol, alanine amino transferase (ALT. EC 2.6.1.1) and aspartate amino transferase (AST, EC 2.6.1.1) activities were measured using commercial clinical investigation kits (Wako, Osaka, Japan).

## 3. STATISTICAL ANALYSIS

Data collected were subjected to a two-way factorial analysis and Duncan's multiple range test to determine the significant difference at the 0.05 level [11].

## 4. RESULTS AND DISCUSSION

Laying performance of hens was affected by dietary CSC treatments at the end of the feeding trial (**Table 2**). There were no significant differences ($P > 0.05$) in feed intake, weight gain and egg weight due to treatments. However, hen-day egg production was significantly reduced ($P < 0.05$) among dietary treatments compared with the control. The highest egg production (56.7%) in CSC diets achieved by hens fed fermented CSC + enzyme was lower ($P < 0.05$) than the control (67.00%). Layers feed consumption and body weight gain were unaffected by treatment methods, probably because the fermentation process and the additives used in the diets were unable to cause appreciable reduction in the gossypol level. The feed consumed by the layers appeared to be principally used for body maintenance. This view is similar to that reported by Olorede and Longe [12] that hens can use feed or metabolizable energy intake to fulfill maintenance requirement, thereby leaving little for egg production. In general, the decrease in egg production observed with dietary treatments of CSC might be the consequence of residual effect of gossypol that binds with the cake's protein thereby making it unavailable to the hens for egg production. The marginal improvement found with the fermented cake + enzyme may be attributed to the combination of fermentation process and enzyme degradation of the fibre that made nutrients available to the birds. This is in agreement with Babalola *et al*. [7] who reported improved nutrient availability to pullet chicks fed β-xylanase supplemented castor seed meal diet.

The haematological values are presented in **Table 3**. For all treatments, PCV and Hb were lower than the control with the exception of fermented CSC + enzyme which had similar PCV value with the control. No significant differences ($P > 0.05$) were observed for RBC and WBC. The decreases in PCV and Hb values in hens fed diets containing unfermented CSC or fermented CSC + Vitamin E, or ferrous sulphate, suggest inadequate nutrient utilisation in the hens. This may be partly due to the residual effect of gossypol on blood variables. A reduction in haematological parameter has been reported by Apata [13] and Rinchard *et al*. [5] in chicks and fish fed diets containing high levels of legume seed meal and cotton seed meal, respectively. However, Mitruka and

**Table 2.** Performance of laying hens fed diets containing different treatments of unfermented or fermented cotton seed cake (CSC).

| Dietary treatments | Feed intake (g per hen $day^{-1}$) | Weight gain (g per hen $day^{-1}$) | Hen-day Egg production (%) | Egg weight (g) |
|---|---|---|---|---|
| Unfermented CSC + enzyme | $83.0^{a}$ | $3.4^{a}$ | $45.7^{b}$ | $54.6^{b}$ |
| Unfermented CSC + Vit. E | $86.7^{a}$ | $3.5^{a}$ | $44.3^{b}$ | $54.4^{b}$ |
| Unfermented CSC + $FeSO_4$ | $81.0^{a}$ | $2.9^{a}$ | $43.5^{b}$ | $53.2^{b}$ |
| Fermented CSC + enzyme | $85.8^{a}$ | $3.9^{a}$ | $56.7^{ab}$ | $56.7^{b}$ |
| Fermented CSC + Vit. E | $82.5^{a}$ | $3.1^{a}$ | $53.1^{b}$ | $56.4^{b}$ |
| Fermented CSC + $FeSO_4$ | $78.6^{a}$ | $3.4^{a}$ | $52.8^{b}$ | $55.0^{b}$ |
| Control | $106.5^{b}$ | $5.6^{b}$ | $67.0^{a}$ | $60.1^{a}$ |
| F X A | NS | NS | NS | NS |
| SEM | 7.2 | 1.1 | 6.4 | 3.8 |

Values in a column with the same letter do not differ significantly at $P < 0.05$. NS = not significant; SEM = standard error of means; F = test ingredient form; A = additives

**Table 3.** Haematological values of laying hens fed diets containing different treatments of unfermented or fermented cotton seed cake (CSC).

| Dietary treatments | PVC (%) | Hb (g/dL) | RBC ($\times 10^{12}$/L) | WBC ($\times 10^{9}$/L) |
|---|---|---|---|---|
| Unfermented CSC + enzyme | $24.0^{ab}$ | $8.1^{ab}$ | 4.37 | 9.67 |
| Unfermented CSC + Vit. E | $18.0^{b}$ | $4.3^{a}$ | 3.50 | 11.30 |
| Unfermented CSC + $FeSO_4$ | $18.5^{b}$ | $5.1^{a}$ | 4.09 | 10.85 |
| Fermented CSC + enzyme | $27.0^{a}$ | $7.8^{ab}$ | 4.00 | 12.20 |
| Fermented CSC + Vit. E | $20.0^{b}$ | $6.0^{ab}$ | 4.03 | 9.97 |
| Fermented CSC + $FeSO_4$ | $22.3^{b}$ | $7.1^{ab}$ | 4.10 | 9.60 |
| Control | $30^{a}$ | $9.1^{b}$ | 4.90 | 9.10 |
| F x A | Ns | Ns | Ns | Ns |
| SEM | 1.6 | 0.41 | 0.19 | 1.02 |

Values in a column with the same letter do not differ significantly at $p < 0.05$; PCV = packed cell volume; Hb = haemoglobin; RBC = red blood cell; WBC = white blood cell; Ns = not significant; F = tests ingredient form; A = additives; SEM = standard error of means

**Table 4.** Serum biochemical constituents of laying hens fed diets containing different treatments of unfermented or fermented cotton seed cake (CSC).

| Dietary treatments | Total protein (mmol/L) | Albumin (mmol/L) | Cholesterol (mmol/L) | ALT ($IUL^{-1}$) | AST ($IUL^{-1}$) |
|---|---|---|---|---|---|
| Unfermented CSC + enzyme | $23^{b}$ | 5.3 | 1.8 | $8.67^{b}$ | $118^{b}$ |
| Unfermented CSC + Vit. E | $26^{b}$ | 6.0 | 2.5 | $12.00^{b}$ | $116^{b}$ |
| Unfermented CSC + $FeSO_4$ | $22^{b}$ | 5.5 | 1.5 | $20.00^{c}$ | $216^{d}$ |
| Fermented CSC + enzyme | $24^{b}$ | 5.0 | 1.5 | $19.33^{c}$ | $163^{c}$ |
| Fermented CSC + Vit. E | $27^{b}$ | 5.3 | 1.5 | $19.33^{c}$ | $163^{c}$ |
| Fermented CSC + $FeSO_4$ | $26^{b}$ | 5.3 | 2.1 | $13.67^{b}$ | $134^{c}$ |
| Control | $31^{a}$ | 4.0 | 1.1 | $4.00^{a}$ | $90^{a}$ |
| F x A | Ns | Ns | Ns | Ns | Ns |
| SEM | 1.08 | 0.67 | 0.90 | 1.06 | 13.86 |

Values in a column with the same letter do not differ significantly at $p < 0.05$; AST = aspartate aminotransferase activity; ALT = alanine aminotransferase activity; Ns = not significant; F = tests ingredient form; A = additives; SEM = standard error of means

Rawnsley [14] reported 7.00-18.6 g/dl as normal values of Hb in chickens. Thus, a range of 4.3-5.1 g/dl obtained in this study for unfermented CSC + Vitamin E, or $FeSO_4$ fell short of the range consistent with good layers health.

Of the serum biochemical constituents, total protein among dietary treatments was lower ($P < 0.05$) than the control, whereas the ALT and AST were higher ($P < 0.05$) than the control (**Table 4**), with no significant differences ($P > 0.05$) observed in albumin and cholesterol values for all treatments. Decrease in serum total protein concentration irrespective of the treatment method corroborates the inadequacy of these simple technologies in improving the nutritional quality of this feedstuff especially at the level of inclusion. The increase in serum ALT and AST activities indicate release of the aminotransferases from cytoplasm to blood stream which is probably due to liver and/or other tissues damage. A similar observation has been reported by Muhammad and Oloyede [15] for chicks fed femented *Temialia cattapa* seed meal-based diet.

In conclusion, the results of this study show that the different treatment methods employed for CSC could not significantly improve its nutritive value for egg production at 15% level in the diet of laying hen.

## 5. ACKNOWLEDGEMENTS

This study was partly funded by the senate research grant of the University of Ilorin. The author is grateful for this financial assistance.


## REFERENCES


[1] Morgan, S.E. (1989) Gossypol as a toxicant in livestock. *Veterinary Clinics of North America*: *Food Animal Practice*, **5(2)**, 251-262.

[2] Risco, C.A. and Chase, C.C.Jr. (1997) Gossypol. Handbook of plant and Fungal Toxicants, CRS Press, Boca Raton.

[3] Ikurior, S.A. and Fetuga, B.L.A. (1984) Some anti-nutritional components in Nigeria cottonseed and cottonseed meals. *Nigerian Journal of Animal Production*, **11(2),** 110-116.

[4] Calhoun M.C., Huston, J.E., Kulhmann, S.W., Baldwin B.C.Jr., Engdahi, B.C. and Bales, K.W. (1990) Free gossypol intake and erythrocyte fragility of lambs fed cottonseed meal processed by different methods. *Journal of Animal Science*, **68(1)**, 53-57.

[5] Rinchard, J., Lee, K.J., Dabrowski, K., Ciereszko, A., Blom, J.H. and Ottobrc, J.S. (2003) Influence of gossypol from dietary cottonseed meal on haematology, reproductive steroids and tissue gossypol enantiomer concentrations in male rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Nutrition*, **9(4)**, 275-282.

[6] Danicke, S., Dusel, G., Jeroch, H. and Kluge, H. (1999) Factors affecting efficiency of NSP-degrading enzymes in rations for pigs and poultry. *Agribiology Research*, **51(1)**, 1-24.

[7] Babalola, T.O.O., Apata, D.F. and Atteh, J.O. (2006) Effect of β-xylanase supplementation of boiled castor seed meal-based diets on the performance, nutrient absorbability and some blood constituents of pullet chicks. *Tropical Science*, **46(4)**, 216-223.

[8] Risco, C.A., Adams, A.L., Seebohm, S., Thatcher, M.-J., Staples, C.R. Van Horn, H.H., McDowell, L.R., Calhoun, M.C. and Tatcher, W.W. (2002) Effects of Gossypol from Cottonseed on Haematological Responses and Plasma α-Tocopherol Concentration of Dairy Cows. *Journal of Dairy Science*, **85(12)**, 3395-3402.

[9] The National Research Council (NRC), (1995) Nutrient requirements of poultry (9$^{th}$ rev. edn). National Academy Press, Washington, DC.

[10] Dacie, J.V. and Lewis, S.M. (2001) Practical Haematology. 9th Edition, Churchill Livingstone, London, 633.

[11] Steel, R.G.D. and Torrie, J.H. (1980) Principles and Procedures of Statistics. 2nd Edition, McGunfermented-Hill, New York.

[12] Olorede, B.R. and Longe, O.G. (2000) Effect of replacing palm kernel cake with sheabutter cake on egg quality characteristics, haematology and serum chemistry of laying hens. *Nigerian Journal of Animal production*, **27(1)**, 19-23.

[13] Apata, D.F. (2004) Egg production and haematological profile of laying hens fed dietary unfermented or processed *Prosopis africana* seeds. *Journal of Agricultural Research and Development*, **3(3)**, 99-104.

[14] Mitruka, B.M. and Rawnsley, H.M. (1977) Clinical, Biochemical and Haematological refrence values in norma experimental animals. Masson Publishing USA Inc., New York.

[15] Muhammad, N.O. and Oloyede, O.B. (2010) Effects of *Aspergillus niger*-fermented *Terminalia cattapa* seed meal-based diet on selected enzymes of some tissues of broiler chicks. *Food and Chemical Toxicol*, **48(5)**, 1250-1254.

**Vol.1, No.2, 56-61 (2010)**
doi:10.4236/as.2010.12008

**Agricultural Sciences**

# The HLP mutation confers enhanced resistance to leaf-rust in different wheat genetic backgrounds

## ——Lesion-mimic mutation confers resistance in wheat

**Cristina Andrea Kamlofski[1], Alberto Acevedo[2]***

[1]Estación Experimental Agropecuaria Concepción del Uruguay, CRER, INTA, Concepción del Uruguay, Entre Ríos, Argentina
[2]Centro de Investigación de Recursos Naturales, INTA Castelar, Castelar, Buenos Aires, Argentina;
*Corresponding author: aacevedo@cnia.inta.gov.ar

Received 8 July 2010; revised 19 July 2010; accepted 20 July 2010.

## ABSTRACT

**In several plant species, lesion-mimic mutants simulate the disease-resistance response in the absence of pathogens. Interestingly, some of these mutants confer broad-spectrum resistance to diverse pathogens. We previously demonstrated that the HLP (hypersensitive-like phenotype) mutant of bread wheat (*Triticum aestivum* L.) exhibited spontaneous hypersensitive response (HR) in the absence of any pathogen input. However, when HLP plants showing HR phenotype were challenged with leaf-rust (*Puccinia triticina*) they were more resistant than plants of the mother-line of comparable developmental stage that did not show spontaneous HR, suggesting that the HLP mutation may confer enhanced resistance to the fungus. In this paper we validate the aforementioned finding in several wheat genetic backgrounds. Two-way crosses were performed among the HLP mutant and eight wheat commercial stocks, and third backcross progenies with and without spontaneous HR were challenged with leaf-rust to investigate the response to the fungus. Backcrosses to cv. Sinvalocho M.A., the mother-line, and cv. Purplestraw, highly susceptible to leaf-rust attack, were used as controls. Third backcross progenies of cvs. Sinvalocho M.A., Purplestraw, Buck Guaraní and Pro INTA Imperial bearing spontaneous HR phenotype were more resistant to the fungal pathogen than third backcross progenies that did not carry the HLP mutation. Other four wheat stocks were as healthy as the HLP mutant. As expected, backcross to the mother-line demonstrated that the HLP mutation conferred an additional resistance to the already healthy performance displayed by the mother-line at adult plant stage. The introgression of the HLP mutation conferred heightened leaf-rust resistance and caused no kernel weight reduction on the backcrossed progenies. Taken together, these data validate the direct use of this type of mutations in disease-resistance breeding.**

**Keywords:** Hypersensitive Response; Lesion-Mimic Mutant; Puccinia Triticina, Wheat Commercial Stocks

## 1. INTRODUCTION

Plants have evolved several defense mechanisms to overcome pathogen attack. One of the most common and effective defense responses in plants is the hypersensitive response (HR), which results in localized cell death at the site of pathogen infection [1]. Mutants have been identified in several plant species that spontaneously form localized areas of dead tissue resembling those seen in the HR. In these class of mutants, hereafter referred to as lesion-mimic mutants, disease symptoms or HR cell death occur in the absence of pathogens.

Several lesion-mimic mutants have been identified and characterized in Arabidopsis: *acd* [2-4]; *dll* [5]; *hlm* [6]; *len* [7]; *lsd* [8]; *ssi* [9,10]. The *LSD*1 gene coding for a novel zinc finger protein functions as a negative regulator of plant cell death. Interestingly, the *lsd*1 mutants were resistant to the bacterial pathogen *Pseudomonas syringae* and the oomycete *Peronospora parasitica* [11]. The *HLM*1 gene encodes a cyclic nucleotide-gated channel that is permeable to $K^+$ and $Na^+$ ions and is activated by cGMP and cAMP. The *hlm*1 mutants also exhibited increased resistance to a virulent strain of *Pseudomonas syringae* pv *tomato* [6].

An underlying agronomic feature of some graminaceous lesion-mimic mutants is that they confer broad-spectrum resistance to different pathogens. Mutations of the barley *Mlo* gene conferred expression of a lesion-mimic phenotype which provided resistance to all known races of the fungus responsible for powdery mildew (*Blumeria graminis* f.sp. *hordei*). However, barley seedlings carrying the *mlo* mutation showed enhanced susceptibility to the rice blast fungus *Magnaporthe grisea* [12], and resistance genes ml-o5, ml-o6, and ml-10 were responsible for grain yield reduction in chromosome-doubled haploid lines of barley tested in a disease-free field trial [13]. In rice, some lesion-mimic mutants displayed enhanced resistance to pathogens: *cdr*1, *cdr*2 and *cdr*3 exhibited heightened resistance to *M. grisea* [14], *spl*4, *spl*5-1, *spl*5-2, *spl*7, *spl*10, *spl*11, *spl*12, *spl*13, *spl*14 and *Spl*15 conferred non-race-specific resistance to rice blast and bacterial blight [15,16] and *ebr3* showed increased resistance to *M. grisea* and the bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* [17]. Co-segregation analyses of blast and bacterial blight resistance and lesion-mimic phenotypes in segregating populations of *spl*17 and *Spl*26 demonstrated that enhanced resistance to the two diseases was conferred by mutations in the lesion-mimic genes [18]. The wheat mutant M66 showed enhanced resistance to powdery mildew and yellow and brown rusts although its yield was 50% that of the control, (cv. Guardian) [19-21]. Conversely, the HLP mutant of wheat that exhibited spontaneous HR lesions showed also heightened resistance to leaf-rust (*Puccinia triticina*) and similar yield to that of its mother line (cv. Sinvalocho M.A.) [22]. Because enhanced resistance coincided with the presence of spontaneous HR, the HLP mutant was backcrossed to different wheat genetic stocks and challenged with leaf-rust to genetically validate the phenomenon.

We report in this paper that the introgression of the HLP mutation into different wheat genetic stocks confers heightened levels of resistance to leaf-rust and does not affect kernel weight.

## 2. MATERIALS AND METHODS

### 2.1. Plant Material and Backcross Program

A backcross program was performed among the HLP mutant of bread wheat (*Triticum aestivum* L.) and eight wheat cultivars to incorporate the mutation induced in HLP into the eight different genetic backgrounds. Cultivar Sinvalocho M.A., mother-line of HLP, and cv. Purplestraw, highly susceptible to *Puccinia triticina*, were selected because they have been used for many years in Argentina as differential hosts in genetic studies involving leaf rust [23-25]. The remaining six wheat cultivars (Pro INTA Don Alberto, Pro INTA Isla Verde, Granero INTA, Pro INTA Imperial, Buck Guaraní, and Klein Cacique) were selected from the large public (INTA) and private (Buck and Klein) breeding programs because the average genetic diversity within each of these programs was very similar to the total genetic diversity present in the complete Argentine germplasm [26].

Since the mutation induced in HLP controls the expression of visible and spontaneous HR lesions, its introgression into the wheat genetic stocks was traced, by the naked eye, simply by selfing the plant receptor and selecting the HR phenotypes in the plant receptor progeny.

### 2.2. Pathogen Infection Study and Kernel Weight Evaluation

A spontaneous leaf-rust infection study was carried out in the experimental field at the Agricultural Experimental Station of Concepción del Uruguay, Entre Ríos, Argentina. There were two sowing dates, 30 May 2006 and 23 May 2007, each with two repetitions. Seeds of third backcross progenies corresponding to the aforementioned crosses were sown in the field in 1.2-m rows separated by 0.2-m. For each cross, natural leaf-rust infection was evaluated by scoring the number of pustules on the flag leaf of 10 plants that carried the HLP mutation and 10 plants that did not. Evaluated plants were chosen at random. Results were the means of two independent experiments.

The type of infection was also recorded according to the classification described by Mains and Jackson [27].

The evaluation of the kernel weight trait was performed by using the same experimental design described for the spontaneous leaf-rust study. Results were the means of two independent experiments.

### 2.3. Statistical Analyses

The Mann-Whitney test was used to statistically analyze the leaf-rust infection response and the kernel weight trait in third backcross progenies with and without spontaneous HR.

## 3. RESULTS

### 3.1. Expression of Resistance of HLP Mutation to Leaf Rust in Different Wheat Stocks

Preferentially, lesion mimic mutations have been characterized only in the genetic background where they were isolated. Even though it is highly desirable to know as well if the candidate mutation is functional in other genetic stocks different from the original one, knowledge about this matter appears to be scarce. Moreover, this information is mandatory to decide whether the mutation is a useful genetic factor to be considered in plant breeding programs.

In a previous investigation we demonstrated that adult HLP plants that exhibited spontaneous HR lesions were more resistant to leaf-rust attack than Sinvalocho M.A. plants of a comparable developmental stage, indicating that the HLP mutation, traced by the naked-eye by the presence of spontaneous HR, was phenotypically associated with fungal resistance.

In an effort to validate at the genetic level, whether the HLP mutation also conferred pathogen resistance in other genetic backgrounds, the HLP mutant was backcrossed to two wheat controls and six commercial cultivars, and field-grown plants of progenitors and third backcrossed progenies were challenged with natural leaf-rust infections. Two-way backcrosses to cv. Sinvalocho M.A., the mother-line, and cv. Purplestraw, highly susceptible to leaf-rust attack, demonstrated that adult plants of progenitor HLP exhibiting spontaneous HR accumulated lower numbers of leaf-rust pustules per $cm^2$ of flag leaf (3.00 ± 0.89) that in general terms were also smaller (infection type 1) than those observed on control plants of progenitors Sinvalocho M.A. (12.00 ± 2.58; U = 1; $P < 0.001$; infection type 2–2++) (**Figure 1(a)**) and Purplestraw (38.50 ± 4.58; U = 0; $P < 0.001$; infection type 2–3) (**Figure 1(b)**), of a comparable developmental stage (**Table 1, Figure 1**). Third backcrossed progenies derived from HLP × Sinvalocho M.A. cross demonstrated that adult plants carrying the HLP mutation were more resistant (4.00 ± 2.16 pustules/flag leaf $cm^2$) to the fungal pathogen than adult plants that did not carry the mutation (12.75 ± 3.09 pustules/flag leaf $cm^2$; U = 1; $P < 0.001$) and therefore they did not exhibit spontaneous HR lesions (**Figure 1**). Accordingly, third backcrossed progenies derived from HLP × Purplestraw cross showed that adult plants bearing spontaneous HR phenotype were more resistant (4.10 ± 2.30 pustules/flag leaf $cm^2$) to leaf-rust than adult plants that did not exhibit spontaneous HR lesion (43.40 ± 5.15 pustules/flag leaf $cm^2$; U = 0; $P < 0.001$) (**Figure 1**). On average, the accumulation of rust pustules/flag leaf area allowed the classification of naturally infected plants in two groups. In one group the number of pustules ranged from 33.92 to 48.55 and it was composed of plants of cv. Purplestraw and plants of third backcrossed progenies that exhibited no spontaneous HR. The other group was composed of plants of the HLP mutant and plants of third backcrossed progenies that showed spontaneous HR, and the accumulation of pustules ranged from 1.80 to 6.40. Interestingly, to 6.40. Interestingly, not only the latter group accumulated a small number of rust pustules compared to the first one, but it also showed a common feature, *i.e.*, all their members carried the HLP mutation. Since an elevated number of leaf-rust pustules were detected in the susceptible wheat control, this meant that the experiments were performed under conditions of good spontaneous leaf-rust infection (**Table 1**, **Figure 1(b)**).

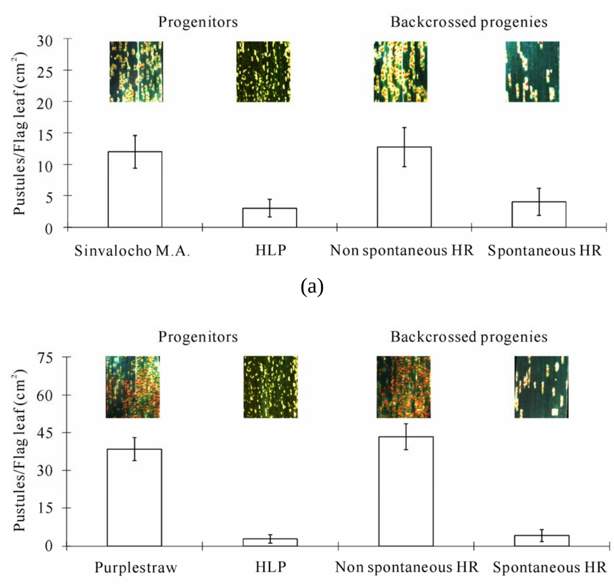


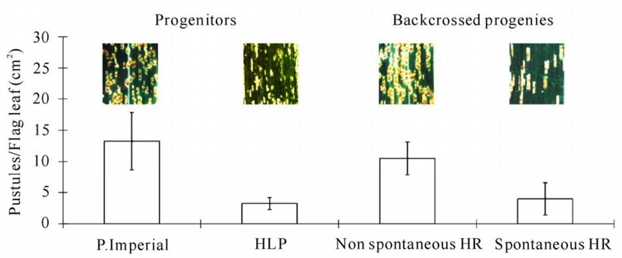

**Figure 1.** Differential response of HLP, Sinvalocho M.A. (a), Purplestraw (b), and their corresponding backcrossed progenies to leaf rust attack. Note that field-grown plants from HLP and backcrossed progenies that carry the HLP mutation accumulated less rust pustules than plants of Sinvalocho M.A., Purplestraw, and backcrossed progenies that did not carry the mutation.

Adult plants of four commercial cultivars (Klein Cacique, Pro INTA Don Alberto, Pro INTA Isla Verde, and Granero INTA) out of the six cultivars analyzed did not differ from the response displayed by the HLP mutant to leaf-rust attack (**Table 1**); however, flag leaves of cvs. Pro INTA Imperial (U = 1; $P < 0.001$; infection type 2–2++) (**Figure 2(a)**) and Buck Guaraní (U = 1; $P < 0.001$; infection type 2–2++) (**Figure 2(b)**) accumulated higher numbers of leaf-rust pustules compared to HLP (**Table 1, Figure 2**). Third backcrossed progenies derived from both HLP × P. Imperial and HLP × B. Guaraní crosses showed that adult plants that carried the HLP mutation were more resistant (4.00 ± 2.58 pustules/flag leaf $cm^2$, and 4.25 ± 1.50 pustules/flag leaf $cm^2$, respectively) to leaf-rust than adult plants that did not carry the mutation (10.50 ± 2.64 pustules/flag leaf $cm^2$; U = 1; $P < 0.001$, and 14.00 ± 2.94 pustules/flag leaf $cm^2$ ; U = 1; $P < 0.001$, respectively), and thus they did not exhibit spontaneous HR phenotypes (**Figure 2**).

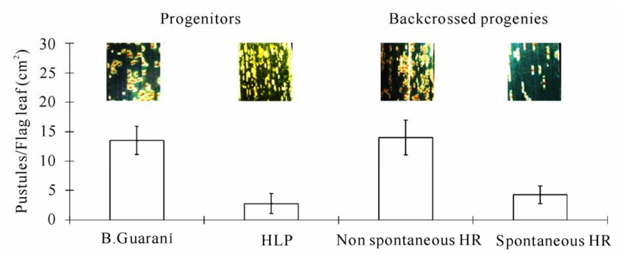
**Table 1.** Natural leaf-rust infection levels in field-grown plants of several wheat genetic stocks.

| Progenitors | Pustules/Flag leaf (Number cm$^{-2}$) |
|---|---|
| Mutant | |
| HLP | 3.00 ± 0.89 |
| Controls | |
| Sinvalocho M.A. | 12.00 ± 2.58 |
| Purplestraw | 38.50 ± 4.58 |
| Commercial cultivars | |
| Pro INTA Imperial | 13.25 ± 4.57 |
| Buck Guaraní | 13.50 ± 2.38 |
| Klein Cacique | 3.10 ± 1.43 |
| Pro INTA Don Alberto | 2.80 ± 1.14 |
| Pro INTA Isla Verde | 2.15 ± 0.93 |
| Granero INTA | 2.35 ± 0.90 |

Notes: Four commercial cultivars were as healthy as the HLP mutant. Data represent the average of two independent experiments with repetitions.




(a)




(b)

**Figure 2.** Differential response of HLP, Pro INTA Imperial (a), Buck Guaraní (b), and their corresponding backcrossed progenies to natural leaf-rust infection. Note that field-grown plants from the HLP mutant and from backcrossed progenies that carry the HLP mutation showed less rust pustules than developmentally comparable plants of P. Imperial, B. Guaraní, and backcrossed progenies that did not carry the mutation.

### 3.2. Kernel Weight Evaluation

Because wheat is both nutritionally and economically an important crop worldwide, it is desirable that the mutation induced in the HLP mutant causes no detrimental pleiotropic effects in agronomic traits that are components of the cereal yield. Field-grown plants that were naturally infected with leaf-rust demonstrated, for each cross, that no kernel weight differences ($P > 0.05$) were detected between plants of third backcrossed progenies that showed spontaneous HR and plants that did not (**Table 2**), indicating that the HLP mutation, traced by the naked-eye by the presence of spontaneous HR lesions, did not have a substantial effect on the kernel weight of the different genetic backgrounds examined.

## 4. DISCUSSION

The fact that most lesion-mimic mutations have been solely studied in the genetic background of the wild-type indicates that any putative association between the lesion-mimic mutation and a genetic trait of agronomic

**Table 2.** Kernel weight values in progenitors and backcross progenies carrying and not carrying the HLP mutation.

| Plant material | 100 Kernel weight (g) |
|---|---|
| Progenitors | |
| HLP | 3.74 ± 0.16 |
| Sinvalocho M.A. | 3.72 ± 0.19 |
| Purplestraw | 3.72 ± 0.21 |
| Buck Guaraní | 3.82 ± 0.26 |
| Pro INTA Imperial | 3.49 ± 0.59 |
| Backcrosses | |
| HLP × Sinvalocho M.A. (spontaneous HR) | 3.75 ± 0.15 |
| HLP × Sinvalocho M.A. (without HR) | 3.71 ± 0.18 |
| Purplestraw × HLP (spontaneous HR) | 3.70 ± 0.24 |
| Purplestraw × HLP (without HR) | 3.70 ± 0.23 |
| Buck Guaraní × HLP (spontaneous HR) | 3.84 ± 0.32 |
| Buck Guaraní × HLP (without HR) | 3.48 ± 0.29 |
| HLP × Pro INTA Imperial (spontaneous HR) | 4.15 ± 0.22 |
| HLP × Pro INTA Imperial (without HR) | 3.42 ± 0.35 |

Notes: The introgression of the HLP mutation, traced by the observation of spontaneous HR lesions, caused no kernel weight reduction. Data represent the average of two independent experiments with repetitions.

interest represents a phenotypic correlation that should be validated at the genetic level by crossing the genotype that carries the lesion mimic mutation to other genetic backgrounds and verifying if the genetic correlation between the mutation and the agronomic trait exists. This validation is deemed important to consider the direct use of this type of mutations in breeding programs.

We previously demonstrated that adult plants of HLP showing spontaneous HR were more resistant to leaf-rust attack compared with plants of the mother-line of comparable developmental stage that did not show spontaneous HR. This finding indicated that the mutation induced in HLP was phenotypically associated with fungal resistance [22].

In this study we have genetically validated the aforementioned finding by challenging with leaf-rust infections the backcross-mediated introgression of the HLP mutation into several wheat genetic backgrounds besides the mother-line. We have demonstrated that adult plants of third backcrossed progenies bearing spontaneous HR phenotype were more resistant to leaf-rust attack than adult plants of third backcrossed progenies that did not carry the HLP mutation (**Figures 1** and **2**). The introgression of the mutation induced in HLP conferred similar levels of resistance to the fungal pathogen in different wheat genetic backgrounds. Interestingly, similarity coefficient among cultivars used as progenitors ranged from 0.7784 (P. Imperial and P. Don Alberto) to 0.6296 (Granero INTA and P. Isla Verde), and it was 0.6625 in the specific case of P. Imperial and B. Guaraní (Manifesto, pers. comm.), indicating that the HLP mutation proved to be functionally efficient against the fungal pathogen in genetically diverse wheat germplasm.

Even though the mother-line of the HLP mutant carries at least two leaf-rust (*Lr*) genes [25] that may explain the durable resistance that Sinvalocho M.A. has shown for decades, the possibility that those genes confer the enhanced resistance observed in plants of third backcross progenies that showed HR phenotype is fairly low.

Diseases are a leading cause of crop losses, primarily leaf-rust that may reduce yield greatly [28]. Currently, the introgression of disease resistance genes into wheat breeding programs requires DNA-molecular-marker-assisted selection to trace those genes in the plant breeding materials. However, because the mutation induced in HLP controls the expression of visible and spontaneous HR lesions, its introgression into wheat genetic backgrounds has the advantage that it can be traced, by the naked eye, simply by selfing the plant receptor and selecting the HR phenotypes in the plant receptor progeny. Indeed, this visual method represents a simpler and more economic way of tracing the mutation, compared to DNA-molecular-marker-assisted selection method.

On the other hand, several investigations have demonstrated that resistance mediated by lesion-mimic mutations is accompanied, in most plants, by lower yield, stunted growth or other abnormal characteristics that prevent the direct use of these mutations in disease-resistance breeding. Yield of the wheat mutant M66 was 50% lower than that of the control [19]. Mutagen induced resistance genes *ml-o*5, *ml-o*6, and *ml*-10 conferred a four per cent reduction in grain yield caused mainly by lower thousand grain weight in a population of 198 chromosome-doubled haploid lines of spring barley tested in a disease-free field trial [13]. In contrast to the *suppressor of SA insensitivity*1 (*ssi*1), *accelerated cell death*6 (*acd*6) and *lsd*6 mutations of *Arabidopsis*, and to most *spl* and *cdr* mutations of rice, which reduced final plant height [3,9,15,29] and shorter life cycle [14] and resulted in a lower yield [16], the mutation induced in HLP caused no detrimental pleiotropic effects that affected the agronomic performance of the plant [22] and did not affect kernel weight when it was backcrossed-incorporated in several genetic stocks (**Table 2**). The latter finding, coupled with the fact that backcrossed-mediated introgression of the HLP mutation in several wheat genetic stocks heightened leaf-rust resistance validates the direct use of this mutation in disease-resistance breeding.

## 5. CONCLUSIONS

The introgression of the HLP mutation into different wheat genetic stocks confers heightened levels of resistance to leaf-rust, similar to the reported for the introgression of the mutation into the genetic background of the mother-line. To our knowledge this is the first report of a lesion-mimic mutation that enhances pathogen resistance without affecting kernel weight in genetically diverse wheat germplasm. Taken together, both findings validate the direct use of this mutation in disease-resistance breeding.

## 6. ACKNOWLEDGEMENTS

We thank Dr Marcela M. Manifesto (Instituto de Recursos Biológicos, INTA, Argentina) for providing wheat similarity coefficient data. This research was supported by a grant from the Secretariat of Science and Technology (BID 1201 OC-AR PICT N° 08-9555) to AA.

## REFERENCES

[1] Dangl, J.L., Dietrich, R.A. and Richberg, M.H. (1996) Death don't have no mercy: Cell death programs in plant-microbe interactions. *The Plant Cell*, **8(10)**, 1793-1807.

[2] Greenberg, J.T. and Ausubel, F.M. (1993) *Arabidopsis* mutants compromised for the control of cellular damage during pathogenesis and aging. *The Plant Journal*, **4(2)**, 327-342.

[3] Rate, D.N., Cuenca, J.V., Bowman, G.R., Guttman, D.S. and Greenberg, J.T. (1999) The gain-of-function *Arabidopsis acd* 6 mutant reveals novel regulation and function of the salicylic acid signaling pathway in controlling cell death, defenses, and cell growth. *The Plant Cell*, **11(2)**, 191-206.

[4] Yao, N. and Greenberg, J.T. (2006) Arabidopsis accelerated cell death 2 modulates programmed cell death. *Plant Cell*, **18(2)**, 397-411.

[5] Pilloff, R.K., Devadas, S.K., Enyedi, A. and Raina, R. (2002) The *Arabidopsis* gain-of-function mutant *dll1* spontaneously develops lesions mimicking cell death associated with disease. *The Plant Journal*, **30(1)**, 61-70.

[6] Balagué, C., Lin, B., Alcon, C., Flottes, G., Malmstrom, S., Kohler, C., Neuhaus, G., Pelletier, G., Gaymard, F. and Roby, D. (2003) HLM1, an essential signaling component in the hypersensitive response, is a member of the cyclic nucleotide-gated channel ion channel family. *Plant Cell*, **15(2)**, 365-379.

[7] Ishikawa, A., Tanaka, H., Nakai, M. and Asahi, T. (2003) Deletion of a chaperonin 60 gene leads to cell death in the *Arabidopsis lesion initiation* 1 mutant. *Plant and Cell Physiology*, **44(3)**, 255-261.

[8] Dietrich, R.A., Delaney, T.P., Uknes, S.J., Ward, E.R., Ryals, J.A. and Dangl, J.L. (1994) *Arabidopsis* mutants simulating disease resistance response. *Cell*, **77(4)**, 565-577.

[9] Shah, J., Kachroo, P. and Klessig, D.F. (1999) The *Arabidopsis ssi1* mutation restores pathogenesis-related gene expression in *npr1* plants and renders defensin gene expression salicylic acid dependent. *The Plant Cell*, **11(2)**, 191-206.

[10] Kachroo, P., Shanklin, J., Shah, J., Whittle, E.J. and Klessig, D.F. (2001) A fatty acid desaturase modulates the activation of defense signaling pathways in plants. *Proceedings of the National Academy of Sciences*, **98(16)**, 9448-9453.

[11] Dietrich, R.A., Richberg, M.H., Schmidt, R., Dean, C. and Dangl, J.L. (1997) A novel zinc finger protein is encoded by the arabidopsis *LDS1* gene and functions as a negative regulator of plant cell death. *Cell*, **88(5)**, 685-694.

[12] Jarosch, B., Kogel, K. and Schaffrath, U. (1999) The ambivalence of the barley *mlo* locus: Mutations conferring resistance against powdery mildew (*Blumeria graminis* f.sp. *hordei*) enhance susceptibility to the rice blast fungus *Magnaporthe grisea*. *Molecular Plant-Microbe Interactions*, **12**, 508-514.

[13] Kjær, B., Jensen, H.P., Jensen, J. and Helms, J.J. (1990) Associations between three ml-o powdery mildew resistance genes and agronomic traits in barley. *Euphytica*, **46(3)**, 185-193.

[14] Takahashi, A., Kawasaki, T., Henmi, K., Shi, I.K., Kodama, O., Satoh, H. and Shimamoto, K. (1999) Lesion mimic mutants of rice with alterations in early signaling events of defense. *The Plant Journal*, **17(5)**, 535-545.

[15] Yin, Z., Chen, J., Zeng, L., Goh, M., Leung, H., Khush, G.S. and Wang, G.L. (2000) Characterizing rice lesion mimic mutants and identifying a mutant with broad-spectrum resistance to rice blast and bacterial blight. *Mol Plant Microbe Interact*, **13(8)**, 869-876.

[16] Mizobuchi, R. and Hirabayashi, H. (2002) Isolation and characterization of rice lesion mimic mutants with enhanced resistance to rice blast and bacterial blight. *Plant Science*, **163(2)**, 345-353.

[17] Campbell, M.A. and Ronald, P.C. (2005) Characterization of four rice mutants with alterations in the defence response pathway. *Molecular Plant Pathology*, **6(1)**, 11-12.

[18] Wu, C., Bordeos, A., Madamba, M.R., Baraoidan, M., Ramos, M., Wang, G.L., Leach, J.E. and Leung, H. (2008) Rice lesion-mimic mutants with enhanced resistance to diseases. *Molecular Genetics and Genomics*, **279**, 605-619.

[19] Kinane, J.T. and Jones, P.W. (2001) Isolation of wheat mutants with increased resistance to powdery mildew from small induced variant populations. *Euphytica*, **117(3)**, 251-260.

[20] Boyd, L.A. and Minchin, P.N. (2001) Wheat mutants showing altered adult plant disease resistance. *Euphytica*, **122(2)**, 361-368.

[21] Boyd, L.A., Smith, P.H., Wilson, A.H. and Minchin, P.N. (2002) Mutations in wheat showing altered field resistance to yellow and brown rust. *Genome*, **45(6)**, 1035-1040.

[22] Kamlofski, C.A., Antonelli, E., Bender, C., Jaskelioff, M., Danna, C.H., Ugalde, R. and Acevedo, A. (2007) A lesion-mimic mutant of wheat with enhanced resistance to leaf rust. *Plant Pathol*, **56(1)**, 46-54.

[23] Sacco, F., Suárez, E.Y. and Naranjo, T. (1998) Mapping of the leaf rust resistance gene *Lr*3 on chromosome 6B of Sinvalocho M.A. *Genome*, **41(5)**, 686-690.

[24] Campos, P. (2004) Diferencias en la población patógena de *Puccinia triticina* que afecta a los cultivos de trigo pan (*Triticum aestivum*) y trigo candeal (*Triticum turgidum var: durum*). *Actas VI Congreso Nacional de Trigo*, Bahía Blanca, Buenos Aires.

[25] Ingala, L.R., Saione, H.A., Nisi, J., Helguera, M. and Sacco, F. (2004) Identificación de genes para resistencia a roya de la hoja y de marcadores de ADN asociados a los mismos en un cultivar de trigo con resistencia durable. *Actas XXXIII Congreso Argentino de Genética*, Sociedad Argentina de Genética, Mendoza, 112.

[26] Manifesto, M.M., Schlatter, A.R., Hopp, H.E., Suarez, E.Y. and Dubcovsky, J. (2001) Quantitative evaluation of genetic diversity in wheat germplasm using molecular markers. *Crop Science*, **41(3)**, 682-690.

[27] Mains, E.B. and Jackson, H.S. (1926) Physiological specialization in the leaf rust of wheat *Puccinia triticina* Erikss. *Phytopathology*, **16**, 89-120.

[28] Roelfs, A.P., Singh, R.P. and Saari, E.E. (1992) Rust diseases of wheat: concepts and methods of disease management. CIMMYT, Mexico, 80.

[29] Weymann, K., Hunt, M., Uknes, S., Neuenschwander, U., Lawton, K., Steiner, H.Y. and Ryals, J. (1995) Suppression and restoration of lesion formation in *Arabidopsis lsd* mutants. *The Plant Cell*, **7(12),** 2013-2022.

**Vol.1, No.2, 62-67 (2010)**
doi:10.4236/as.2010.12009

**Agricultural Sciences**

# Physico-chemical properties, fatty acid and mineral content of some walnuts (*Juglans regia* L.) types

**Mehmet Musa Özcan[1]*, Cesari İman[2], Derya Arslan[1]**

[1]Department of Soil Science, Faculty of Agriculture, University of Selçuk, Konya, Turkey; *Corresponding Author: mozcan@selcuk.edu.tr
[2]Food Engineering, Kırşehir, Turkey

Received 12 June 2010; revised 28 June 2010; accepted 13 July 2010.

## ABSTRACT

**Some physical and chemical properties, mineral content and fatty acid compositions of kernel and oils of several walnut types (Büyük Oba, Kaman-2, Kaman-5) were determined. The oil yields from these kernels changed between 61.4% to 72.8%. The crude fibre contents of kernels ranged between 3.77% and 3.80%. In addition, crude protein contents of kernels ranged between 7.05% and 8.10%. While the peroxide values of kernel oils change between 3.18 meq/Kg and 3.53 meq/Kg, acidity values ranged between 0.35% and 0.56%. The main fatty acids of walnut kernel oils were oleic, linoleic, linolenic and palmitic acids. Linoleic acid contents of kernel oils varied between 49.7% and 55.5%. On the other hand, oleic acid contents ranged between 20.5% and 26.4%. As a result, the present study showed the walnut kernels of the researched species of walnut kernels from Turkey are a potential source of valuable oil which might be used for edible and other industrial applications.**

**Keywords:** Walnut; Kernel; Oil; Fatty Acid Composition; Mineral Contents

## 1. INTRODUCTION

Walnut (*Juglans regia* L) a member of Juglandaceae family is one of the finest nuts of temperate regions. It is the oldest cultivated fruit in the world and grown spontaneously almost all over Turkey. Fifty percent of production is consumed on- form and the remainder is marketed [1-3].

Ripe walnuts are mostly eaten as dessert nuts or used in cakes, desserts and confectionery of all kinds from ice cream to Baklava. The walnut plant has a high nutriational value and high quality wood. In turkey, walnut has a special value in Turkish foods and is very common in traditional Turkish foods [2]. Although walnuts are rich in fat, a diet supplemented with walnuts had a beneficial effect on blood lipids, lowering blood cholesterol and lowering the ratio of serum concentrations of low deroity lipoprotein: high density lipoprotein by 12% [4,5]. Oil contents of walnut kerrels can generally vary from 52 to 70 % depending on the cultivar, location grown and irrigation rate [2,3,6-8]. Most nuts are rich in manounsaturated fat (oleic acid) while walnuts are also high in two polyunsaturated fatty acids linoleic acid and α-linolenic acids. The major fatty acids found in walnut oil are oleic, linoleic and linolenic acids [3,5,8]. The fatty acid profile of walnut oil varies between cultivars. It is important to a identify these differences in locally grown cultivars and to identify which fatty acids give the best nutritional qualities [8,9]. Some fruit seeds such as cherry, apricot, citrus and apple can be used as sources of oils. Some seed oils are already used for several purposes: blending with highly saturated edible oils to provide new oils with modified nutritional values as ingredients in paint and varnish formulations, surface coatings and oleo-chemicals, and as oils for cosmetic purposes [10].

The aim of this study was to determine their physical and chemical properties, mineral contents and fatty acid composition of some walnut types collected from Kırşehir province in Turkey.

## 2. MATERIAL AND METHOD

### 2.1. Material

The kernels of some walnut cultivars (Büyük Oba, Kaman-2, Kaman-5) were obtained by hand processing from walnuts growing in Kırşehir province of Turkey in August 2008. Kernels were kept in glass jars until analyses at refrigerator. In all stages of trials, dry and mature kernels have been used.

## 2.2. Physical Analyses

**Shelled weights of walnuts:** It was used 25 unit walnut for each walnut variety. Each walnut was weighted separately, and average hulled fruit weights for each one were found.

**Shelled diameters of walnuts:** Mean diameter of each hulled walnut was measured by using electronic cumpas.

**Yields of walnuts:** Five walnut were used for yield analysis of each walnut. Yield was calculated as the percentage rate to the shelled walnut fruit of kernel weight.

**Determination of dry matter:** Air dried and ground walnut samples were waited in incubator calibrated to 105℃ for 24 h, and about 10 g samples from each dried walnut was weighted.

## 2.3. Chemical Analyses

The some chemical compositions (crude oil, crude protein, crude fiber, and crude ash, acidity, peroxide value, refractive index and saponification value) were analyses according to AOAC [11]. For oil analyses, each samples was homogenized and subjected to extraction for 6 h with petroleum ether (boiling range 30-60℃) in a Soxhlet apparatus. The extracted oil was dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure in a rotary film evaporator. Oil percentages were determined by weight difference. Ash was determined in a muffle furnace at 900℃ for 8 h [11]. The nitrogen content estimated by the Kjeldahl method and was converted to protein content by using the conversion factor 6.25.

## 2.4. Determination of Fatty Acids

Fatty acid composition for walnut kernel samples were determined using a modified fatty acid methyl ester method as described by Hışıl [12]. The oil was extracted three times for 2 g air-dried seed sample by homogenization with petrolium ether. The oil samples (50-100 mg) were converted to its fatty acid methyl esters (FAME). The methyl esters esters of the fatty acids (1 µl) were analysed in a gas chromotography (Shimadzu GC-2010) equipped with a flame ionising detector (FID), a fused silica capillary column (60 m × 0.25 mm i.d.; film thickness 0.20 mikrometere). It was operated under the following conditions: oven temperature program. 90℃ for 7 min. Raised to 240℃ at a rate 5℃/min and than kept at 240℃ for 15 min); injector and detector temperatures, 260 and 260℃; respectively, carrier gas. nitrogen at flow rate of 1.51 ml/min; split ratio 1/50 µl/min.

A Standard fatty acid methyl ester mixture (Sigma Chemical Co.) was used to identify sample peaks. Commercial mixtures of fatty acid methyl esters were used as reference data for the relative retention times [13]. Quantitative analyses of the fatty acids were performed using the heptadecanoic acid methyl ester as internal standard. The results are mean values of three replicates.

## 2.5. Determiation of Mineral Contents

About 0.5 g of dried and walnut kernels were put into burning cup with 15 ml of pure $NHO_3$. The sample was incinerated in a MARS 5 microwave oven (CEM corporation Manufactura) at 200℃. Distilled deionized water and ultrahigh-purity commercial acids were used to prepare all reagents, standards and walnut kernel samples. After digestion treatment, samples were filtrated through whatman No 42. The filtrates were collected in 50 ml Erlenmayer flasks and analysed by ICP-AES (Varian). The mineral contents of the samples were quantified against standard solutions of known concentrations which were analysed concurrently [14].

Working conditions of ICP-AES:
Instrument: ICP-AES (Varian-Vista
RF Power: 0.7-1.5 kw (1.2-1.3 kw for Axial)
Plasma gas flow rate (Ar): 10.5-15 L/min. (radial) 15 (axial)
Auxilary gas flow rate (Ar): 1.5
Viewing height: 5-12 mm
Copy and reading time: 1-5 s (max. 60 s)
Copy time: 3 s (max. 100 s)

## 2.6. Statistical Analyses

Results of the research were analysed for statistical significance by analysis of variance [15]. This research was performed by three duplicates with a replicate.

# 3. RESULTS AND DISCUSSION

The physical and chemical properties of some walnut varieties (Büyük Oba, Kaman-2, Kaman-5) collected from Kırşehir province in Turkey are given in **Table 1**. The weight with hull, diameter, hull weight, kernel weight, yield, dry matter, crude fibre, crude ash, crude protein, crude oil, saponification, refraxtive index, acidity, peroxide value of walnut kernels were determined. According to variance analyses, important differences were found between physical properties and their weighs and kinds as statistical, $p < 0.01$ level.

The oil yields of kernels varied from 53% (Büyük Oba and Kaman-5) to 60% (Kaman-2 cv) of the dry weight. The oil contents of kernels changed among the varieties to more than about 60% of each. However, because of economical value of the oil, these kernels could be used as potential sources of oils. Büyükoba cultivar had the highest oil (72.87%) content, followed by Kaman-5 cv (72.13%) and Kaman-2 cv (61.38%). The

**Table 1.** Some physical and chemical properties of walnut kernel and oils.

| | Walnut types | | | | | |
|---|---|---|---|---|---|---|
| **Properties** | **Kaman–5** | | **Büyük Oba** | | **Kaman–2** | |
| Weight with shelled (g) | 12.96 | ± 1.00 b | 15.74 | ± 0.02 a | 13.6326 | ± 0.03 b |
| Diameter with shelled (mm) | 36.72 | ± 1.22 b | 41.02 | ± 0.98 a | 39.79 | ± 0.9 a |
| Shell weight (g) | 6.6882 | ± 0.01 a | 7.7585 | ± 0.0005 a | 4.3256 | ± 0.01 b |
| Kernel weight (g) | 7.815 | ± 0.005 | 8.9289 | ± 0.02 | 6.5872 | ± 0.0198 |
| Yield (%) | 53 | ± 0.4 b | 53 | ± 2 b | 60 | ± 2.5 a |
| Drt matter (%) | 99.58667 | ± 0.015275 | 98.58333 | ± 1.440498 | 99.58 | ± 0.04359 |
| Crude oil (%) | 72.13 | ± 4.681047 | 72.865 | ± 8.619632 | 61.375 | ± 10.3450 |
| Peroxide value (meq $O_2$/kg) | 3.5294 | ± 0.0004 | 3.1849 | ± 0.0999 | 3.4482 | ± 0.0103 |
| Crude fiber (%) | 3.90 | ± 0.60 | 3.77 | ± 0.32 | 3.87 | ± 0.31 |
| Acidity (%) | 0.5628 | ± 0.0101 | 0.35 | ± 0.015 | 0.5575 | ± 0.01015 |
| Saponification value | 114.60 | ± 0.01 a | 106.96 | ± 0.01 b | 102.09 | ± 1.00 c |
| Ash (%) | 1.985 | ± 0.431335 | 1.71 | ± 0.028284 | 2.525 | ± 1.15259 |
| Crude Protein[a] (%) | 8.10125 | ± 0.055225 | 7.0489 | ± 1.099834 | 7.24155 | ± 1.878 |
| Refractive index (nD20) | 1.535 | ± 0.0005 | 1.534 | ± 0.00005 | 1.537003 | ± 0.0001 |

[a]Nx6.25

crude fibre contents ranged between 3.77% (Kaman-5 cv) and 3.90% (Büyükoba). While crude ash contents changed between 1.99% (Kaman-5 cv) and 2.53% (Kaman-2), crude proteins of kernels ranged between 7.05 % (Büyükoba cv) to 8.10% (Kaman-5 cv). In addition, kernel weights changed between 6.59 g/unit (Kaman-2 and 8.93 g/unit (Büyükoba cv). These results are comparable to data previously reported in the literature [7,16]. Nuts and oils intended to be cooked may require a low polyunsaturated fatty acid content [17].

Some physical and chemical properties of walnut kernels and oils are given in **Table 1**. According to variance analyses, differences between varieties to saponification values were found statistically important at the $p < 0.01$ level. While the peroxide values of kernel oils change between 3.18 (Büyükoba) and 3.53 meq/Kg (Kaman-5 cv), acidity values ranged between 0.35% (Büyükoba cv) and 0.56% (Kaman-5 and Kaman-2). Refractive index was determined between 1.534 (Büyükoba) and 1.537 (Kaman-2 cv). In addition, saponification values of kernel oils were measured between 102.09 (Kaman-2 cv) and 114.60 (Kaman-5 cv). Differences among the values of walnut varieties can probably be because of growing conditions, climatic, environmental conditions and analytic conditions.

Fatty acid compositions of walnut kernel oils are given in **Table 2**. Results showed that the oils of all varieties used in this experiment had higher linoleic and oleic acid contents. Linoleic acid contents of kernel oils ranged between 49.7% (Kaman-5 cv) and 55.5% (Kaman-2 cv). The proportions of the most abundant fatty acids (linoleic acid) of the kernel oils varied among different varieties. This proportion was also higher than that in other fruit seed oils; mahaleb (35.4%), cherry laurel (53.7%), date pit (49.54%), walnut (13.8-33.0%) [8,18-20]. Stearic and palmitic acids are the main saturated components in all walnut cultivars. Palmitic acid is differed in the different walnut cultivars. Its percentage was found between 6.3% (Kaman-5) and 6.3% (Büyükoba and Kaman-2). These results are in good agreement with

**Table 2.** Fatty acid composition of walnut oils (%).

| | Walnut types | | |
|---|---|---|---|
| **Fatty Acids** | **Kaman-5** | **Büyük Oba** | **Kaman-2** |
| Palmitic (C16:0) | 6.5 | 6.3 | 6.3 |
| Stearic C18:0) | 2.6 | 2.5 | 2.6 |
| Oleic (C:18:1) | 26.4 | 22.2 | 20.5 |
| Linoleic (C18:2) | 49.7 | 53.6 | 55.5 |
| Linolenic (C:18:3) | 14.3 | 14.5 | 14.8 |

in fatty acid composition for several walnut kernels [2,3,5,6-8]. Our results are similar in fatty acid composition when compared to the values in the literature.

Palmitic, stearic, oleic, linoleic and linolenic acid contents of walnut oil were established as 7.22%, 1.07%, 28.51%, 52.46% and 10.50%, respectively [221]. Özkan and Koyuncu [3] found that the contents of the main fatty acids of walnut genotypes were 5.24-7.62% palmitic, 2.56-3.67% stearic, 21.18-40.20% oleic, 43.94-60.12% linoleic and 6.91-11.52% linolenic. Zwarts *et al.* [8] reported as 6.7-8.2% palmitic, 1.4-2.5% stearic, 13.8-33.0% oleic, 49.3-62.3% linoleic and 8.0-14.2% linolenic acids. The oleic acid content of walnut oil was lower than that of walnut oil reported by Zwarts *et al.* [8], Özkan and Koyuncu [3] and Koyuncu and Aşkın [22]. The walnut fatty acid composition shows high contents of linoleic acid and linolenic acid which are beneficial to human health and linoleic acid and especially linoleinic acid play important roles for human health regarding the cardio vascular system [3,4,22].

The mineral contents of walnut kernels were determined by ICP-AES. The mineral compositions of kernels were summarized in **Table 3**. Mineral elements were found to vary widely depending on different walnut cultivar kernels. According to variance analyses, differences between walnut cultivars to Ca, Cu, Fe, K, Mg, Mn, Na and P were found statistically important at $p < 0.01$ level.

Ca, K, Mg, Na and P contents of all the walnut cultivar kernels were generally found very high. In addition, other minerals were determined very low. The levels of Ca of samples ranged between 2462.3 mg/Kg (Büyükoba cv) and 2757.9 mg/Kg (Kaman-5 cv), K contents were determined between 3478.8 mg/Kg (Büyükobacv) and 5476.2 mg/Kg (Kaman-5). While Mg contents are established between 4163.4 mg/Kg (Büyükoba cv) and 5488.1 mg/Kg (Kaman-2 cv), P contents of kernels were found between 2226.2 mg/Kg (Büyükoba cv) and 2604.3 mg/Kg (Kaman-2 cv). Walnut kernels were found to be rich in some minerals such as Ca (1108.6 mg/kg), K (4627.6 mg/kg), P (3621.9 mg/kg), Na (44.7 mg/kg) Mn (46.3 mg/kg and Mg (1089.9 mg/kg) [23]. Çağlarırmak [2] reported as 280-380 mg/100 g P, 230-340 mg/100 g K, 81-99 mg/100 g Mg and 67-105.5 mg/100 g Ca in fresh walnut kernels. Our results were found differences compared with mineral values reported by Çağlarırmak [2]. These differencies of cultivars minerals may be due to growth conditions, varieties, genetic factors, harvesting time, soil properties, geographical variations and analytical procedures [2,24]. Calcium is the major component of bone and assists in teeth development [25]. Other elements which may contribute to biological processes, but which have not been established as essential are barium, cadmium [24]. The high quantity of potassium, phosphorus, magnesium, and calcium, together with the small proportion of sodium plus the content of

**Table 3.** Mineral contents of walnut kernels (mg/Kg)[b].

| Minerals | Walnut types | | | | | |
|---|---|---|---|---|---|---|
| | Kaman–5 | | Büyük Oba | | Kaman–2 | |
| **B** | 15.114 | ± 1.503[b] C | 11.985 | ± 2.001 | 13.057 | ± 1.107 C |
| **Ca** | 2757.883 | ± 10.436 B | 2462.315 | ± 76.754 D | 2637.618 | ± 37.460 B |
| **Cr** | 1.695 | ± 0.360 C | - | - | 3.323 | ± 2.820 C |
| **Cu** | 5.676 | ± 1.099 C | 9.333 | ± 0.801 E | 5.944 | ± 0.325 C |
| **Fe** | 18.584 | ± 1.542 C | 17.875 | ± 1.252 E | 21.815 | ± 3.514 C |
| **K** | 5476.201 | ± 663.718 Aa | 3478.757 | ± 482.96 Ab | 5380.995 | ± 160.96 Aa |
| **Mg** | 4375.513 | ± 925.221 A | 4163.363 | ± 368.281 B | 5488.101 | ± 218.072 A |
| **Mn** | 21.991 | ± 4.977 C | 22.201 | ± 1.413 E | 17.585 | ± 0.134 C |
| **Mo** | - | - | - | - | 1.671 | ± 0.537 C |
| **Na** | 617.713 | ± 65.545 C | 667.416 | ± 74.322 D | 833.433 | ± 26.601 C |
| **Ni** | 1.651 | ± 0.774 C | - | - | 1.915 | ± 4.564 C |
| **P** | 2241.411 | ± 653.820 B | 2226.221 | ± 230.554 C | 2604.255 | ± 45.318 B |
| **Zn** | 17.981 | ± 0.523 C | 20.623 | ± 1.185 E | 18.353 | ± 4.206 C |

a[Dryweight]; [b]mean±standard deviation the essential elements as iron, manganese, copper, and zinc and allows the apricot, as well as the almond, to be considered as an excellent source of bioelements [26].

## 4. CONCLUSIONS

The accurate quantification of these analyses has very important applications for the nutrition sciences, because fatty acids, protein, oil and mineral contents in particular seed have a very important effect on health. These results of the experiment presented have shown that apricot cultivars have some distinctive chemical and physiccal properties, fatty acid and mineral content profiles. Kernels in apricot varieties can be good source oil due to their abundance in the kernels and their high oil content. Such utilization of apricot fruits processing wastes could provide extra income and at the same time help minimize a waste disposal problem. The mineral contents of apricot cultivar kernels collected from Malatya province of Turkey were established by ICP-AES. The contents of most minerals such as Ca, K, Mg and P are at adequate levels. Mineral elements were found to vary widely depending on different apricot kernels. Apricot kernels were found to be important sources of nutrients and essential elements. In addition, it is apparent that apricot kernels are good sources of micro and macro minerals, and consumed as a food ingredient to provide the human nutrient.

In this study, Kaman walnut varieties have got standard walnut properties. When these varieties were grown modified conditions, it was estimated to be having more quality. While walnuts have about 13.2 g fruit weight, 38 mm diameter with hull, 6.5 g hull weight, 7 g kernel weight, 55% yield due to physical properties, as a chemical properties walnut contained 98% dry matter, 65% crude oil, 2% ash, 7.4% crude protein and 3.75% crude fiber. The oils of walnut varieties are more yellowish-clear. Mean peroxide value, free fatty acidity, density, saponification value, refractive index values are 3.2 meq/kg, 0.4%, 13.9 g/ml, 107.5, 1.535 $n_{D,}$ respecttively. The major fatty acids of walnut oils were established as 6.4% palmitic, 2.5% stearic, 23% oleic, 51.5% linoleic and 14.4% linolenic. The highest minerals were Ca, Mg, K and P. Walnut is one of important foods need found in daily diets. High polyunsaturated fatty acid contents are the most important properties. At the same time, due to walnut's nutrition important was thought to be found between strategic foods in future.

## 5. ACKNOWLEDGEMENTS

This work was supported by Selçuk University Scientific Research Project (S.U.-BAP. Konya-Turkey).

## REFERENCES

[1] Şen, S.M. (1986) Ceviz Yetiştiriciliği. Eser Matbaası, Samsun.

[2] Çağlarırmak, N. (2003) Biochemical and physical properties of some walnut genotypes (*Juglans regia* L.). *Nahrung/Food*, **47(1)**, 28-32.

[3] Özkan, G. and Koyuncu, M.A. (2005) Physical and chemical comparision of some walnut (*Juglans regia* L.) genotypes grown in Turkey. *Grasas y Aceites* **56(2)**, 142-147.

[4] Sabate, J., Fraser, G.E., Burke, K., Knutsen, S.F., Benett, H., Linstead, K.D. (1993) Effects of walnuts on serum lipid levels and blood pressure in normal men. *The New England Journal of Medicine*, **328(9)**, 603-607.

[5] Savage, G.P. (2001) Chemical composition of walnuts (*Juglans regia* L.) grown in New Zealand. *Plant Foods for Human Nutrition*, **56(1)**, 75-82.

[6] Olez, H. (1971) Marmara bölgesi cevizlerinin (*Juglans regia* L.) seleksiyon yoluyla ıslahı üzerine araştırmalar. *Yalova Bahçe Kültürleri Araştırma Merkezi Dergisi*, **4(8)**, 1-4.

[7] Beyhan, O.E., Kaya, I., Şen, S.M. Doğan, M. (1995) Fatty acid composition of walnut (*Juglans regia* L.) types selected in Darende. *Turkish Journal of Agricultural Forestry* **19(4)**, 299-302.

[8] Zwarts, L., Savage, G.P. and McNeil, B.L. (1999) Fatty acid content of New Zealand-grown walnuts (*Juglans regia* L.). *International Journal of Food Science Nutrition*, **50(3)**, 189-194.

[9] Greve, C., Mc Granahan, G., Hasey, J., Synder, R., Kelly, K., Gold, H. D. and Labavitch, J. (1992) Variationin polyunsaturated fatty acids composition of Persian walnut. *Journal of American Social Hart Science*, **117(3)**, 518-522.

[10] Helmy, H.E. (1990) Studies on the pigments of some citrus, prune and cucurbit seed oils when processed with or without cottonseed oil. *Journal of American Oil Chemist Society*, **67(6)**, 376-380.

[11] Association of Official Analytical Chemists (1984) Official Methods of Analysis. Association of Official Chemistry, 14th Edition, Arlington.

[12] Hışıl, Y. (1998) Instrumental Analysis Techniques. Ege University Engineering Faculty Publication, Bornova-İzmir.

[13] AOCS. (1990) Official Methods and Recommended Practices. 4th Edition, American Oil Chemists Society, Champaign. II.

[14] Skujins, S. (1998) Handbook for ICP-AES (Varian-Vista). A short Guide to Vista Series. *ICP-AES Operation. VarianInt.* AG. Zug. Version 1.0., Switzerland.

[15] Püskülcü, H. and İkiz, F. (1989) Introduction Statistic (İstatistiğe Giriş). Bilgehan Press. Bornova-İzmir.

[16] Garcia, J.M., Agar, I.I. and Streit, J. (1994) Lipid characterijation in kerrels from different walnut cultivars. *Turkish Journal of Agricultural Forestry*, **18**, 195-198.

[17] Gunstore, F.D. and Norris, F.A. (1983) Lipids in Foods: Chemistry. *Biochemistry and Technology*, 1st Edition, Oxford.

[18] Colombini, M., Vanoni, M.C. and Amelotti, G. (1979) Olio di noci. nocciole. mandorle. avocado: Composizione sterolica. *La Rivista Italiana delle Sostanze Grasse*, **56**, 392-393.

[19] Allam, S.S.H. (2001) Utilization of some untraditional sources of high oleic acid oils for improving vegetable oils stability. *La Rivista Italiana Delle Sostanze Grasse* **78(6)**, 337-341.

[20] Yücel, S.O. (2005) Determination of conjugated linolenic acid content of selected oil seeds grown in Turkey. *Journal of American Oil Chemist Society*, **82(12)**, 893-897.

[21] Koyuncu, M. and Aşkın, M.A. (1999) Van Gölü çevresinde yetiştiriciliği yapılan bazı ceviz tiplerinin depolanması üzerine çalışmalar. *Turkish Journal of Agricultural Forestry*, **23(4)**, 785-796.

[22] Abbey, M., Noaks, M., Belling, G.B. and Nestel, P.J. (1994) Partial replacement of saturated fatty acids with almonds or walnuts lowers total plasma cholesterol and low-density lipoprotein cholesterol. *American Journal Clinical Nutrition*, **59(5)**, 995-999.

[23] Özcan, M. (2009) Some proximate characteristics of fruit and oil of walnut (*Juglans regia* L.) growing in Turkey. *Iranian Journal of Chemistry & Chemical Engineering* **28(1)**, 7-62.

[24] Macrae. R., Robinson, R.K. and Sadler, M.J. (Eds.), (1993) Encyclopaedia of Food Science. *Food Technology and Nutrition*, Academic Press INC, San Diego, 3126-3131.

[25] Brody, T. (1994) Nutritional Biochemistry. Academic Press, San Diego.

[26] Saura-Calixto, F. and Canellas, J. (1982) Correlations between lipid and protein contents in nuts and oil seeds. *Anal Bromatol*, **34**, 23-31.

**Vol.1, No.2, 68-75 (2010)**
doi:10.4236/as.2010.12010

Agricultural Sciences

# Evaluation of wheat ear insects in large scale field in central Germany

## ——Evaluation of wheat ear insects in winter wheat scale field

**Nawal Gaafar*, Christa Volkmar**

Institute of Agricultural & Nutritional Sciences, Martin-Luther-University Halle-Wittenberg, Halle, Wittenberg, Germany
*Corresponding authors: nawal_gaafar@yahoo.com

Received 16 June 2010; revised 3 July 2010; accepted 15 July 2010.

## ABSTRACT

**Wheat ear insects in large scale winter wheat field in Salzmünde (Saxsony-Anhalt) central Germany were evaluated. The present study aimed at studying the abundance of wheat blossom midges WBM, *Sitodoplosis mosellana* (Géhin), C*ontarinia tritici* (Kirby) and thrips, *Limothrips cerealium* (Haliday) and *Haplothrips tritici* (Kurdjumov). Infestation in winter wheat during the growing seasons 2007, 2008 and 2009 was evaluated. Three methods were used to determine population densities and damage of wheat midges and thrips; pheromone traps, inspection of ear insects and water traps. A strong correlation between midge's catches and weather conditions was obtained in field observations. A positive correlation between pheromone catches and ear infestation levels was recorded; it was higher in 2008 than in 2009. On the other hand, in 2007 there was no synchronization; *S. mosellana* hibernated emerged too late to coincide with the susceptible wheat growth stages. The chemical treatment applied at 2008 for highly infestation; there were significant differences in thrips and midge numbers between treated and untreated. Thrips and midge numbers were lower in the treated than in control. The high midge populations in water traps were recorded at growth stages 77-79 and 83 and the low populations were recorded at GS 75 and 75-77. This gives a reliable base for decision making to midges control.**

**Keywords:** Winter Wheat; Thrips; Wheat Midges; Population Densities

## 1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important cereal grain crops in the world and it is cultivated over a wide range of climatic conditions [1]. Yields can be improved if producers take time to inspect their fields and control the insect pests during the growing season [2]. Important pests that may reduce wheat yields are wheat blossom midges and thrips. The orange wheat blossom midge *Sitodiplosis mosellana* (Géhin) and the yellow wheat blossom midge *Contarinia tritici* (Kirby) (Diptera: Cecidomyiidae), have a very patchy spatial distribution and infestations vary from year to year, because they have the capacity for extended diapauses and only a portion of the larvae in the soil develop and pupate each spring, depending on climatic conditions [3]. *S. mosellana* and *C. tritici* cause direct damage by the larvae feeding on developing grain, and secondary fungal attack by *Fusarium graminearium* and *Septoria nodorum* may occur [3]. During the past decade, infestations of wheat midge seriously reduced the yield and quality of wheat in the major wheat-producing provinces in Germany [4,5], UK [6] Canada [7] and Finland [8]. The highest wheat midge populations can be found in fields where wheat was grown in previous years and in fields that are next to them.

Pheromone traps gave a reliable indication of peak midge emergence, onset of flight and abundance of midges throughout the season. The wheat plants are susceptible growth stages (GS) from the flag leaf sheath opening up to the flowering half complete (GS 47-65), [10]. Also weather conditions have to be favorable for the insect to lay eggs within the florets [6,11,12]. The critical risk factors are the proportion of diapausing midge larvae that might develop in any given season, the coincidence between emergence of adult midges and susceptible stages [13] and the suitability of the weather during adult midge activity coinciding with susceptible growth stages for flight and oviposition [14,15]. A strong correlation between maximum trap catches and crop infestation levels has resulted in many studies [3,5].

White water traps are often used to sample migrating and flying insects. Larvae are caught in their migrating way from wheat ears to soil at the end of the season. Insects are attracted visually by colour of the traps and are then captured in the water. Studies have demonstrated the preferences of a certain cultivar of insect to a particular coloured trap, as well as weather condition, especially rainfall [16].

Thrips infesting cereals are usually found behind the sheath of the flag leaf, feeding on the stem; however, leaves, and heads also were attacked [17]. Adults and nymphs can cause damage and, if present in large numbers, may cause the tissue on which they are feeding, to turn into a silver coloration. The stage of growth at the infestation time seems to determine the extent of yield loss [18]. The most important thrips species in the world, damaging wheat and barley heads are *Limothrips cerealium* (Haliday) and *Haplothrips tritici* (Kurdjumov). They are species of wide ecological plasticity, and able to build up populations with notable individual numbers in cooler zones of Europe [19-21].

The objective was to determine the abundance of WBM and thrips infestation in large scale wheat fields through three monitoring methods to establish economic thresholds. To address the growers need for monitoring systems against wheat ear insects to prepare an expert system should help wheat farmers in dry region in central Germany.

## 2. MATERIAL AND METHODS

### 2.1. Winter Wheat Fields

The winter wheat varieties Tommi, Manager and Impression were chosen to cultivate in 2007, 2008 and 2009, respectively. These varieties are commonly cultivated and with high quality properties [22], they were sown in sandy loam soil in the previous October every year in Salzmünde (Latitude 51°4'N, Longitude 11°55'E) central Germany. The crop rotation in the experiment sites was winter wheat after winter wheat and the plots size was 7.5 hectares.

#### 2.1.1. Monitoring WBM Adults Using Pheromone Traps

Pheromone monitoring kits were obtained from AgriSense™ (UK). Each trap consisted of a pheromone lure; Dispenser: Septa; Material: Natural rubber; Packaging: Individually Sachet Packed; Sachet Material: Foil Lined Laminate [23]. Two traps were set up when winter wheat was at growth stages 45 (flag leaf sheath swollen) and were taken off at GS 77 (late milky) in the studied years. The traps were placed at the same height as the wheat ears at a distance of 20 m from field borders and separated by 10 m [24,25]. Trap catches were recorded twice a week. Trapped WBM adults and debris were removed from the traps; and depending on the density of the caught insects the cards were changed.

#### 2.1.2. Inspection of Thrips and Midges in Wheat Ears

Ten ears were collected in method of liner observation [26] at flowering stage (GS 65) and milky stage (GS 73) [27] when the most larvae are already practically grown up, but still not left the spikes, they transported in sealed bags and stored at –20℃. By mean of a binocular the numbers of larvae per ear was counted and classified as *S. mosellana* or *C. tritici* and thrips *Limothrips cerealium* (Haliday) and *Haplothrips tritici* (Kurdjumov). In addition, kernel damage was registered as reformatted, cherviled or cracked.

#### 2.1.3. Surveying WBM Larvae Using Water Traps

The migrated midge's larvae from wheat ear were monitored using white water traps as expectation factor for the following years. The traps consisted of white plastic dishes; 12.5 cm diameter and 6.5 cm deep. Two traps were placed on the ground among wheat plants at milky stage (GS 73) and were taken off at gold dough (GS 89), and were partly filled with water (200 ml) plus 1ml of detergent (Fit). Traps were examined twice a week and larvae were counted using a magnifying glass.

### 2.2. Chemical Control

The wheat midge's management was conducted by using Karate (Lambda cyhalothrin), a pyrethroid insecticide, at a rate of 0.75l/ ha [28]; insecticide application was sprayed on 3rd June 2008 (GS 59), and only a 4/5 of the wheat field was sprayed. Insect populations were sampled before the insecticide application, thereafter, 3, 7, 10, 15 and 20 days after treatment.

### 2.3. Statistical Analysis

Numbers of captured insects and ear insect's evaluation were analyzed by linear model (a repeated measures analysis of variance (Statistix 9) [29]. Tukey test was used to compare means of varieties. Significances were noted at $P < 0.05$ for all trials. Thrips and midge numbers per ear were correlated with infested kernels by using the Pearson's correlation coefficient.

## 3. RESULTS

### 3.1. Monitoring *S. Mosellana* Adults Using Pheromone Traps

Populations of *S. mosellana* adults started slowly till milky stage and the first peak was recorded at GS 73 (1496 midges/trap) in 2007 (**Figure 1**). In 2008 large variations in numbers of midges in the pheromone traps and in time of peak catches were found; the highest number of males was 173 midges/trap recorded in GS (55-59) (**Figure 2**). There was one peak in 2009 (32 midges/trap) at GS 59-61 (**Figure 3**).

The lowest number of midges were1, 13 and 2.5 midges/trap in 2007, 2008, and 2009, respectively (**Figures 1-3**). Coincidence of adult activity and susceptible growth stages was more obvious in 2008 than in 2009 as shown in oval shape in **Figures 2** and **3**. The susceptible stages of wheat coincided with suitability for flight and oviposition. There was also a strong correlation between peak pheromone trap catches and weather conditions, rainfall and temperature ($r$ = +0.892 and $r$ = +0.742) in 2008 & 2009, respectively. On the other hand, there was no correlation ($r$ = +0.38) in 2007, possibly because the midge activity started later than the susceptible stage.

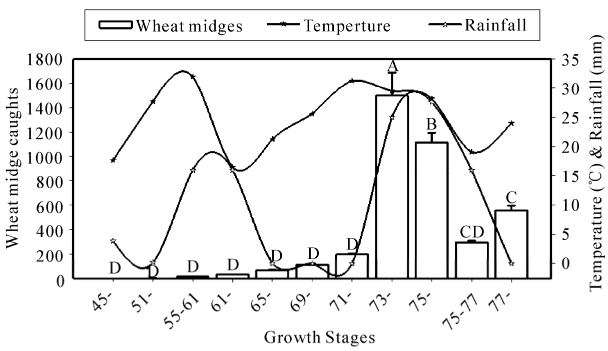



**Figure 1.** Mean *Sitodiplosis mosellana* adults caught in pheromone one trapsamd their realtion with temperature and rainfall in Salzmünde 2007.

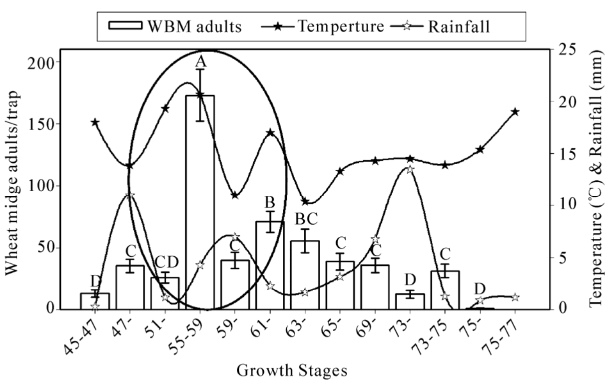



**Figure 2.** Mean ± SE of *Sitodiplosis mosellana* adults catches in pheromone traps and their relation with temperature and rainfall in 2008. Oval refers to coincidence of adult activity and susceptible growth stages. Different letters indicate significant differences.

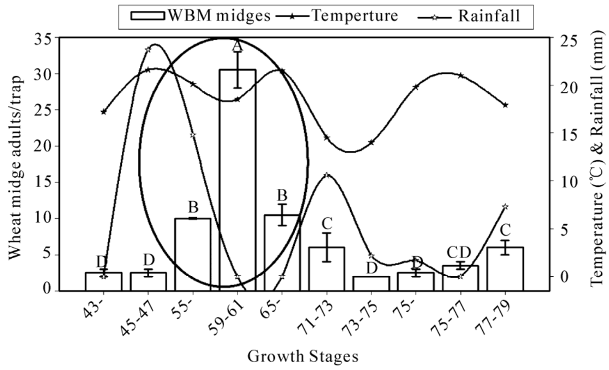



**Figure 3.** Mean ± SE of *Sitodiplosis mosellana* adults catches in pheromone traps and their relation with temperature and rainfall in 2009. Oval refere to coincidence of adult activity and susceptible growth stages. Different letters indicate significant differences.

## 3.2. Inspection of Thrips and Midges in Wheat Ears

### 3.2.1. 2007

#### 3.2.1.1. Total Thrips

In the most important growth stage GS 65&73 there was significant difference in thrips populations ($P$ = 0.0047) ($P$ = 0.0484) and ($P$ = 0.0451) in thrips adults, larvae and total thrips, respectively. The thrips adults were 0.7 and 1.5/ear in the same way. The corresponding records in thrips larvae were 1.3 and 2.0/ear. The total thrips/ear were 2.1 and 3.5, respectively (**Figure 4**).

#### 3.2.1.2. Wheat Midges

There was a significant difference ($P$ = 0.0357) in total midges between both growth stages (flowering and milky). Total midges (*S. mosellana & C. tritici*) were 0.2 and 1.8 larvae/ ear, respectively (**Figure 4**).

#### 3.2.1.3. Infested Kernels by Thrips and Midges

There was significant difference ($P$= 0.0391) in infested kernels (deformated, cherviled or cracked kernels) between the growth stages 65 and 73, these values were 0.2 and 1.8 infested kernels/ear, respectively (**Figure 4**).

### 3.2.2. 2008

#### 3.2.2.1. Total Thrips

Thrips population was 10.0 thrips/ear before the insecticide application, while after 3 days post treatment; they were 8.8 and 26.0 thrips/ear in the treated and control, respectively.

**In flowering stage (GS 65):** Significant differences were found ($P$ = 0.0083) in the number of total thrips between treated and control. On the 7$^{th}$ day, thrips number in control plants were higher than in treated 26.4 and 6.8/ear, respectively; the corresponding numbers on the

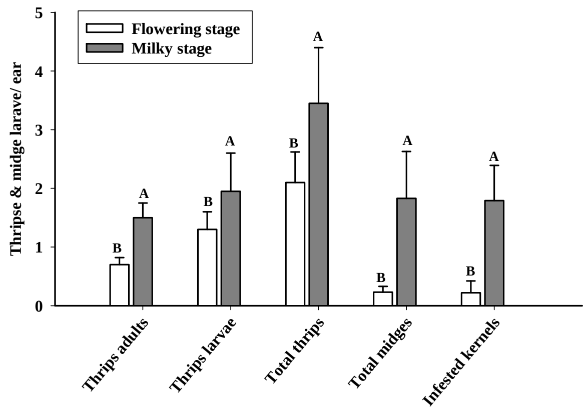



**Figure 4.** Mean ± SE of thrips (adults, larvae & total) and total midges in two growth stages in 2007. Different letters indicate significant differences.

10th day were 27.6 and 6.8 thrips/ear (**Figure 5(a)**).

**In milky stage (GS 73):** There was significantly different ($P$ = 0.0041) in thrips number between treated and untreated. Thrips numbers were lower in the treated than control. They were 6.8 and 31.6 thrips/ear, respectively after 15 days post treatment; the corresponding records on 20th day were 18.4 and 34.4 thrips/ear (**Figure 5(a)**).

### 3.2.2.2. Wheat Midges

There was no wheat midge larvae recorded before treatment (Figure 5(b)), while 3 days after treatment; they were 0.0 and 4.4 midge larvae/ear in treated and control plots, respectively.

**In flowering stage (GS 65):** There was no significant difference ($P$ = 0.0672) in the number of midge larvae (*S. mosellana & C. tritici*) between treated and untreated plots. On the 7th day, midge larvae numbers in treated were lower than in control 0.8 and 2.0/ ear, correspondingly; the equivalent records on the 10th day were 2.4 and 3.6 thrips/ear (Figure 5(b)).

**In milky stage (GS 73):** There was significantly different ($P$ = 0.0245) in wheat midge larvae between treated and untreated. Midge larvae numbers were higher in control than in treated plants. They were 4.0 and 1.2 larvae/ear, respectively after 15 days post treatment; the corresponding numbers on 20th day were 4.8 and 2.4 larvae/ear; this mean that treated had an half population which recorded in control (**Figure 5(b)**).

### 3.2.2.3. Correlation between Thrips, Midges and Infested Kernels

There was significant difference ($P$ = 0.0485) in infested kernels by thrips and wheat midge. Treated wheat had lower infested kernels than control plants. There was a positive correlation coefficient between wheat midge larvae and infested kernels ($r$ = +0.56 and +0.76) in GS 65 and 73 stages, respectively; while there was no

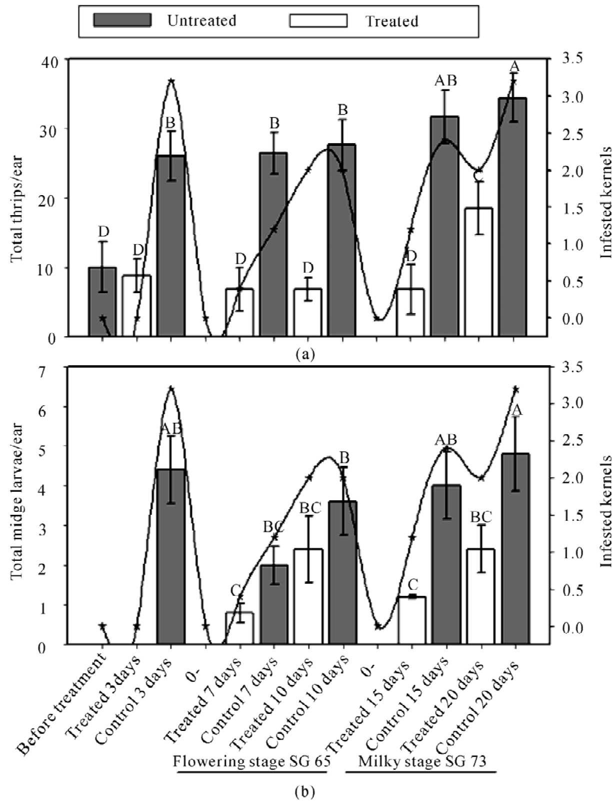



**Figure 5.** Mean ± SE of total thrips (a) andl midge larvae (b) in treated and untreated winter wheat during season 2008. Different letters indicate significant differences.

significantly correlation between total thrips and infested kernels ($r$ = +0.121 and +0.175) in both stages (**Figures 5 (a)** and **(b)**).

## 3.2.3. 2009

### 3.2.3.1. Total Thrips

There was a significant difference in thrips populations in GS 65 and 73; the significant value was ($P$ = 0.0030) in thrips adults and ($P$ = 0.0484) in total thrips. While there was no significant difference ($P$ = 0.891) in thrips larvae between both stages. The thrips adult were 0.3 in GS 65 and 1.1/ear in 73, respectively. The corresponding records in thrips larvae were 3.2 and 3.4/ear. The total thrips were 3.5 and 4.5/ear in GS 65 and 73, respectively (**Figure 6**).

### 3.2.3.2. Wheat Midges

There was a significant different ($P$ = 0.0263) in total midges populations (*S. mosellana & C. tritici*) between GS 65 and 73. The total midges were 0.3 and 0.8 larvae/ ear in GS 65 and 73, respectively (**Figure 6**).

### 3.2.3.3. Infested Kernels by Thrips and Midges

There was significant difference ($P$ = 0.0169) in infested kernels between growth stages 65 and 73, these values were 0.3 and 0.8 infested kernels/ear, respectively (**Figure 6**).

## 3.3. Monitoring WBM Larvae Using Water Traps

### 3.3.1. 2007

Yellow wheat midge larvae were only recorded on GS 75 (1 larva/trap). *S. mosellana* larvae were significantly higher ($P$ = 0.039) on growth stage 85 than other growth stages. The population densities of *S. mosellana* were 6, 4 and 13 midge larvae/trap at growth stages 75, 83 and 85, respectively. The last WBM larvae were caught on growth stage 87-89 (1 larva/trap) (**Figure 7**).

### 3.3.2. 2008

Populations of wheat midge larvae (*S. mosellana & C. tritici*) were significantly higher ($P$ = 0.0023) on control than treated. Population density was significantly lower ($P$ = 0.0353) on the first two stages (75 and 75-77 (18th & 22nd June)) than other two growth stages (77-79 and 83 (26th & 30th June)). The results indicated that *S. mosellana & C. tritici* populations could be divided into two groups; the high populations were recorded on 26th & 30th June and the low populations were recorded on 18th & 22nd June. Mean of low populations of *S. mosellana & C. tritici* 1 and 2 midge larvae/trap in treated and untreated plants. Mean of high populations of both wheat midge larvae were 4 and 12 larvae/trap in treated and control plants, respectively (**Figure 8**).

### 3.3.3. 2009

Yellow wheat midge was only recorded on GS 77 & 87 (1 & 2 larvae/trap, respectively). Population density of orange wheat midge was significantly higher ($P$ = 0.028) on growth stages 83 and 89 than the others. *S. mosellana* numbers were 4 larvae/trap in both stages. The last WBM larvae were caught on growth stage 89 (**Figure 9**).

## 4. DISCUSSION

Large variations in adult midge's numbers caught in the pheromone traps and in timing of peak catches were found between years (ca. fivefold) in farm scale studies under-

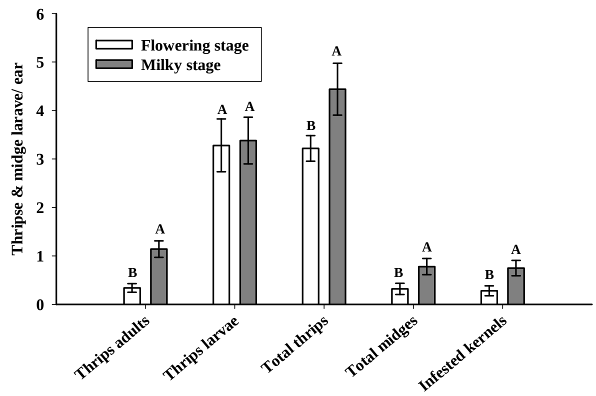



**Figure 6.** Mean ± SE of thrips (adults, larvae & total) and total midges in two growth stages in 2009. Different letters indicate significant differences.

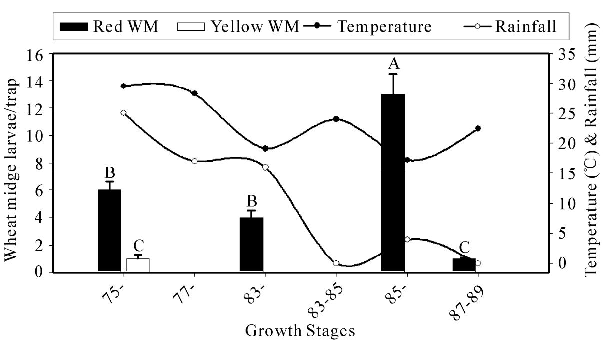



**Figure 7.** Mean ± SE of red-orange and yellow midge larvae by white water traps and their relation to temperature and rainfall during winter wheat season 2007. Different letters indicate significant differences.

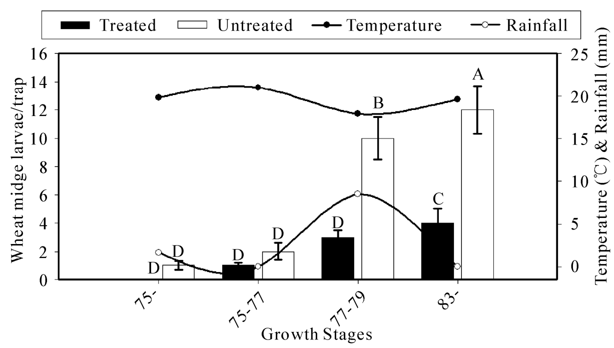



**Figure 8.** Mean ± SE of red-orange and yellow midge larvae catches in treated and untreated plots by white water traps and their relation to temperature and rainfall during winter wheat season 2008. Different letters indicate significant differences.

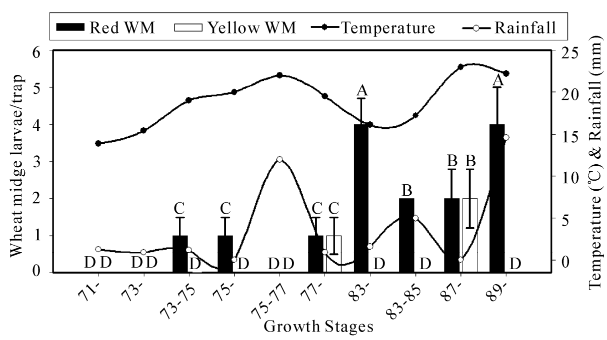



**Figure 9.** Mean ± SE of red-orange and yellow midge larvae by white water traps and their relation to temperature and rainfall during winter wheat season 2009. Different letters indicate significant differences.

taken in Salzmünde. This suggests that it is more useful for farmers to put traps in neighboring fields which were cultivated wheat in the year. There was no coincidence in 2007 between wheat midge activity and susceptible stages of wheat. Therefore wheat plants had escaped from midge's infestation, because the hibernated midges emerged later due to the warm weather in spring; it was ca. > 10℃ as stated by Oakley *et al.* [12]. In general, in 2008 and 2009 the peak of midge flight synchronized with the susceptible stage of the crop, it was more adequately in 2008 than in 2009, damage levels tended to be higher in 2008 than in 2009, because there was correlation between total numbers of males caught during the susceptible period and infestation as confirmed by Ellis *et al.* [30]. Pheromone traps were very valuable in indicating midge's emergence and for decision making. This is a significant benefit with other systems for monitoring wheat midges as mentioned by Gaafar and Volkmar [5,32]

The peaks of pheromone trap catch for the whole season occurred when the wheat was past the susceptible growth stage ex. 2007, but for setting the economic threshold the peak catch during the susceptible period is more relevant. There was also a strong correlation between peak pheromone trap catches and temperature and high rainfall. These results agree with those obtained by Oakley *et al.* [6], Bruce *et al.* [3], Volkmar *et al.* [31], Gaafar and Volkmar [32], who studied pheromone traps in different sites. Routine use of this monitoring method should eliminate most unnecessary applications of insecticides, and help assure that the benefits of insecticide applications exceed the cost.

Levels of midge infestation were higher in 2008 than in 2009 in both methods; evaluation ear insects and white water traps. This meant that there was a good relation between pheromone trap catches and midge's infestation. Therefore, chemical control was applied in 2008 and did not apply in 2007 or 2009 in case of low levels of midge infestation in 2007 and 2009. In terms of the impact of meteorological conditions, although soil moisture levels were favourable, weather conditions (temperature > 10℃ and 1mm rainfall) in the 2007 season were warmer than usual, which delayed the emergence of wheat midges in wheat ears as well as in white water traps. As a consequence, although the pheromone traps indicated that WBM had emerged, the time of arrival of many of the egg-laying females in the crop was not synchronized with the susceptible growth stage. This explains why there was a poorer correlation between pheromone trap catches and subsequent infestation in 2007 or 2009 than in 2008. Similar results were recorded by Ellis *et al.* [30], who reported that difference in weather condition between 2004 and 2005 had direct affected on wheat midge's populations.

Although levels of midge infestation were generally higher in 2008 than in 2007 and 2009, there is evidence to suggest that the proposed thresholds to the control decision are a good basis with which to predict the risk of midge attack. If cumulative trap catches exceed 30 midges/trap/day after heading (GS 59-65), then this indicates an economic risk to the wheat crop and an insecticide application may be necessary. The corresponding record in water trap was more than 10 midge larvae/trap (at late milky stage). As well as for ear evaluation, three or four maggots per kernel will destroy the kernels in that ear. Similar results were found by Olfert *et al.* [33,10], Oakley *et al.* [6] and Ellis *et al.* [30] in Canada and UK, they confirmed that if one or more adult midges are observed for every 4-5 heads or 3-4 midge larvae/ear; insecticide treatment is recommended. An economic threshold for *L. cerealium* was 25 thrips/ear, while Larsson [18] reported that this value was 35 thrips/tiller in his studies on winter barley. Pheromone traps, ear inspection and midges captured indicate the class of risk, while the threshold indicates the need for control.

## 5. CONCLUSIONS

The sequential sampling plans (pheromone traps, ear insect's evaluation and water traps) described in this paper should provide a method for more efficient midges monitoring. If pheromone trap catches indicate that a significant number of adults and suitable weather (temperature is > 16℃ and heavy rain is ca. 8 mm) during the susceptible stage of the wheat crop, need to be closely monitored at growth stages 47-65 [34]. Ear insects evaluation should be conducted in the milky stage (GS 73-75, when most larvae are already practically grown up, but have still not left the spike), while water traps should be also monitored carefully after the heavy rain, especially at late milky stage. A strong correlation between midge's catches and weather conditions was obtained in field observations; this gives a reliable base for decision making to midges control.

## 6. ACKNOWLEDGEMENTS

The authors want to thank to Dr. H. Hussein (Academy of Science, Czech Republic) for his beneficial notes on this manuscript, and Dr. N. El-Wakeil for his help in statistical analysis.

## REFERENCES

[1] Varshney, R.K., Balyan H.S. and Langridge, P. (2006) Genome mapping and molecular breeding in plants. *Cereals and Millets*, **1(2)**, 79-134.

[2] Wheat Insect Control Recommendations (WICR). http://www.utextension.utk.edu/fieldcrops/cotton/cotton_insects/pubs/PB1768-Wheat.pdf

[3] Bruce, T.J.A., Hooper, A. M., *et al.* (2007) Development of a pheromone trap monitoring system for *Sitodiplosis mosellana*, in the UK. *Pest Management Science*, **63(1)**, 49-56.

[4] Gaafar, N., Cöster, H. and Volkmar, C. (2009) Evaluation of ear infestation by thrips and wheat blossom midges in winter wheat cultivars. In: Feldmann, F., Alford, D.V. and Furk, C., Eds., *Proceedings* 3rd *International Symposium on Plant Protection & Plant Health in Europe*, Berlin, 349-359.

[5] Gaafar, N. and Volkmar, C. (2010) Assessment of wheat ear insects in winter wheat varieties in central Germany. *Journal of Pest Science* (Accepted).

[6] Oakley, J. N., Talbot, G., Dyer, C., *et al.* (2005) Integrated control of wheat midge: Variety choice, use of pheromone traps & treatment thresholds. *HGCA Project Report*, **363**, 65.

[7] Lamb, R.J., Sridhar, P. Smith, M.A.H. and Wise, I.L. (2003) Oviposition preference and offspring performance of a wheat midge *Sitodiplosis mosellana* on defended and less well defended wheat plants. *Environmental Entomology*, **32(2),** 414-420.

[8] Kurppa, S.L.A. (1989) Wheat blossom midges, *Sitodiplosis mosellana* Géhin and *Contarinia tritici* (Kirby) in Finland during 1981-1987. *Annales agriculturae Fenniae*, **28**, 87-96.

[9] Oakley, J.N. (1994) Orange wheat blossom midge: A literature review and survey of the 1993 outbreak. *Research Review No.* 28*. Home-Grown Cereals Authority London*, 51.

[10] Olfert, O., Elliott, R.H., Hartley, S. and Zeleny, K. (2004) Forecast of wheat midge in Alberta and Saskatchewan for 2004, 2003 Crop variety highlights and insect pest forecasts. *Saskatoon Research Centre Technical Bulletin No.* 2004-01, 14-15.

[11] Pivnick, K.A. (1993) Response of males to female sex pheromone in the orange wheat blossom midge, *Sitodiplosis mosellana*. *Journal of Chemical Ecology*, **19(8),** 1677-1689.

[12] Oakley, J.N., Cumbleton, P.C., Corbett, S.J., Saunders, P. *et al*. (1998) Prediction of orange wheat blossom midge activity and risk of damage. *Crop Protection*, **17(2)**, 145-149.

[13] Basedow, T. and Gillich H. (1982) Untersuchungen zur Prognose des Aufretens *Contarinia tritici* und *Sitodiplosis mosellana*. II. Faktoren, die ein Schadauftreten der Mücken verhindern können. *Anzeiger für Schädlingskunde Pflanzenschutz*, **55(2)**, 84-89.

[14] Gries, R., Gries, G., Khaskin, G., King, S., Olfert, O. *et al.* (2000) Sex pheromone of orange wheat blossom midge, *Sitodiplosis mosellana*. *Naturwissenschaften*, **87**, 450-454.

[15] Smith, M. and Lamb, R. (2001) Factors influencing oviposition by *Sitodiplosis mosellana* (Diptera: Cecidomyiidae) on wheat spikes. *Canadian Entomologist*, **133(4),** 533-548.

[16] Barker, A.M., Sanbrooke, K.J., Aebischer, N.J. (1997) The water trap colour preferences of farmland sawflies. *Entomologia Experimentalis et Applicata*, **85(1)**, 83-86.

[17] Kakol, E. and Kucharczyk, H. (2004) The occurrence of Thrips (Thysanoptera, Insecta) on winter and spring wheat in chosen regions of Poland. *Acta Phytopathologica et Entomologica Hungarica*, **39(1-3)**, 263-269.

[18] Larsson, H. (2005) Economic damage by *Limothrips denticornis* in rye, triticale and winter barley. *Journal of Applied Entomology*, **129(7)**, 386-392.

[19] Zawirska, I. and Walkowski, W. (2000) Fauna and importance of thrips (*Thysanoptera*) for rye and winter wheat in Poland. *Journal of Plant Protection Research*, **40(1),** 36-55.

[20] Andjus L. (2004) The thrips fauna on wheat and on plants of the spontaneous flora in the bordering belt surrounding it. *Acta Phytopathologica et Entomologica Hungarica*, **39(1-3)**, 255-261.

[21] Volkmar, C., Schröder, A., Gaafar, N., Cöster, H. and Spilke, J. (2009) Evaluierungsstudie zur Befallssituation von Thripsen in einem Winterweizensortiment. *Mitteilunge der Deutschen Gesellschaft für Allgmeine und Angewandte Entomolgie*, **17(1)**, 227-230.

[22] Anonymous, Beschreibende Sortenliste Getreide, Mais Ölfrüchte Leguminosen (großkörnig) Hackfrüchte (außer Kartoffeln) (2009) *Bundessortenamt*, *Osterfelddamm* 80, 30627 *Hanover*, 80-131.

[23] AgriSense™ (2007) Orange wheat blossom midge, Pheromone monitoring kit. AgriSense-BCS LTD, Treforest Industrial Estate, pontypridd, South Wales.

[24] Rieckmann, W., Block, T., Frosch, M., Heimbach, U., Lein, K.-Lauenstein, G., Matthes, P., Steck, U. and Volkmar C. (2001) Deutscher Vorschlag für eine EPPO-Richtlinie zur Prüfung von Insektiziden gegen Gallmücken an Getreide. *BBA* 1.35.

[25] Petersen, G. and Heimbach, U. (2009) Ergebnisprotokoll der 19. Tagung des DPG-Arbeitskreises Integrierter Pflanzen-schutz, Arbeitsgruppe, Schädlinge in Getreide und Mais. *Journal Für Kulturpflanzen*, **61(3)**, 178-179.

[26] Hübner, M. and Wittkopf, R. (2010) Pflanzenschutzempfehlung des amtlichen Pflanzenschuttz dienstes. Ackerbau & Grü-nland, LLFG Jahresbericht. www.llfg.sachsen-anhalt.de

[27] Tottman, D.R. (1987) The decimal code for the growth stages of cereals, with illustrations. *Annals of Applied Biology*, **110(2)**, 441-454.

[28] Anonymous, List of authorized plant protection products in Germany with information on terminated authorizations. *Bundesamt für Verbraucherschutz und Lebensmittelsicherheit* 56, Auflage 2008.

[29] Thomas, C.R. and Maurice, S.C. (2008) Statistix 9. *Managerial Economics McGraw-Hill/Irwin*, More information at http://www.statistix.com

[30] Ellis, S.A., Bruce, T.J.A., Smart, L.E., Martin, J.L., Snape, J. and Self, M. (2009) Integrated management strategies for varieties tolerant and susceptible to wheat midge. *HGCA Project Report*, **451**.

[31] Volkmar, C., Werner, C. and Matthes, P. (2008) On the occurrence and crop damage of wheat blossom midges (*Contarinia tritici* (Kby.) and *Sitodiplosis mosellana* (Geh.) in Saxony-Anhalt. *Mitteilunge der Deutschen Gesellschaft für Allgmeine und Angewandte Entomolgie*, **16(1)**, 305-308.

[32] Gaafar, N. and Volkmar, C. (2009) Monitoring system of orange wheat blossom midge, *Sitodiplosis mosellana*

(Géhin) using pheromone trap in the central Germany. *Mitteilunge der Deutschen Gesellschaft für Allgmeine und Angewandte Entomolgie*, **17(1)**, 221-225.

[33] Olfert, O., Mukerji, M.k. and Doane, J.F. (1985) Relationship between infestation levels and yield loss caused by wheat midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecido-myiidae), in spring wheat in Saskat-chewan. *Canadian Entomologist*, **117(5)**, 593-598.

[34] Pivnick, K.A. and Labbe, E. (1993) Daily patterns of activity of females of the orange wheat blossom midge, *Sitodiplosis mosellana*. *Canadian Entomologist*, **125(4)**, 725-736.

**Vol.1, No.2, 76-94 (2010)**
doi:10.4236/as.2010.12011 **Agricultural Sciences**

# The wonders of earthworms & its vermicompost in farm production: Charles Darwin's 'friends of farmers', with potential to replace destructive chemical fertilizers from agriculture

**Rajiv K. Sinha[1], Sunita Agarwal[2], Krunal Chauhan[3], Dalsukh Valani[3]**

[1]Visiting Senior Lecturer, School of Engineering (Environment), Griffith University, Nathan Campus, Brisbane, Australia; Corresponding Author: Rajiv.Sinha@griffith.edu.au
[2]Home Science, University of Rajasthan, Jaipur, India
[3]Research Assistant Worked on Vermiculture Projects, Griffith University, Brisbane, Australia

Received 15 April 2010; revised 2 June 2010; accepted 30 June 2010.

## ABSTRACT

**Earthworms and its excreta (vermicast) promises to usher in the 'Second Green Revolution' by completely replacing the destructive agrochemicals which did more harm than good to both the farmers and their farmland. Earthworms restore & improve soil fertility and significantly boost crop productivity. Earthworms excreta (vermicast) is a nutritive 'organic fertilizer' rich in humus, NKP, micronutrients, beneficial soil microbes—'nitrogen-fixing & phosphate solubilizing bacteria' & 'actinomycets' and growth hormones 'auxins', 'gibberlins' & 'cytokinins'. Both earthworms and its vermicast & body liquid (vermiwash) are scientifically proving as both 'growth promoters & protectors' for crop plants. In our experiments with corn & wheat crops, tomato and egg-plants it displayed excellent growth performances in terms of height of plants, color & texture of leaves, appearance of flowers & fruits, seed ears etc. as compared to chemical fertilizers and the conventional compost. There is also less incidences of 'pest & disease attack' and 'reduced demand of water' for irrigation in plants grown on vermicompost. Presence of live earthworms in soil also makes significant difference in flower and fruit formation in vegetable crops. Composts work as a 'slow-release fertilizer' whereas chemical fertilizers release their nutrients rather quickly in soil and soon get depleted. Significant amount of 'chemical nitrogen' is lost from soil due to oxidation in sunlight. However, with application of vermicompost the 'organic nitrogen' tends to be released much faster from the excreted 'humus' by worms and those mineralised by them and the net overall efficiency of nitrogen (N) is considerably greater than that of chemical fertilizers. Availability of phosphorus (P) is sometimes much greater. Our study shows that earthworms and vermicompost can promote growth from 50 to 100% over conventional compost & 30 to 40% over chemical fertilizers besides protecting the soil and the agroecosystem while producing 'nutritive and tasty food' at a much economical cost (at least 50-75% less) as compared to the costly chemical fertilizers.**

**Keywords:** A Slow Release Fertilizer; Vermicompost – Miracle Growth Promoter; Rich in Nutrients; Humus & Hormones; Vermicompost Induce Biological Resistance in Plant; Suppress & Repel Pest Attack

## 1. INTRODUCTION

A revolution is unfolding in vermiculture studies for vermicomposting of diverse organic wastes by waste eater earthworms into a nutritive 'organic fertilizer' and using them for production of 'chemical-free safe food', both in quantity & quality without recourse to agrochemicals. Heavy use of agro-chemicals since the 'green-revolution' of the 1960's boosted food productivity, but at the cost of environment & society. It killed the beneficial soil organisms & destroyed their natural fertility, impaired the power of 'biological resistance' in crops making them more susceptible to pests & diseases. Chemically grown foods have adversely affected human health. The scientific community all over the world is desperately looking for an 'economically viable, socially safe & environmentally sustainable' alternative to the agro-chemicals.

Vermicomposts work as a 'slow-release organic fertilizer'. With their continued application the 'organic nitrogen' & other nutrients in compost tends to be released at constant rate from the accumulated 'humus' and the net overall efficiency of NPK over a period of years is considerably greater than 50% of that of chemical fertilizers. Our study shows that it can promote growth from 50 to 100% over conventional compost & 30 to 40% over chemical fertilizers besides protecting the soil and the agro-ecosystem while producing 'nutritive and tasty food' at a much economical cost (at least 50-75% less) as compared to the costly chemical fertilizers. Study found that maximum benefit from vermicompost is obtained when it constitutes between 10 to 40% of the growing medium [1].

The best part is that the use of earthworms and vermicompost in farm production provides dual-benefit to crops. While promoting excellent growth it also protects the crops from pests and diseases and thus significantly reduce the use of chemical pesticides.

Several farms in world especially in North America, Australia and Europe are going organic as the demand for 'organic foods' are growing in society. In 1980, the U.S. Board of Agriculture published a '*Report and Recommendations on Organic Farming*' based on case studies of 69 organic farmers in U.S. and reported that over 90,000 to 1,00,000 farmers in U.S. had already switched over to organic farming [2]. This must have gone in millions now. Earthworms will provide the answer [3]. They have over 600 million years of experience in land management, soil improvement & farm production. No wonder, Sir Charles Darwin called them as the 'unheralded soldiers of mankind and farmer's friend working day and night under the soil' [4,5]. Importance of earthworms in growth of crop plants was indicated by the ancient Indian scientist Surpala as early as in the 10th Century A.D. in his epic 'Vrikshayurveda' (Science of Tree Growing) who suggested to add earthworms in pomegranate plants to get good quality of fruits [6].

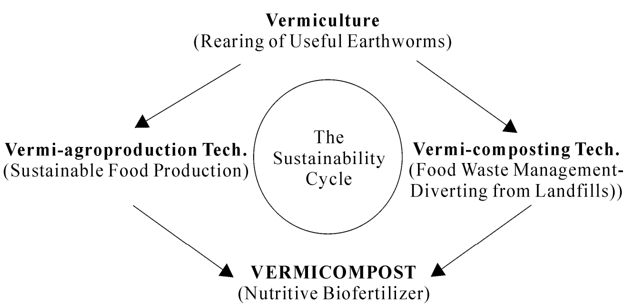



**Figure 1**. The sustainability cycle of vermiculture technology: from food waste to food again.

## 2. EARTHWORMS: THE SOIL MANAGER

Earthworms restore & improve soil fertility and boost crop productivity by the use of their excretory products - 'vermicast'. They excrete beneficial soil microbes, and secrete polysaccharides, proteins and other nitrogenous compounds into the soil. They promote soil fragmentation and aeration, and bring about 'soil turning' and dispersion in farmlands. Worm activity can increase air-soil volume from 8-30%. One acre of land can contain up to 3 million earthworms the activities of which can bring up to 8-10 tons of 'top soil' to the surface (in the form of vermicast) every year. Presence of worms regenerate compacted soils and improves water penetration in such soils by over 50%. [7-9]. U.S. study indicate that 10,000 worms in a farm plot provides the same benefit as three farmers working 8 hours in shift all year round with 10 tons of manure applied in the plot [10].

Indian study showed that an earthworm population of 0.2-1.0 million per hectare of farmlands can be established within a short period of three months. On an average 12 tons/hectare/year of soil or organic matter is ingested by earthworms, leading to upturning of 18 tons of soil/year, and the world over at this rate it may mean a 2 inches of fertile humus layer over the globe [11]. Studies at CSIRO, Australia found that introductions of earthworms in disturbed lands can yield substantial benefits to agricultural productivity and amelioration of soil degradation.

## 3. CHEMICAL FERTILIZERS-A BANE, COMPOST THE BOON: REDISCOVERING THE VALUE OF 'COMPOST' FOR SAFE FOOD PRODUCTION

Chemical fertilizers which ushered the 'green revolution' in the 1950-60's came as a 'mixed blessing' for mankind. It dramatically increased the 'quantity' of the food produced but decreased its 'nutritional quality' and also the 'soil fertility' over the years. The soil has become addict and increasingly greater amount of chemical fertilizers are needed every year to maintain the soil fertility and food productivity at the same levels. There is evidence that a plateau has been reached in global efforts to increase the yield per hectare through agro-chemicals. The early response to chemical fertilizers is 'levelling off' after a 3% annual increase 1950-1984. Over the years it has worked like a 'slow poison' for the soil with a serious 'withdrawal symptoms'. The farmers today are caught in a 'vicious circle' of higher use of agrochemicals to boost food productivity at the cost of declining soil fertility. The excessive use of 'nitrogenous fertilizer' (urea) has also led to increase in the level of 'inorganic nitrogen' content in groundwater (through leaching effects) and in the human food with grave consequences for the human health.

Organic farming systems with the aid of various nutrients of biological origin such as compost (conventional microbial compost or vermicompost made by earthworms) are thought to be the answer for the 'food safety and security' in future. Among them 'composts' made from biodegradation of organics of MSW (municipal solid waste) which is being generated in huge amount every day all over the world are most important. The organic fraction of the MSW (about 70-80%) containing plenty of nitrogen (N), potash (K) and phosphorus (P) is a good source of macro and micronutrients for the soil. Also, there is always greater economic as well as ecological wisdom in converting as much 'waste into compost'.

## 4. AGRONOMIC VALUES OF COMPOST (CONVENTIONAL OR VERMICOMPOST)

Composts (conventional or vermicompost) are aerobically decomposed products of organic wastes such as the cattle dung and animal droppings, farm and forest wastes and the municipal solid wastes (MSW). Some believe it is a 'miracle' plant growth promoter [12]. They supply balanced nutrients to plant roots and stimulate growth; increase organic matter content of the soil and thus improve their physical and chemical properties; add useful micro-organisms to the soil and provide food for the existing soil micro-organisms and thus increase their biological properties and capacity of fertility renewal. One ton of conventional compost may contain 10 lbs of nitrogen (N), 5 lbs of phosphorus ($P_2O_5$) and 10 lbs of potash ($K_2O$). Compost made from poultry droppings contain highest nutrient level among all compost [13].

There are other agronomic benefits of composts application, such as high levels of soil-borne disease suppression and removal of soil salinity. One study reported that mean root disease was reduced from 82% to 18% in tomato and from 98% to 26% in capsicum in soils amended with compost [14]. Other reported that with application of compost in vineyards, levels of exchangeable sodium (Na) under vine were at least reduced to 50% [15]. Biological properties of soil were also improved with up to ten-fold increase in total microbial counts. Most significant was three-fold increase in the population of earthworms under the vine with long-term benefits to the soil.

## 5. VERMICOMPOST VS CONVENTIONAL COMPOST

Our studies at Griffith University, Australia has conclusively proved that the indigenously prepared earthworms vermicompost is 'exceptionally superior' over all brands of conventionally prepared & marketed composts certified by Compost Australia. Studies confirm that vermicompost is at least 4 times more nutritive than conventional cattle dung compost [16]. In Argentina, farmers who use vermicompost consider it to be seven (7) times richer than conventional composts in nutrients and growth promoting values [17,18]. This is mainly due to 'humus' content in vermicompost excreted by earthworms which otherwise takes very long time to form humus in conventional composting system through slow decay of organic matter. The 'humic acid' in vermicompost stimulate plant growth even in small amount [19]. Vermicompost retains nutrients for long time than the conventional compost & while the latter fails to deliver the required amount of macro and micronutrients including the vital NKP (nitrogen, potassium & phosphorus) to plants in shorter time, the vermicompost does. Vermicompost also has very 'high porosity', 'aeration', 'drainage' and 'water holding capacity' than the conventional compost and this again due to humus contents. [16].

Earthworm participation enhances natural biodegradation and decomposition of organic materials from 60 to 80% by promoting the growth of 'beneficial decomposer aerobic bacteria' in the waste biomass. The quality of compost is significantly better, rich in key minerals & beneficial soil microbes. It is also disinfected and free of any pathogens as the worms release anti-pathogenic coelomic fluid in the waste biomass [20]. In fact in the conventional aerobic composting process which is thermophilic (temperature rising up to 55℃) many beneficial microbes are killed and nutrient especially nitrogen is lost (due to gassing off of nitrogen). Some studies found that while the conventional compost was higher in 'ammonium', the vermicompost tended to be higher in 'nitrates', which is the more bio-available form of nitrogen for plants [21]. They also found that vermicompost has higher N availability than the conventional compost on a weight basis and the supply of several other plant nutrients e.g. phosphorus (P), potassium (K), sulfur (S) and magnesium (Mg), were significantly increased by adding vermicompost as compared to conventional compost to soil. Study found the NPK value of vermicompost processed by worms from the same feedstock (cattle dung) significantly increases by 3 to 4 times. It also enhances several micronutrients [22].

Studies have found that if 100 kg of soil organics (with say containing 2 kg of plant nutrients) are processed through the earthworms, there is a production of about 300 kg of 'fresh living soil' with 6% of NPK and several trace elements that are equally essential for healthy plant growth. This magnification of plant nutrients is possible because earthworms produce extra nutrients from grinding rock particles with organics and by enhancing atmospheric nitrogen fixation. Earthworms activate this ground mix in a short time of just one hour. When 100 kg of the same organic wastes are composted conventionally unaided by earthworms, about 30 kg compost is derived with 3% NPK [11].

Although the conventional composting process is completed in about 8 weeks, but additional 4 weeks is required for 'curing'. Curing involves the further aerobic decomposition of some compounds, organic acids and large particles that remain after composting. Less oxygen and water is required during curing. Compost that has had insufficient curing may damage crops. Vermicompost do not require any curing and can be used straightway after production. It retains nutrients for long time and while the conventional compost fails to deliver the required amount of macro and micronutrients including the vital NKP (nitrogen, potassium & phosphorus) to plants in shorter time, the vermicompost does. [12,23]. This was also verified by us [24].

**Table 1.** NPK value of vermicompost compared with conventional cattle dung compost made from cattle dung.

| Nutrients | | Cattle Dung Compost | Vermicompost |
|---|---|---|---|
| 1. | N | 0.4-1.0% | 2.5-3.0% |
| 2. | P | 0.4-0.8% | 1.8-2.9% |
| 3. | K | 0.8-1.2% | 1.4-2.0% |

**Source**: Agarwal [22]

**Table 2.** Comparison between nutritive values of the end products of conventional composting and vermicomposting systems (CNP in %; Others in mg/100 gm of compost).

| Parameter | Conventional Composting | Vermicomposing |
|---|---|---|
| Total Carbon (C) | 9.34% | 13.5% |
| Total Nitrogen (N) | 1.05% | 1.33% |
| Available Phosphorus (P) | 0.32% | 0.47% |
| Iron (Fe) | 587.87 | 746.2 |
| Zinc (Zn) | 12.7 | 16.19 |
| Manganese (Mn) | 35.25 | 53.86 |
| Copper (Cu) | 4.42 | 5.16 |
| Magnesium (Mg) | 689.32 | 832.48 |

Source: Jadia & Fulekar [25]

## 6. SOIL PROTECTIVE COMPOST VS SOIL DESTRUCTIVE CHEMICAL FERTILIZERS

Upon successive application, all composts condition the soil with rich population of 'beneficial soil microbes' & and 'essential nutrients' thus reinforcing its natural fertility, whereas, the chemical fertilizers destroy the beneficial microbes and impair the natural fertility of soil while also affecting soil pH and porosity. Composts work as a 'slow-release fertilizer' whereas chemical fertilizers release their nutrients rather quickly in soil and soon get depleted. Nitrogen and phosphorus particularly are not all available to plant roots from the conventional composts in the first year because N & P in organic matter are resistant to decay. Nitrogen is about one half effective as compared to chemical fertilizer, but phosphorus & potassium are as effective as chemical fertilizers. However, with continued application of compost over the years the 'organic nitrogen' (N) from the accumulated 'humus' (through a long decay process) tends to be released and the net overall efficiency of nitrogen (N) over a period of years is considerably greater than 50% of that of chemical fertilizers. Availability of phosphorus is sometimes much greater [12,26]. But vermicompost releases nitrogen (N) much faster and even after single application as 'humus' is directly excreted by worms and they also mineralise nitrogen from the waste organics to make it bio-available to plants. The net overall efficiency of nitrogen (N) is considerably greater than that of chemical fertilizers [27]. All compost (including vermicompost), are produced from some 'waste materials' of society which is converted into a 'valuable resource'. It is like 'killing two birds in one shot'. More significant is that it is of biological origin *i.e.* a 'renewable resource' and will be readily available to mankind in future. Whereas, chemical fertilizers are made from petroleum products which are 'non-renewable' and a 'depleting' resource. While in the use of compost the environment is 'benefited' at all stages - from production (salvaging waste & diverting them from landfills and reducing greenhouse gases) to application in farms (adding beneficial microbes to soil & improving biochemical properties), in the use of chemical fertilizers the environment is 'harmed' at all stages - from procurement of raw materials from petroleum industries to production in factories (generating huge amount of chemical wastes and pollutants) and application in farms (adversely affecting beneficial soil micro-organisms and soil chemistry). And with chemical fertilizers, there is yet another problem. A significant amount of 'nitrogen' (N) is lost from the soil due to oxidation in sunlight. Studies indicate that upon application of 100 kg urea (N) in farm soil, 40-50 kg gets oxidised and escapes as 'ammonia' ($NH_3$) into the air, about 20-25 kg leaches underground polluting the groundwater, while only 20-25 kg is available to plants [16]

**Properties of Farm Soil Using Compost Vis-a-vis Chemical Fertilizers**

Suhane [16], studied the chemical and biological properties of soil under organic farming (using various types of composts) and chemical farming (using chemical fertilizers - urea (N), phosphates (P) and potash (K)). Results are given in **Table 1**.

## 7. ADVANTAGES OF USE OF VERMICOMPOST OVER CHEMICAL FERTILIZERS

The biggest advantage of great socio-economic significance is that the food produced is completely organic, 'safe & chemical-free'. Use of vermicompost enhances size, color, smell, taste, flavour and keeping quality (storage value) of flowers, fruits, vegetables and food grains. Studies indicate that vermicompost gives 30-40% higher yield of crops over chemical fertilizers. Of greater agronomic significance is that the minerals in the vermicompost are 'readily & immediately bio-available' to the plants. Chemical fertilizers (and also manures) have to be broken down in the soil before the plants can absorb. Vermicompost also has greater 'water holding capacity' due to humus contents and hence reduces the requirement of water for irrigation by 30-40%. Use of chemical fertilizers require high amount of water for irrigation.

Another big advantage of great economic & environmental significance is that over successive years of application, vermicompost 'build-up the soils natural fertility' and also regenerates a rich population of earthworms in the farm soil from the cocoons which further help improve soil fertility and subsequently lesser amount of vermicompost is required to maintain a good yield and productivity. On the contrary, with the continued application of chemical fertilizers over the years the 'natural fertility of soil is destroyed' and it becomes 'addict to chemicals'. Subsequently greater amount of chemicals are required to maintain the same yield & productivity of previous years. More uses of agro-chemicals to boost food productivity are in fact a 'self-defeating' proposition.

**Table 3.** Farm soil properties under organic farming and chemical farming.

| Chemical & Biological Properties of Soil | Organic Farming (Use of Composts) | Chemical Farming (Use of Chemical Fertilizers) |
|---|---|---|
| 1) Availability of Nitrogen (kg/ha) | 256.0 | 185.0 |
| 2) Availability of Phosphorus (kg/ha) | 50.5 | 28.5 |
| 3) Availability of Potash (kg/ha) | 489.5 | 426.5 |
| 4) *Azatobacter* (1000/gm of soil) | 11.7 | 0.8 |
| 5) Phospho Bacteria (100,000/kg of soil) | 8.8 | 3.2 |
| 6) Carbonic Biomass (mg/kg of soil) | 273 | 217 |

Source: Suhane [16]

## 8. EARTHWORMS IMPROVES TOTAL PHYSICAL, CHEMICAL & BIOLOGICAL QUALITY OF SOIL

Earthworms are regarded as 'biological indicator' of soil fertility and a 'soil conditioner'. They lead to total improvement in the physical (soil porosity & softness), chemical (good pH and essential plant nutrients) and biological (beneficial soil microbes & organisms) quality of soil and land where they inhabit. They swallow large amount of soil with organics (microbes, plant & animal debris) everyday, grind them in their gizzard and digest them in their intestine with aid of enzymes. Only 5-10 percent of the chemically digested and ingested material is absorbed into the body and the rest is excreted out in the form of fine mucus coated granular aggregates called 'vermicastings' which are rich in NKP (nitrates, phosphates and potash), micronutrients and beneficial soil microbes [28].

## 9. EARTHWORMS & VERMICOMPOST: MIRACLE PLANT GROWTH PROMOTER & PROTECTOR

Earthworms vermicast is a highly nutritive 'organic fertilizer' rich in humus, NKP (nitrogen 2-3%, potassium 1.85-2.25% and phosphorus 1.55-2.25%), micronutrients, beneficial soil microbes like 'nitrogen-fixing bacteria' and 'mycorrhizal fungi' and are scientifically proving as 'miracle growth promoters'. [29-31]. One study reports as high as 7.37% nitrogen (N) and 19.58% phosphorus as $P_2O_5$ in worms vermicast [32]. Another study showed that exchangeable potassium (K) was over 95% higher in vermicompost [16]. There are also good amount of calcium (Ca) and magnesium (Mg). Vermicompost has very 'high porosity', 'aeration', 'drainage' and 'water holding capacity'. More important is that it contains 'plant-available nutrients' and appears to increase & retain more of them for longer period of time. A matter of still greater agronomic significance is that worms & vermicompost also increases 'biological resistance' in plants (due to Actinomycetes) and protect them against pest and diseases either by repelling or by suppressing them [1,34,35].

### 9.1. High Levels of Bio-Available Nutrients for Plants

Earthworms mineralize the nitrogen (N) and phosphorus (P) and all essential organic & inorganic elements in the compost to make it bio-available to plants as nutrients [36]. They recycle nitrogen in soil in very short time ranging from 20 to 200 kg N/ha/year & increase nitrogen contents by over 85% [37]. After 28 weeks the soil with living worms contained 75 ppm of nitrate nitrogen (N), compared with the controlled soil which had only 45 ppm [38]. Worms increase nitrogen levels in soil by adding their metabolic & excretory products (vermicast), mucus, body fluid, enzymes and decaying tissues of dead worms [39,40]. Lee [41] suggests that the passage of organic matter through the gut of worm results in phosphorus (P) converted to forms which are more bio-available to plants. This is done partly by worm's gut enzyme 'phosphatases' and partly by the release of phosphate solubilizing microorganisms in the worm cast [42].

### 9.2. High Level of Beneficial and Biologically Active Soil Microorganisms

Among beneficial soil microbes stimulated by earthworms are 'nitrogen-fixing & phosphate solubilizing bacteria', the 'actinomycetes' & 'mycorrhizal fungi'. Studies found that the total bacterial count was more than $10^{10}$/gm of vermicompost. It included *Actinomycetes*, *Azotobacter*, *Rhizobium*, *Nitrobacter* & Phos-phate Solubilizing Bacteria ranges from $10^2$-$10^6$ per gm of vermicompost [16].

### 9.3. Rich in Humus: Key to Growth and Survival of Plants

Vermicompost contains 'humus' excreted by worms which makes it markedly different from other organic fertilizers. It takes several years for soil or any organic matter to decompose to form humus while earthworms secrete humus in its excreta. Without humus plants cannot grow and survive. The humic and fulvic acids in humus are essential to plants in four basic ways – 1) Enables plant to extract nutrients from soil; 2) Help dissolve unresolved minerals to make organic matter ready for plants to use; 3) Stimulates root growth; and, 4) Helps plants overcome stress. Presence of humus in soil even help chemical fertilizers to work better [43]. This was also confirmed by other study [44]. One study found that humic acids isolated from vermicompost enhanced root elongation and formation of lateral roots in maize roots. Humus in vermicast also extracts 'toxins', 'harmful fungi & bacteria' from soil & protects plants [19].

### 9.4. Rich in Plant Growth Hormones

Some studies speculated that the growth responses of plants from vermicompost appeared more like 'hormone-induced activity' associated with the high levels of nutrients, humic acids and humates in vermicompost [21, 45]. Researches show that vermicompost use further stimulates plant growth even when plants are already receiving 'optimal nutrition'. It consistently improved seed germination, enhanced seedling growth and development, and increased plant productivity significantly much more than would be possible from the mere conversion of mineral nutrients into plant-available forms. Some studies have also reported that vermicompost contained growth promoting hormone 'auxins', 'cytokinins' and flowering hormone 'gibberlins' secreted by earthworms [16,47,48].

### 9.5. Enzymes for Improving Soil Nutrients & Fertility

Vermicompost contain enzymes like amylase, lipase, cellulase and chitinase, which continue to break down organic matter in the soil (to release the nutrients and make it available to the plant roots) even after they have been excreted. [30,31]. They also increases the levels of some important soil enzymes like dehydrogenase, acid and alkaline phosphatases and urease. Urease play a key role in N-cycle as it hydrolyses urea and phosphatase bioconvert soil phosphorus into bio-available form for plants.

## 10. EARTHWORMS REDUCE SOIL SALINITY & IMPROVE FERTILITY OF SODIC SOILS

Studies indicate that *Esinea fetida* can tolerate soils nearly half as salty as seawater *i.e.* 15 gm/kg of soil and also improve its biology and chemistry. (Average seawater salinity is around 35 g/L). Farmers at Phaltan in Satara district of Maharashtra, India, applied live earthworms to their sugarcane crop grown on saline soils irrigated by saline ground water. The yield was 125 tones/hectare of sugarcane and there was marked improvement in soil chemistry. Within a year there was 37% more nitrogen, 66% more phosphates and 10% more potash. The chloride content was less by 46% [27].

Ansari [49] studied the production of potato (*Solanum tuberosum*) by application of vermicompost in a reclaimed sodic soil in India. With good potato growth the sodicity (ESP) of the soil was also reduced from initial 96.74 to 73.68 in just about 12 weeks. The average available nitrogen (N) content of the soil increased from initial 336.00 kg/ha to 829.33 kg/ha.

## 11. EARTHWORMS PROTECTS PLANTS AGAINST PESTS AND DISEASES & SIGNIFICANTLY REDUCE USE OF CHEMICAL PESTICIDES

Earthworms are both 'plant growth promoter and protector'. There has been considerable evidence in recent years regarding the ability of earthworms and its vermicompost to protect plants against various pests and diseases either by suppressing or repelling them or by inducing biological resistance in plants to fight them or by killing them through pesticidal action. The actinomycetes fungus excreted by the earthworms in their vermicast produce chemicals that kill parasitic fungi such as *Pythium* and *Fusarium* [34]. Another study confirmed that application of vermicompost reduced the damage by stripted cucumber beetle (*Acalymma vittatum*), spotted cucumber beetle (*Diabotrica undecimpunctata*) on cucumber and larval hornworms (*Manduca quinquemaculata*) on tomatoes in both greenhouse and field experiments [50].

### 11.1. Ability to Induce Biological Resistance in Plants

Vermicompost contains some antibiotics and actinomycetes which help in increasing the 'power of biological resistance' among the crop plants against pest and diseases. Spray of chemical pesticides was significantly reduced by over 75% where earthworms and vermicompost were used in agriculture [13,16].

### 11.2. Ability to Repel Crop Pests

There seems to be strong evidence that worms varmicastings sometimes repel hard-bodied pests [1,33]. Studies reported statistically significant decrease in arthropods (aphids, buds, mealy bug, spider mite) populations, and subsequent reduction in plant damage, in tomato, pepper, and cabbage trials with 20% and 40% vermicompost additions [34]. George Hahn, doing commercial vermicomposting in California, U.S., claims that his product repels many different insects pests. His explanation is that this is due to production of enzymes 'chitinase' by worms which breaks down the chitin in the insect's exoskelton [17].

### 11.3. Ability to Suppress Plant Disease

Studies reported that vermicompost application suppressed 20-40% infection of insect pests *i.e.* aphids (*Myzus persicae*), mearly bugs (*Pseudococcus spp.*) and cabbage white caterpillars (*Peiris brassicae)* on pepper (*Capiscum annuum*), cabbage (*Brassica oleracea*) and tomato (*Lycopersicum esculentum*) [51].

Studies have also found that use of vermicompost in crops inhibited the soil-born fungal diseases. They also found significant suppression of plant-parasitic nematodes in field trials with pepper, tomatoes, strawberries and grapes [34]. The scientific explanation behind this concept is that high levels of agronomically beneficial microbial population in vermicompost protects plants by out-competing plant pathogens for available food resources *i.e.* by starving them and also by blocking their excess to plant roots by occupying all the available sites. This concept is based on 'soil-foodweb' studies pioneered by Dr. Elaine Ingham of Corvallis, Oregon, U.S. (http://www.soilfoodweb.com).

Edwards and Arancon [27] also reported the disease suppressing effects of applications of vermicompost, on attacks by fungus *Pythium* on cucumber, *Rhizoctonia* on radishes in the greenhouse, by *Verticillium* on strawberries and by *Phomposis* and *Sphaerotheca fulginae* on grapes in the field. In all these experiments vermicompost applications suppressed the incidence of the disease significantly. They also found that the ability of pathogen suppression disappeared when the vermicompost was sterilized, convincingly indicating that the biological mechanism of disease suppression involved was 'microbial antagonism.

Studies also reported considerable suppression of root knot nematode (*Meloidogyne incognita*) and drastic suppression of spotted spider mites (*Tetranychus* spp.) and aphid (*Myzus persicae*) in tomato plants after application of vermicompost teas (vermiwash liquid) [52]. They are serious pests of several crops.

## 12. SOME KEY STUDIES SUPPORTING SOIL FERTILITY IMPROVEMENT AND GOOD CROP PRODUCTION BY EARTHWORMS AND VERMICOMPOST OVER CHEMICAL FERTILIZERS

There have been several reports that earthworms and its vermicompost can induce excellent plant growth and enhance crop production.

### 12.1. Cereal Crops

Glasshouse studies made at CSIRO Australia found that the earthworms (*Aporrectodea trapezoids*) increased growth of wheat crops (*Triticum aestivum*) by 39%, grain yield by 35%, lifted protein value of the grain by 12% & also resisted crop diseases as compared to the control. The plants were grown in a 'red-brown earth' with poor nutritional status and 60% moisture. There was about 460 worms $m^{-2}$ [53]. They also reported that in Parana, Brazil invasion of earthworms significantly altered soil structure and water holding capacity. The grain yields of wheat and soybean increased by 47% and 51%, respectively [54].

Some studies were made on the impact of vermicompost and garden soil in different proportion on wheat crops in India. It was found that when the garden soil and vermicompost were mixed in 1:2 proportions, the growth was about 72-76% while in pure vermicompost, the growth increased by 82-89% [55]. Another study reported that earthworms & its vermicast improve the growth and yield of wheat by more than 40% [56]. Other studies also reported better yield and growth in wheat crops applied with vermicompost in soil. [57-59].

Studies made on the agronomic impacts of vermicompost on rice crops (*Oryza sativa*) reported greater population of nitrogen fixers, actinomycetes and mycorrhizal fungi inducing better nutrient uptake by crops and better growth [60]. Another study was made on the impact of vermicompost on rice-legume cropping system in India. Integrated application of vermicompost, chemical fertilizer and biofertilizers (*Azospirillum* & phosphobacteria) increased rice yield by 15.9% over chemical fertilizer used alone. The integrated application of 50% vermicompost, 50% chemical fertilizer and biofertilizers recorded a grain yield of 6.25 and 0.51 ton/ha in the rice and legume respectively. These yields were 12.2% and 19.9% higher over those obtained with 100% chemical fertilizer when used alone [61]. Studies made in the Philippines also reported good response of upland rice crops grown on vermicompost [62].

### 12.2. Fruit Crops

Study found that worm-worked waste (vermicompost) boosted grape yield by two-fold as compared to chemical fertilizers. Treated vines with vermicompost produced 23% more grapes due to 18% increase in bunch numbers. The yield in grapes was worth additional value of AU $ 3,400/ha [63]. Farmer in Sangli district of Maharashtra, India, grew grapes on 'eroded wastelands' and applied vermicasting @ 5 tons/ha. The grape harvest was normal with improvement in quality, taste and shelf life. Soil analysis showed that within one year pH came down from 8.3 to 6.9 and the value of potash increased from 62.5 kg/ha to 800 kg/ha. There was also marked improvement in the nutritional quality of the grape fruits [27].

Study was made on the agronomic impacts of vermicompost and inorganic (chemical) fertilizers on strawberries (*Fragaria ananasa*) when applied separately and also in combination. Vermicompost was applied @ 10 tons/ha while the inorganic fertilizers (nitrogen, phosphorus, potassium) @ 85 (N)-155 (P)-125 (K) kg/ha. Significantly, the 'yield' of marketable strawberries and the 'weight' of the 'largest fruit' was 35% greater on plants grown on vermicompost as compared to inorganic fertilizers in 220 days after transplanting. Also there were 36% more 'runners' and 40% more 'flowers' on plants grown on vermicompost. Also, farm soils applied with vermicompost had significantly greater 'microbial biomass' than the one applied with inorganic fertilizers [7]. Studies also reported that vermicompost increased the yield of strawberries by 32.7% and also drastically reduced the incidence of physiological disorders like albinism (16.1%→ 4.5%), fruit malformations (11.5% → 4%), grey mould (10.4% → 2.1%) and diseases like Botrytis rot. By suppressing the nutrient related disorders, vermicompost use increased the yield and quality of marketable strawberry fruits up to 58.6% [64].

Studies made on the agronomic impact of vermicompost on cherries found that it increased yield of 'cherries' for three (3) years after 'single application' inferring that the use of vermicompost in soil builds up fertility and restore its vitality for long time and its further use can be reduced to a minimum after some years of application in farms. At the first harvest, trees with vermicompost yielded an additional $ 63.92 and $ 70.42 per tree respectively. After three harvests profits per tree were $ 110.73 and $ 142.21 respectively [65].

### 12.3. Vegetable Crops

Studies on the production of important vegetable crops like tomato (*Lycopersicum esculentus*), eggplant (*Solanum melangona*) and okra (*Abelmoschus esculentus*) have yielded very good results [27,66-68]. Another study was made on the growth impacts of earthworms (with feed materials), vermicompost, cow dung compost and chemical fertilizers on okra (*A. esculentus*). Worms and vermicompost promoted excellent growth in the vegetable crop with more flowers and fruits development. But the most significant observation was drastically less incidence of 'Yellow Vein Mosaic', 'Color Rot' and 'Powdery Mildew' diseases in worm and vermicompost applied plants [69]. Study was made on the production of potato (*Solanum tuberosum*) by application of vermicompost in a reclaimed sodic soil in India. The overall productivity of potato was significantly high (21.41 tons/ha) on vermicompost applied @ 6 tons/ha as compared to control which was 04.36 tons/ha. The sodicity of the soil was also reduced and nitrogen (N) contents increased significantly [49]. Study was made on the growth impacts of organic manure (containing earthworm vermicasts) on garden pea (*Pisum sativum*) and compared with chemical fertilizers. Vermicast produced higher green pod plants, higher green grain weight per plant, higher percentage of protein content and carbohydrates and higher green pod yield (24.8-91%) as compared to chemical fertilizer [70].

Studies made on the effects of vermicompost & chemical fertilizer on hyacinth beans (*Lablab purpureas*) found that all growth & yield parameters e.g. total chlorophyll contents in leaves, dry matter production, flower appearance, length of fruits and fruits per plant, dry weight of 100 seeds, yield per plot and yield per hectare were significantly higher in those plots which received vermicompost either alone or in combination with chemicals. The highest fruit yield of 109 ton/ha was recorded in plots which received vermicompost @ 2.5 tons/ha [71].

### 12.4. Herbage Production

A study was made on the impact of earthworms on soil properties and herbage production in a field micro-plot experiment in Ireland. Study site was reclaimed after industrial peat mining, and seeded with perennial ryegrass and clover. The presence of earthworms had little effect on herbage production in the first year. But total herbage yield was 25% greater in the second year and 49% greater in the third year in plots receiving annual topdressing of cattle slurry with earthworms compared to similarly-treated plots with cattle slurry but without earthworms. Ironically, no effect of earthworms on herbage yield was detected in plots receiving chemical fertilizers only [54].

The conclusion drawn from such study is that it is the earthworms in soil which matters in plant productivity and not the organic manure (cattle slurry) alone. In the first year, it took the earthworm to restore and condition the disturbed mined soil. However, the cattle slurry (dung) provided the necessary feed materials for the worms to act with vigor and excrete nutritive 'vermicast' in soil which promoted higher herbage yield in the second year (25%). In the third year, the worm population in soil increased significantly leading to higher excretion of vermicast, higher soil fertility and higher plant production (49%).

In a bucket experiment they found that the cumulative herbage yields over a period of 20 months was 89% higher in buckets with earthworms added with cattle manure as compared to those without earthworms but only with cattle manure, and only 19% higher in buckets receiving exclusive chemical fertilizers. These results were as compared to control.

## 13. OUR EXPERIMENTAL STUDIES SUPPORTING EARTHWORMS AND ITS VERMICOMPOST AS SUPERIOR CROP NUTRIENT OVER CONVENTIONAL COMPOST & CHEMICAL FERTILIZERS

### 13.1. Cereal Crops

#### 13.1.1. Farm Wheat Crops (Agriculture Research Institute, Jaipur, India)

This facility was provided by ARI at Jaipur, India. Results are given in **Table 4**.

**Key Observations, Findings and Discussion**

In the farm experiment the highest growth and yield in wheat crop was achieved where reduced dose (3/4) of chemical fertilizer (NPK- 90:75:60) were supplemented with full dose of vermicompost (@ 2.5 tons/ha. Although vermicompost alone can work as 'driving force' but when chemical fertilizers are added as 'helping hand' it can do even better. However, the total yield of the grain (grain/ear) as well as the ear length of crops grown on vermicompost were as good as those grown on full doses of chemical fertilizers (NPK- 120:100:80).

#### 13.1.2. Farm Wheat Crops (Rajendra Agriculture University, Bihar, India)

This facility was provided by RAU, Pusa, India under a collaborative research program. Cattle dung compost was applied four (4) times more than that of vermicompost. Results are given in **Table 5**.

**Table 4.** Agronomic impacts of earthworms, vermicompost vis-a-vis chemical fertilizers on farm wheat crops.

| | Treatments | Shoot Length (cm) | Ear Length (cm) | Root Length (cm) | Wt. of 1000 grains (In grams) | Grains/Ear |
|---|---|---|---|---|---|---|
| 1. | Vermicompost (@ 2.5 t/ha) | 83.71 | 13.14 | 23.51 | 39.28 | 32.5 |
| 2. | Earthworms (1000 Nos.) In 25 × 25 m farm land | 67.83 | 9.85 | 18.42 | 36.42 | 30.0 |
| 3 | NPK (90:75:60) (Reduced Dose) + VC (Full Dose) (2.5 t/ha) | 88.05 | 13.82 | 29.71 | 48.02 | 34.4 |
| 4 | NPK (120:100:80) (Full Dose) | 84.42 | 14.31 | 24.12 | 40.42 | 31.2 |
| 5. | CONTROL | 59.79 | 8.91 | 12.11 | 34.16 | 27.7 |

Source: Ph. D Thesis (Reena Sharma [72]); University of Rajasthan, Jaipur, INDIA; Key: VC = Vermicompost; N = Urea; P = Phosphate; K = Potash (In Kg/hectare).

**Table 5.** Agronomic impacts of vermicompost, cattle dung compost & chemical fertilizers in exclusive applications & in combinations on farmed wheat crops.

| Treatment | Input/Hectare | Yield/Hectare |
|---|---|---|
| 1) CONTROL | (No Input) | 15.2 Q/ha |
| 2) Vemicompost (VC) | 25 Quintal VC/ha | 40.1 Q/ha |
| 3) Cattle Dung Compost (CDC) | 100 Quintal CDC/ha | 33.2 Q/ha |
| 4) Chemical Fertilizers (CF) | NPK (120:60:40) kg/ha | 34.2 Q/ha |
| 5) CF + VC | NPK (120:60:40) kg/ha + 25 Q VC/ha | 43.8 Q/ha |
| 6) CF + CDC | NPK (120:60:40) kg/ha + 100 Q CDC/ha | 41.3 Q/ha |

Source: Sinha *et al*. [27]; Key: N = Urea; P = Phosphate; K = Potash (In Kg/ha)

**Key Observations, Findings & Discussion**

Exclusive application of vermicompost supported yield comparable to rather better than chemical fertilizers. And when same amount of agrochemicals were supplemented with vermicompost @ 25 quintal/ha the yield increased to about 44 Q/ha which is over 28% and nearly 3 times over control. On cattle dung compost applied @ 100 Q/ha (4 times of vermicompost) the yield was just over 33 Q/ha. Application of vermicompost had other agronomic benefits. It significantly reduced the demand for irrigation by nearly 30-40%. Test results indicated better availability of essential micronutrients and useful microbes in vermicompost applied soils. Most remarkable observation was significantly less incidence of pests and disease attacks in vermicompost applied crops.

### 13.1.3. Potted Corn Crops (Griffith University, Australia)

**Study 1:** This was designed to compare the agronomic impacts of earthworms, vermicompost & worms with chemical fertilizers on corn plants. Results are given in **Table 6**.

**Key Observations, Findings and Discussion**

Corn plants with earthworms and vermicompost in soil achieved very good growth and were better over chemical fertilizers studied until week 19. While the plants on chemicals grew only 5 cm (87 cm to 92 cm) in 7 weeks those on vermicompost grew by 15 cm (90 cm to 105 cm) within the same period. But plants with earthworms only (without feed) failed to perform. Most significant finding was that plants on vermicompost demanded less water for irrigation.

**Study 2:** This was designed to test the growth promoting capabilities of earthworms added with feed materials and 'vermicompost', as compared to 'conventional compost'. The doses of vermicompost & conventional compost were 'doubled' (400 gm). Crushed dry leaves were used as feed materials (400 gm). Results are given in **Table 7**.

**Key Observations, Findings and Discussion**

Corn plants with vermicompost in soil achieved rapid and excellent growth and attained maturity very fast. Plants in soil with conventional compost could not achieve maturity until the period of study (week 14). Plants with worms (provided with feed) performed better than those of conventional compost. A significant finding was that when the dose of vermicompost was doubled from 200 grams (Study 1) to 400 grams (Study 2), it simply enhanced total plant growth to almost two-fold (from average 58 cm on 200 gm vermicompost to average 104 cm on 400 gm vermicompost) within the same period of study *i.e.* 6 weeks. Corn plants with double dose of vermicompost achieved maturity in much shorter time. However, our subsequent studies on potted and farmed wheat crops showed that once the 'natural fertility' of the soil is restored with vermicompost application it no long requires higher doses of vermicompost subsequently to maintain or enhance productivity [27].

**Table 6.** Agronomic impacts of earthworms, worms with vermicompost vis-a-vis chemical fertilizers on corn plants (average growth in cm).

| Parameters Studied | CONTROL (No Input) | Treatment 1 EARTHWORMS Only (25 Nos.) (Without Feed) | Treatment 2 Soluble CHEMICAL FERTILIZERS | Treatment 3 EARTHWORMS + VERMICOMPOST (200 gm) |
|---|---|---|---|---|
| **Seed Sowing** | 29th July 2007 | Do | Do | Do |
| **Seed Germination** | 9th Day | 7th Day | 7th Day | 7th Day |
| **Avg. Growth in 4 wks** | 31 | 40 | 43 | 43 |
| **Avg. Growth in 6 wks** | 44 | 47 | 61 | 58 |
| **App. Of Male Rep. Organ (In wk 12)** | None | None | **Male Rep. Organ** | **Male Rep. Organ** |
| **Avg. Growth in 12 wks** | 46 | 53 | 87 | 90 |
| **App. Of Female Rep. Organ (In wk 14)** | None | None | None | **Female Rep. Organ** |
| **Avg. Growth in15 wks** | 48 | 53 **(App. Of Male Rep. Organ)** | 88 | 95 |
| **App. Of New Corn (In wk 16 )** | None | None | None | **New Corn** |
| **Avg. Growth in 19 wks** | 53 | 56 | 92 | 105 |
| **Color & Texture of Leaves** | Pale & thin leaves | Green & thin | Green & stout leaves | Green, stout & broad leaves |

Source: Sinha *et al*. [27]

**Table 7.** Agronomic impacts of earthworms (with feed), vermicompost vis-a-vis conventional compost on corn plants (average growth in cm)

| Parameters Studied | Treatment 1 Earthworms (25) With Feed (400 gm) | Treatment 2 Conventional COMPOST (400 gm) | Treatment 3 VERMICOMPOST (400 gm) |
|---|---|---|---|
| **Seed Sowing** | 9th Sept. 2007 | Do | Do |
| **Seed Germination** | 5th Day | 6th Day | 5th Day |
| **Avg. Growth In 3 wks** | 41 | 42 | 53 |
| **Avg. Growth In 4 wks** | 49 | 57 | 76 |
| **App. of Male Rep. Organ (In wk 6)** | None | None | **Male Rep. Organ** |
| **Avg. Growth In 6 wks** | 57 | 70 | 104 |
| **Avg. Growth In 9 wks** | 64 | 72.5 | 120 |
| **App. of Female Rep. Organ (In wk 10)** | None | None | **Female Rep. Organ** |
| **App. of New Corn (In wk 11)** | None | None | **New Corn** |
| **Avg. Growth In 14 wks** | 82 | 78 | 135 |
| **Color & Texture of Leaves** | Green & thick | Light green & thin | Deep green, stout, thick & broad leaves |

Source: Sinha *et al*. [27]

### 13.1.4. Potted Wheat Crops (Griffith University, Australia)

This was designed to compare the agronomic impacts of vermicompost with conventional compost & chemical fertilizers on wheat crops. Results are given in **Table 8**.

**Key Observations, Findings & Discussion**

Wheat crops maintained very good growth on vermin-compost & earthworms from the very beginning & achieved maturity in 14 weeks. The striking rates of seed germination were very high, nearly 48 hours (2 days) ahead

**Table 8.** Growth of wheat crops promoted by vermicompost, conventional compost and chemical fertilizers

| Treatments | Week 1 | Week 5 | Week 10 | Week 12 |
|---|---|---|---|---|
| 1) Control | 17 | 22 | 26 | 26 |
| 2) CC | 17 | 31 | 32 | 32 |
| 3) CF | 16 | 36 | 39 | 43 |
| 4) VC + EW | 19 | 39 | 43 | 47 |

(VC 500 gm; EW 25 Nos.; CC 500 gm; CF 5 gm x 3 times; Av. Growth in cm); **Key:** CC = Conventional Compost; CF = Chemical Fertilizer; VC = Vermicompost; EW = Earthworms Source: Sinha *et al.* [27]

of others and the numbers of seed germinated were also high by nearly 20%.

Plants were greener and healthier over others, with large numbers of tillers & long seed ears were formed at maturity. Seeds were healthy and nearly 35-40% more as compared to plants on chemical fertilizers. What they achieved in just 5 weeks, was achieved by others in 10 weeks. More significant was that the pot soil with vermincompost was very soft & porous and retained more moisture. Pot soil with chemicals were hard and demanded more water frequently [27].

## 13.2. Vegetable Crops

This was designed to compare the growth impacts of earthworms, worms with vermicompost and chemical fertilizers on egg plants. Results are given in **Table 9**.

### 13.2.1. Potted Egg Plants (University of Rajsthan, Jaipur, India 1998

**Key Observations, Findings and Discussion**

Potted egg-plants grown on vermicompost with live earthworms in soil bored on average 20 fruits/plant with average weight being 675 gm. Whereas, those grown on chemical fertilizers (NPK) bored only 14 fruits/plant with average weight being only 500 gm. Total numbers of fruits obtained from vermicompost (with worms) applied plants were 100 with maximum weight being 900 gm while those on chemicals were 70 fruits and 625 gm as maximum weight of a fruit. Interestingly, egg-plants grown on exclusive vermicompost (without worms) did not perform as with those with worms, but were signifycantly better over those on chemical fertilizers.

### 13.2.2. Potted Okra Plants (University of Rajasthan, Jaipur, India 1998

This was designed to compare the growth impacts of earthworms, worms with vermicompost and chemical fertilizers on okra plants. Results are given in **Table 10**.

**Key Observations, Findings and Discussion**

Potted okra plants grown on vermicompost (with live worms in soil) bored on average 45 fruits/plant with average weight being 48 gm. Whereas, those grown on chemical fertilizers (NPK) bored only 24 fruits/plant with average weight being only 40 gm. Total numbers of fruits obtained from vermicompost (with worms) applied plants were 225 with maximum weight being 70 gm while those on chemicals were 125 fruits and 48 gm as maximum weight of a fruit. Again, okra plants grown on exclusive vermicompost (without worms) did not perform as with those with worms, but were significantly better over those on chemical fertilizers.

### 13.2.3. Potted Tomato Plants (Griffith University, Australia 2009)

This was designed to compare the agronomic impacts of vermicompost & worms with composted cow manure from market & chemical fertilizers on tomato plants. Results are given in **Table 11**.

**Key Observations, Findings and Discussion**

Tomato plants on vermicompost & vermicompost with worms maintained very good growth from the very beginning. Number of flowers and fruits per plant were also significantly high as compared to those on agrochemicals and conventional compost. Presence of earthworms in soil made a significant difference in 'flower and fruit formation' in tomato plants. This was obviously

**Table 9.** Agronomic impacts of vermicompost, earthworms & vermicompost vis-a-vis chemical fertilizer on growth & development of egg plants.

| | Treatments | Av. Vegetative Growth (In Inches) | Av. No. of Fruits/Plant | Av. Wt. of Fruits/Plant | Total No. of Fruits | Max. Wt. of One Fruit |
|---|---|---|---|---|---|---|
| 1. | Earthworms (50 Nos.) + VC * (250 gm) | 28 | 20 | 675 gm | 100 | 900 gm |
| 2. | Vermicompost (250 gm) | 23 | 15 | 525 gm | 75 | 700 gm |
| 3. | Chemical Fertilizer (NPK) (Full dose) | 18 | 14 | 500 gm | 70 | 625 gm |
| 4. | CONTROL | 16 | 10 | 425 gm | 50 | 550 gm |

(N.B. Value of vegetative growth was taken that was achieved on the 90$^{th}$ day of the study, while the fruiting was estimated from the 45th day & ending with over 120 days); Source: Sinha *et al.* [27]; Key: VC = Vermicompost

**Table 10.** Agronomic impacts of vermicompost, worms with vermicompost vis-a-vis chemical fertilizer on growth & development of okra plants.

| | Treatment | Av. Vegetative Growth (In Inches) | Av. No. of Fruits/Plant | Av. Wt. of Fruits/Plant | Total No. of Fruits | Max. Wt. of One Fruit |
|---|---|---|---|---|---|---|
| 1. | Earthworms (50 Nos.) + VC* | 39.4 | 45 | 48 gm | 225 | 70 gm |
| 2. | Vermicompost (250 gm) | 29.6 | 36 | 42 gm | 180 | 62 gm |
| 3. | Chemical Fertilizer (NPK) (Full dose) | 29.1 | 24 | 40 gm | 125 | 48 gm |
| 4. | CONTROL | 25.6 | 22 | 32 gm | 110 | 43 gm |

(N.B. Value of vegetative growth was taken that was achieved on the 90th day of the study, while the fruiting was estimated after 45th day and ending with over 120 days); Source: Sinha *et al*. [27]

**Table 11.** Growth of tomato plants promoted by vermicompost, vermicompost with earthworms, conventional compost (composted cow manure) & chemical fertilizers (All seedlings measured 5 cm; Average growth in cm).

| Parameters Studied | Control | Chemical Fertilizers (5 gm × 3 times) | Composted Cow Manure (500 gm) | Vermicompost (250 gm) | Vermicompost (250 gm) + Earthworms (50) |
|---|---|---|---|---|---|
| 1).Avg. Growth in 2 Wks. | 10 | 16 | 16 | 18 | 19 |
| 2). Avg. Growth in 4 Wks. | 30 | 49 | 35 | 60 | 60 |
| **3). Number of flowers (Wk.5)** | 8 | 17 | 10 | 27 | 31 |
| 4). Avg. Growth in 6 Wks. | 40 | 70 | 51 | 118 | 125 |
| 5). Avg. Growth in 8 Wks. | 48 | 108 | 53 | 185 | 188 |
| **6). Number of fruits (Wk. 9)** | 4 | 16 | 6 | 22 | 27 |
| 7). Avg. Growth after 10 Wks. | 50 | 130 | 53 | 207 | 206 |

Source: Sinha & Valani [27]

due to more ‘growth & flowering hormones’ (auxins and gibberlins) available in the soil secreted by live earthworms. Very disappointing was the results of composted cow manure obtained from the market with branded name. It could not compete with vermicompost (indigenously prepared from food waste) even when applied in ‘double dose’.

## 14. VERMIWASH: A NUTRITIVE GROWTH PROMOTING PESTICIDAL LIQUID PRODUCED DURING VERMICOMPOST PRODUCTION

The brownish-red liquid which collects in all vermcomposting practices is also productive and protective for farm crops. This liquid partially comes from the body of earthworms (as worm’s body contain plenty of water) and is rich in amino acids, vitamins, nutrients like nitrogen, potassium, magnesium, zinc, calcium, iron and copper and some growth hormones like ‘auxins’, ‘cytokinins’. It also contains plenty of nitrogen fixing and phosphate solubilising bacteria (nitrosomonas, nitrobacter and actinomycetes). Vermiwash has great ‘growth promoting’ as well as ‘pest killing’ properties. Study reported that weekly application of vermiwash increased radish yield by 7.3% [73,74]. Another study also reported that both growth and yield of paddy increased with the application of vermiwash and vermicast extracts [75].

Farmers from Bihar in North India reported growth promoting and pesticidal properties of this liquid. They used it on brinjal and tomato with excellent results. The plants were healthy and bore bigger fruits with unique shine over it. Spray of vermiwash effectively controlled all incidences of pests and diseases, significantly reduced the use of chemical pesticides and insecticides on vegetable crops and the products were significantly different from others with high market value.

George [76] studied the use of vermiwash for the management of ‘Thrips’ (*Scirtothrips dorsalis*) and ‘Mites’ (*Polyphagotarsonemus latus*) on chilli amended with vermicompost to evaluate its efficacy against thrips and mites. Vermiwash was used in three different dilutions e.g. 1:1, 1:2 and 1:4 by mixing with water both as ‘seedling dip’ treatment and ‘foliar spray’. Six rounds of vermiwash sprays were taken up at 15 days interval commencing at two weeks after transplanting. Among the various treatments, application of vermicompost @ 2.5 ton/ha with 6 sprays of vermiwash at 1:1 dilution showed significantly lower incidence of thrips and mites attack. It registered very low mean population of thrips and mites as 0.35 and 0.64 per leaf respectively. It also registered significantly maximum dry chilli yield @ 2.98 quintal/ha. Giraddi *et al.* [74] also reported significantly lower pest population in chilli applied with vermiwash (soil drench 30 days after transplanting, and foliar spray at 60 and 75 days after transplanting) as compared to untreated crops.

Suthar [77] has reported hormone like substances in vermiwash. He studied its impact on seed germination, root & shoot length in *Cyamopsis tertagonoloba* and compared with urea solution (0.05%). Maximum germination was 90% on 50% vermiwash as compared to 61.7% in urea solution. Maximum root and shoot length was 8.65 cm & 12.42 cm on 100% vermiwash as compared to 5.87 & 7.73 on urea. The seedlings with 100% vermiwash foliar spray showed the maximum level of total protein and soluble sugars in their tissues.

## 15. AMOUNT & APPLICATION TIME OF VERMICOMPOST IN CROPS

Vermicompost can be used in any crop and in any amount as it is ‘completely safe’ for soils and crops in all amounts. However, several studies including ours, indicate that vermicompost is required in much ‘lesser amount’ as compared to all other bulky organic fertilizers e.g. composted cattle dung (cow, horse & pig manures and sheep & goat droppings) composted MSW and composted plant residues to promote optimal growth and yield. This is because they contain ‘high nutrients with growth hormones’ and are 4-5 times more powerful growth promoters than all other organic fertilizers and over 30-40% higher over the chemical fertilizers (NKP).

Study made by Central Research Institute for Dryland Agriculture, Hyderabad, India have provided a report which is given in **Table 12**.

**Table 12.** Recommended quantity and time of application of vermicompost in some crops.

| Crop | Quantity | Time of Application |
|---|---|---|
| 1). Rice (Paddy) | 1 ton/acre | After Transplanting |
| 2). Maize (Corn) | 1 ton/acre | Last Ploughing |
| 3). Sugarcane | 1.5 ton/acre | Last Ploughing |
| 4). Groundnut | 0.5 ton/acre | Last Ploughing |
| 5). Sunflower | 1.5 ton/acre | Last Ploughing |
| 6). Chilli | 1 ton/acre | Last Ploughing |
| 7). Potato | 1-1.5 ton/acre | Last Ploughing |
| 8). Tomato | 1-1.5 ton/acre | Last Ploughing |
| 9). Brinjal | 1-1.5 ton/acre | Last Ploughing |
| 10). Okra | 1-1.5 ton/acre | Last Ploughing |
| 11). Cauliflowers | 1-1.5 ton/acre | Last Ploughing |
| 12). Cabbage | 1-1.5 ton/acre | Last Ploughing |
| 13). Garlic | 1-1.5 ton/acre | Last Ploughing |
| 14). Onion | 1-1.5 ton/acre | Last Ploughing |
| 15). Grape (Vineyards) | 1 ton/acre | Summer time |
| 16). Citrus | 2 kg/tree | At planting time & before flowering |
| 17). Pomegranate | 2 kg/tree | At planting time & before flowering |
| 18). Guava | 2 kg/tree | At planting time & before flowering |
| 19). Mango & Coconut | 2 kg/tree | At planting time |
| | 5 kg/tree | 1-5 years old trees |
| | 10 kg/tree | 6-9 years old trees |
| | 20 kg/tree | Trees older than 10 years |
| 20). Cotton | 1 ton/acre | Last Ploughing |

Source: CRIDA (2009), Hyderabad, India [78]

## 16. THE GLOBAL MOVEMENT FOR USE OF VERMICOMPOST TO REPLACE DESTRUCTIVE CHEMICAL FERTILIZERS FROM AGRICULTURE

Worldwide farmers are desperate to get rid of the vicious circle of the use of chemical fertilizers as their cost have been constantly rising and also the amount of chemicals used per hectare has been steadily increasing over the years to maintain the yield & productivity of previous years. Nearly 3-4 times of agro-chemicals are now being used per hectare what was used in the 1960s. In Australia, the cost of MAP fertilizer has risen from AU $ 530.00 to AU $ 1500.00 per ton since 2006. So is the story everywhere in world because the chemical fertilizers are produced from ‘vanishing resources’ of earth. Farmers urgently need a sustainable alternative which is both economical and also productive while also maintaining soil health & fertility. The new concept is ‘Ecological Agriculture’ which is by definition different from

'Organic Farming' that was focused mainly on production of chemical-free foods. Ecological agriculture emphasize on total protection of food, farm & human ecosystems while improving soil fertility & development of secondary source of income for the farmers. UN has also endorsed it. Vermiculture provides the best answer for ecological agriculture which is synonymous with 'sustainable agriculture'.

A movement is going on in India, China, Philippines, Brazil, Mexico, Argentina, Australia, U.S., Canada, Russia and Japan to vermicompost the organic fractions of all their municipal solid wastes (MSW) and among the farmers to vermicompost their farm wastes and use them as a complete 'organic fertilizer' for crops as an alternative to the chemical fertilizers or supplement them with highly reduced doses of chemical fertilizers. Municipal councils, NGOs and composting companies are also participating in vermicomposting business, composting all types of organic wastes on commercial scale and selling them to the farmers. This has dual benefits. Cutting cost on landfill disposal of waste while earning revenues from sale of worms & vermicompost [17, 27,79]. 'Vermicycle Organics' in the U.S. produces 7.5 million pounds of vermicompost every year in high-tech greenhouses and sell to the farmers. Its sale of vermicompost grew by 500% in 2005. 'Vermitechnology Unlimited' in U.S. has doubled its business every year since 1991 [80].

A 'Vermiculture Movement' is going on in India with multiple objectives of community waste management, highly economical way of crop production replacing the costly chemical fertilizers and poverty eradication programs in villages [81].

## 17. IMPORTANT FEEDBACKS FROM FARMERS USING VERMICOMPOST IN INDIA

Farmers in India are being motivated to embrace vermiculture in farming. This is mainly in the States of Karanatka, Tamil Nadu, Gujarat, Maharashtra, Punjab, Harayana, Himachal Pradesh and Bihar. Apple growers in Himachal are using vermicompost on large scale with very good profit.

A number of villages in the districts of Samastipur, Hazipur and Nalanda in the State of Bihar have been designated as 'Bio-Village' where the farmers have completely switched over to organic farming by vermicompost and have given up the use of chemical fertilizers since 2005. Some of them asserted to have harvested three (3) different crops in a year (reaping 2-3 times more harvest) due to their rapid growth & maturity, and reduced harvest cycle. (*Authors takes pride in motivating farmers in Bihar through personal contacts under a collaborative research program between Griffith University, Australia and Rajendra Agriculture University, Bihar*).

Some of the important revelation by farmers about use of vermicompost were

1) Reduced use of 'water for irrigation';

2) Reduced 'pest attack' (by at least 75%) especially after spray of vermiwash (liquid drained during vermicomposting);

3) Reduced 'termite attack' in farm soil especially where worms were in good population;

4) Reduced 'weed growth';

5) Faster rate of 'seed germination' and rapid seedlings growth and development;

6) Greater numbers of fruits per plant (in vegetable crops) and greater numbers of seeds per ear (in cereal crops), heavier in weight—better in both, quantity and quality as compared to those grown on chemicals;

7) Fruits and vegetables had 'better taste' and texture and could be safely stored up to 6-7 days, while those grown on chemicals could be kept at the most for 2-3 days;

8) Fodder growth was increased by nearly 50% @ 30 to 40 quintal/hectare;

9) Flower production (commercial floriculture) was increased by 30%-50% @ 15-20 quintal/hectare. Flower blooms were more colorful and bigger in size;

## 18. ENVIRONMENTAL-ECONOMICS OF FOOD PRODUCTION BY VERMICOMPOST VIS-À-VIS CHEMICAL FERTILIZERS

A matter of considerable economic and environmental significance is that the 'cost of food production' by vermicompost (produced locally on-farm from organic wastes diverted from landfill disposal at high cost) will be significantly low by more than 60-70% as compared to chemical fertilizers (produced in factories from vanishing petroleum products using huge electricity) and the food produced will be a 'safe chemical-free food' for the society. Due to enhanced colour, taste, smell and flavour of food products farmers gets higher price for their farm products. It is a 'win-win' situation for both producers (farmers) and the consumers (feeders).

And with the growing global popularity of 'organic foods' which became a US $ 6.5 billion business every year by 2000, there will be great demand for vermicompost in future. US Department of Agriculture estimates 25% of Americans purchase organically grown foods at least once a week. The cost of production of vermicompost is simply insignificant as compared to chemical fertilizers. While the vermicompost is produced from 'human waste'—a raw material which is in plenty all over the world, chemical fertilizers are obtained from 'petroleum products' which is a vanishing resource on earth. Vermicompost can be produced 'on-farm' at low-cost by simple devices, while the chemical fertilizers are high-tech & high-cost products made in factories [17, 82].

Use of vermicompost in farm soil eventually leads to increase in the number of earthworm population in the farmland over a period of time as the baby worms grow out from their cocoons. It infers that slowly over the years, as the worms build up the soil's physical, chemical & biological properties, the amount of vermicompost can be slowly reduced while maintaining the same yield. The yield per hectare may also increase further as the soil's natural fertility is restored & strengthened. On the contrary, in chemical agriculture, the amount of chemicals used per hectare has been steadily increasing over the years to maintain the same yield as the soil became 'addict'. Nearly 3-4 times of agro-chemicals are now being used per hectare what was used in the 1960s.

Vermicompost is able to retain more soil moisture and also protects crops from pests & diseases thus reducing the demand of water for irrigation by nearly 30-40% and pest & disease control by almost 75%. This significantly cut down on the cost of production. As it also helps the crops to attain maturity and reproduce faster, it shortens the 'harvesting time'. This further cuts on the cost of production and also adds to the economy of farmers as they can grow more crops every year in the same farm plot.

While vermicompost production & use is an 'environmentally friendly' practices (salvaging waste & improving soil properties), production of chemical fertilizers is 'environmentally damaging' (generating hazardous wastes & pollutants and greenhouse gases) in its entire life-cycle, since harnessing of raw materials from the earth crust, to their processing in factories (generating huge waste and pollution) and application in farms (polluting soil & killing beneficial organisms) with severe economic & environmental implications. Production and use of 1 kg of chemical nitrogen fertilizer emits 2,500 gm of $CO_2$, 10 gm $N_2O$ & 1 gm $CH_4$. Molecule to molecule, $N_2O$ and $CH_4$ are 310 & 22 times more powerful GHG than $CO_2$. Earthworms converts a product of 'negative' economic & environmental value *i.e.* 'waste' into a product of 'highly positive' economic & environmental values *i.e.* 'highly nutritive organic fertilizer' (brown gold) and 'safe food' (green gold). Vermiculture can maintain the global 'human sustainability cycle'—producing food back from food & farm wastes.

Earthworms biomass comes as a valuable by-product in all vermicomposting practices and they are good source of nutritive 'worm meal'. They are rich in proteins (65%) with 70-80% high quality essential amino acids 'lysine' and 'methionine' and are being used as feed material to promote 'fishery' and 'poultry' industry. They are also finding new use as a source of 'bioactive compounds' (enzymes) for production of modern medicines for cardiovascular diseases and cure for cancer in the making of 'antibiotics' from the ceolomic fluid as it has anti-pathogenic properties. On commercial scale tons of worm biomass can result every year as under favorable conditions worms 'double' their number at least every 60-70 days.

If vermi-products (worms, vermicompost & vermiwash) are able to replace agrochemicals in food production and protein rich worms provide nutritive feeds for fishery and poultry production it would truly help achieve greater sustainability in production of 'safe food' for mankind in future [83,84].

## 19. CONCLUSIONS AND REMARKS

Our studies and those of other learned authors have conclusively proved that earthworms and its excreta (vermicast) or even its body fluids (vermiwash) have tremendous crop growth promoting and protecting potential and may work as the main 'driving force' in sustainable food production while maintaining soil health and fertility and can completely replace the use of agro-chemicals from farm production or just require them as 'helping hand' [83,84]. Vermicompost also reinforce plants physiologically to attain maturity and reproduce faster, thus reducing the 'life-cycle' of crops and also shortening the 'harvesting time'. Reduced incidence of 'pest and disease attack', 'controlling pests without pesticides' and 'better taste of chemical-free organic food products especially 'fruits and vegetables' grown with earthworms and vermicompost are matter of great socio- economic and environmental significance.

In case of fruits and vegetable crops presence of earthworms in soil make a big difference in growth performance. It looks worms have more positive impacts on flowering of horticultural crops and significantly aid in fruit development obviously due to secretion of growth hormones 'auxins' and 'gibberlins' [22,69,85]. No wonder then, Surpala, in $10^{th}$ century A.D. recommended to add earthworms in pomegranate plants to obtain good fruits.

Use of vermicompost in farm soil eventually leads to increase in the number of earthworm population in the farmland over a period of time as the baby worms grow out from their cocoons. Slowly over the years, as the worms & vermicompost build up the soil's physical, chemical and biological properties of soil and restore its natural fertility, reduced amount of vermicompost will be required to maintain productivity. This is contrary to those with chemical fertilizers whose amount of use has gradually increased over the years.

More studies is required to develop the potential of vermicompost teas (vermiwash) as a sustainable, non-toxic and environmentally friendly alternative to 'chemical pest control' or at least its application in farming practices can also lead to significant reduction in use of chemical pesticides.

Earthworms are truly justifying the beliefs and fulfilling the dreams of Sir Charles Darwin who called earthworms as '*unheralded soldiers' of mankind*' and '*friends of farmers*'. It is also justifying the beliefs of Dr. Anatoly Igonin one of the great contemporary vermiculture scientist from Russia who said '*Earthworms create soil & improve soil's fertility and provides critical biosphere's functions*: *disinfecting, neutralizing, protective and productive*' [86].

## REFERENCES


[1] Arancon, N. (2004) An interview with Dr. Norman Arancon. *Casting Call*, **9(2)**, http://www.vermico. com

[2] US Board of Agriculture, (1980) *Report* and recommendations on organic farming—case studies of 69 organic farmers in USA. Publication of U.S. Board of Agriculture.

[3] Kale, R.D. (1998) Earthworm cinderella of organic farming. Prism Book Pvt Ltd, Bangalore, 88.

[4] Martin, J.P. (1976) Darwin on earthworms: The formation of vegetable moulds. Bookworm Publishing, Ontario.

[5] Satchell, J.E. (1983) Earthworm ecology-from darwin to vermiculture. Chapman and Hall Ltd., London.

[6] Sadhale, N. (1996) Recommendation to incorporate earthworms in soil of pomogranate to obtain high quality fruits. Surpala's Vrikshayurveda, Verse 131. The Science of Plant Life by Surpala, 10th Century A.D. *Asian Agri-History Bulletin*, Secunderabad.

[7] Bhat, J.V. and Khambata, P. (1996) Role of earthworms in agriculture. Pub. Of Indian Council of Agriculture Research (ICAR), **22**, New Delhi, India, 36.

[8] Ghabbour, S.I. (1973) Earthworm in agriculture: A modern evaluation. *Indian Review of Ecological and Biological Society*, **111(2)**, 259-271.

[9] Capowiez, Y., Cadoux S., Bouchand P., Roger-Estrade, J., Richard G. and Boizard, H. (2009) Experimental evidence for the role of earthworms in compacted soil regeneration based on field observations and results from a semi-field experiment. *Soil Biology & Biochemistry*, **41(4)**, 711-717.

[10] Li, K.M. (2005) Vermiculture Industry in Circular Economy, *Worm Digest*. http://www.wormdigest.org/content/view/135/2/

[11] Bhawalkar, V.U. and Bhawalkar, U.S. (1993) Vermiculture: The bionutrition system. national seminar on indigenous technology for sustainable agriculture. Indian Agriculture Research Institute (IARI), New Delhi, 1-8.

[12] Bombatkar, V. (1996) The miracle called compost. The Other India Press, Pune.

[13] Singh, R.D. (1992) Harnessing the earthworms for sustainable agriculture. Publication of Institute of National Organic Agriculture, Pune, 1-16.

[14] Ayres, M. (2007) Suppression of soil—born plant disease using compost. 3*rd National Compost Research and Development Forum Organized by COMPOST Australia*, Murdoch University, Perth. Ayres. Matthew@saugov.sa. gov.au

[15] Webster, K. (2007) Compost as part of a vineyard salinity remediation strategy. 3rd National Compost Research and Development Forum Organized by COMPOST Australia, Murdoch University, Perth. www.compostforsoils. com.au

[16] Suhane, R. K. (2007) Vermicompost. Publication of Rajendra Agriculture University, Pusa, Bihar, India, 88. info@kvksmp.org

[17] Munroe, G. (2007) Manual of On-farm Vermicomposting and Vermiculture. Publication of Organic Agriculture Centre of Canada, Nova Scotia.

[18] Pajon, S. (2007) The Worms Turn-Argentina. Intermediate Technology Development Group. Case Study Series 4; Quoted in Munroe. http://www.tve.org./ho/doc.cfm?aid=1450&lang=English

[19] Canellas. L.P., Olivares, F.L., Okorokova, A.L. and Facanha, R.A. (2002) Humic Acids Isolated from Earthworm Compost Enhance Root Elongation, Lateral Root Emergence, and Plasma Membrane $H^+$ - ATPase Activity in Maize Roots. *Journal of Plant Physiology*, **130(4)**, 1951-1957.

[20] Pierre, V, Phillip, R. Margnerite, L. and Pierrette, C. (1982) Anti-bacterial activity of the haemolytic system from the earthworms *Eisinia foetida Andrei*. *Invertebrate Pathology*, **40(1)**, 21-27.

[21] Atiyeh, R.M., Subler, S., Edwards, C.A., Bachman, G., Metzger, J.D. and Shuster, W. (2000) Effects of Vermicomposts and Composts on Plant Growth in Horticultural Container Media and Soil. *Pedobiologia*, **44(5)**, 579-590.

[22] Agarwal, S. (1999) Study of vermicomposting of domestic waste and the effects of vermicompost on growth of some vegetable crops. Ph.D. Thesis, University of Rajasthan, Jaipur.

[23] Tognetti, C., Laos, F., Mazzarino, M.J. and Hernandez, M.T. (2005) Composting vs. vermicomposting: A comparison of end product quality. *Journal of Compost Science & Utilization*, **13(1)**, 6-13.

[24] Sinha, R. K. and Gokul, B. (2007) Vermiculture revolution: rapid composting of waste organics with improved compost quality for healthy plant growth while reducing ghg emissions. 3*rd National Compost Research and Development Forum Organized by COMPOST Australia*, Murdoch University, Perth. http:// www. compostforsoils. com.au

[25] Jadia, C.D. and Fulekar, M.H. (2008) Vermicomposting of vegetable wastes: A biophysiochemical process based on hydro-operating bioreactor. *African Journal of Biotechnology*, **7(20)**, 3723-3730.

[26] Reganold, J.P., Papendick, R.I.P. and James, F. (1990) Sustainable Agriculture. *Scientific American*, **262(6)**, 112-120.

[27] Sinha, R.K., Herat, S., Valani, D. and Chauhan, K. (2009) Vermiculture and sustainable agriculture. *American-Eurasian Journal of Agricultural and Environmental Sciences*; IDOSI Publication, 1-55. www.idosi.org

[28] Scheu, S. (1987) Microbial activity and nutrient dynamics in earthworms casts. *Journal of Biological Fertility Soils*, **5(3)**, 230-234.

[29] Binet, F., Fayolle, L. and Pussard, M. (1998) Significance of earthworms in stimulating soil microbial activity. *Biology and Fertility of Soils*, **27(2)**, 79-84.

[30] Chaoui, H.I., Zibilske, L.M. and Ohno, T. (2003) Effects of earthworms casts and compost on soil microbial activity and plant nutrient availability. *Soil Biology and Biochemistry*, **35(2)**, 295-302.

[31] Tiwari, S.C., Tiwari, B.K. and Mishra, R.R. (1989) Microbial populations, enzyme activities and nitrogen, phosphorus, potassium enrichment in earthworm casts and in surrounding soil of a pineapple plantation. *Journal of Biological Fertility of Soils*, **8(1)**, 178-182.

[32] Kale, R.D. and Bano, K. (1986) Field trials with vermicompost, an organic fertilizer. *Proceeding of National Seminar on 'Organic Waste Utilization by Vermicomposting*, GKVK Agricultural University, Bangalore.

[33] Anonymous (2001) Vermicompost as Insect Repellent. *Biocycle*.

[34] Edwards, C.A. and Arancon, N. (2004) Vermicompost Supress Plant Pests and Disease Attacks. *Rednova News*, http://www.rednova.com/display/

[35] Rodríguez, J.A., Zavaleta, E., Sanchez, P. and Gonzalez, H. (2000) The effect of vermicompost on plant nutrition, yield and incidence of root and crown rot of Gerbera (*Gerbera jamesonii* H Bolus). *Fitopathologia*, **35(2)**, 66-79.

[36] Buchanan, M.A., Russel, E. and Block, S.D. (1988) Chemical characterization and nitrogen mineralisation potentials of vermicompost derived from differing organic wastes. In: Edward, C.A. and Neuhauser, E.F., Eds., *Earthworms in Waste and Environmental Management*, SPB Academic Publishing, The Hague, 231-240.

[37] Patil, B.B. (1993) Soil and Organic Farming. *Proceeding of the Training Program on 'Organic Agriculture*, Institute of Natural and Organic Agriculture, Pune.

[38] Barley, K.P. and Jennings A.C. (1959) Earthworms and soil fertility III; The influence of earthworms on the availability of nitrogen. *Australian Journal of Agricultural Research*, **10(3)**, 364-370.

[39] Dash, M.C. and Patra, U.C. (1979) Worm cast production and nitrogen contribution to soil by a tropical earthworm population from a grassland site in Orissa. *Indian Review of Ecological and Biological Society*, **16(4)**, 79-83.

[40] Whalen, J.K., Parmelee, W.R., McCartnney, A.D. and Vanarsdale, J.L. (1999) Movement of nitrogen (n) from decomposing earthworm tissue to soil microbial and nitrogen pools. *Soil Biology and Biochemistry*, **31(4)**, 487-492.

[41] Lee, K.E. (1985) Earthworms, their ecology and relationships with soil and land use. Academic Press, Sydney.

[42] Satchel, J.E. and Martin, K. (1984) Phosphatase activity in earthworm feces. *Journal of Soil Biology and Biochemistry*, **16(2)**, 191-194.

[43] Li, K. and Li, P.Z. (2010) Earthworms helping economy, improving ecology and protecting health. In: Sinha, R.K. *et. al.*, Eds. Special Issue on 'Vermiculture Technology', *International Journal of Environmental Engineering*, Inderscience Pub. (Accepted).

[44] Tomati, V., Grappelli, A. and Galli, E. (1985) Fertility factors in earthworm humus. *Proceedings of International Symposium on 'Agriculture and Environment: Prospects in Earthworm Farming*, Rome, 49-56.

[45] Edwards, C.A. and Burrows, I. (1988) The potential of earthworms composts as plant growth media. In: Edward, C.A. and Neuhauser, E.F. Eds., '*Earthworms in Waste and Environmental Management*', SPB Academic Publishing, The Hague, 21-32.

[46] Neilson, R.L. (1965) Presence of plant growth substances in earthworms. *Nature*, **208(5015)**, 1113-1114.

[47] Tomati, U. Grappelli, A. and Galli, E. (1987) The Presence of Growth Regulators in Earthworm-Worked Wastes. *Proceeding of International Symposium on 'Earthworms'*, Bologna-Carpi, 423-436.

[48] Tomati, V., Grappelli, A. and Galli, E. (1995) The Hormone like Effect of Earthworm Casts on Plant Growth. *Biology and Fertility of Soils*, **5(4)**, 288-294.

[49] Ansari, A.A. (2008) Effect of Vermicompost on the Productivity of Potato (*Solanum tuberosum*) Spinach (*Spinacia oleracea*) and Turnip (*Brassica campestris*). *World Journal of Agricultural Sciences*, **4(3)**, 333-336.

[50] Yardim, E.N., Arancon, N.Q., Edwards, C.A., Oliver, T.J. and Byrne, R.J. (2006) Suppression of tomato hornworm (*Manduca quinquemaculata*) and cucumber beetles (*Acalymma vittatum* and *Diabotrica undecimpunctata*) populations and damage by vermicomposts. *Pedobiologia*, **50(1)**, 23-29.

[51] Arancon, N.Q., Edwards, C.A. and Lee, S. (2002) Management of plant parasitic nematode population by use of vermicomposts. *Proceedings of Brighton Crop Protection Conference-Pests and Diseases*, Brighton, 2002, 705-716.

[52] Edwards, C.A., Arancon, N.Q., Emerson, E. and Pulliam, R. (2007) Suppressing plant parasitic nematodes and arthropod pests with vermicompost teas. *BioCycle*, **48(12)**, 38-39.

[53] Baker, G.H., Williams, P.M., Carter, P.J. and Long, N.R. (1997) Influence of lumbricid earthworms on yield and quality of wheat and clover in glasshouse trials. *Journal of Soil Biology and Biochemistry*, **29(3/4)**, 599-602.

[54] Baker, G.H., Brown, G., Butt K., Curry, J.P. and Scullion, J. (2006) Introduced earthworms in agricultural and reclaimed land: Their ecology and influences on soil properties, plant production and other soil biota. *Biological Invasions*, **8(6)**, 1301-1316.

[55] Krishnamoorthy, R.V. and Vajranabhaiah, S.N. (1986) biological activity of earthworm casts: An assessment of plant growth promoter levels in the casts. *Proceedings of Indian Academy of Sciences* (Animal Science), **95(3)**, 341-351.

[56] Palanisamy, S. (1996) Earthworm and plant interactions. *ICAR Training Program*, Tamil Nadu Agricultural University, Coimbatore.

[57] Roberts, P, Jones, G.E. and Jones, D.L. (2007) Yield re-

sponses of wheat (*Triticum aestivum*) to vermicompost. *Journal of Compost Science and Utilization*, **15(1)**, 6-15.

[58] Suthar, S. (2005) Effect of vermicompost and inorganic fertilizer on wheat (*Triticum aestivum*) production. *Nature Environment Pollution Technology*, **5(2)**, 197-201.

[59] Suthar, S. (2010) Vermicompost: An environmentally safe, economically viable and socially acceptable nutritive fertilizer for sustainable farming; In: Sinha, R.K. *et al*., Eds. Special Issue on 'Vermiculture Technology, *Journal of Environmental Engineering*; Inderscience Pub. (Accepted).

[60] Kale, R.D., Mallesh, B.C., Kubra, B. and Bagyaraj, D.J. (1992) Influence of vermicompost application on the available macronutrients and selected microbial populations in a paddy field. *Soil Biology and Biochemistry*, **24(12)**, 1317-1320.

[61] Jeyabal, A. and Kuppuswamy, G. (2001) Recycling of organic wastes for the production of vermicompost and its response in rice—legume cropping system and soil fertility. *European Journal of Agronomy*, **15(13)**, 153-170.

[62] Guerrero, R.D. and Guerrero, L.A. (2008) Effect of vermicompost on the yield of upland rice in outdoor containers. *Asia Life Sciences*, **17(1)**, 145-149.

[63] Buckerfield, J.C. and Webster, K.A. (1998) Worm-worked waste boost grape yield: Prospects for vermicompost use in vineyards. *The Australian and New Zealand Wine Industry Journal*, **13(1)**, 73-76.

[64] Singh, R., Sharma, R.R., Kumar, S., Gupta, R.K. and Patil, R.T. (2008) Vermicompost substitution influences growth, physiological disorders, fruit yield and quality of strawberry (*Fragaria x ananassa* Duch), *Bioresource Technology*, **99(17)**, 8507-8511.

[65] Webster, K.A. (2005) Vermicompost increases yield of cherries for three years after a single application. *EcoResearch*, South Australia. http://www.ecoresearch.com.au

[66] Atiyeh, R.M., Subler, S., Edwards, C.A. and Metzger, J.D. (1999) Growth of Tomato Plants in Horticultural Potting Media Amended with Vermicompost. *Pedobiologia*, **43(4)**, 1-5.

[67] Gupta, A.K., Pankaj, P.K. and Upadhyava, V. (2008) Effect of vermicompost, farm yard manure, biofertilizer and chemical fertilizers (N, P, K) on growth, yield and quality of lady's finger (*Abelmoschus esculentus*). *Pollution Research*, **27(1)**, 65-68.

[68] Guerrero, R.D. and Guerrero, L.A. (2006) Response of eggplant (*Solanum melongena*) grown in plastic containers to vermicompost and chemical fertilizer. *Asia Life Sciences*, **15(2)**, 199-204.

[69] Agarwal, S, Sinha, R.K. and Sharma, J. (2010) Vermiculture for sustainable horticulture: Agronomic impact studies of earthworms, cow dung compost and vermicompost vis-à-vis chemical fertilizers on growth and yield of lady's finger (*Abelmoschus esculentus*). In: Sinha, R.K. *et al.*, Eds., Special Issue on 'Vermiculture Technology', *International Journal of Environmental Engineering*, Inderscience Pub.

[70] Meena, R.N., Singh, Y., Singh, S.P., Singh, J.P. and Singh, K. (2007) effect of sources and level of organic manure on yield, quality and economics of garden pea (*Pisum sativam* L.) in eastern uttar pradesh. *Vegetable Science*, **34(1)**, 60-63.

[71] Karmegam, N. and Daniel, T. (2008) Effect of vermicompost and chemical fertilizer on growth and yield of Hyacinth Bean (*Lablab purpureas*). *Dynamic Soil, Dynamic Plant*, Global Science Books, **2(2)**, 77-81. kanishkarmegam@gmail.com

[72] Sharma, R. (2001) Vermiculture for sustainable agriculture: study of the agronomic impact of earthworms and their vermicompost on growth and production of wheat crops, Ph.D. Thesis, University of Rajasthan, Jaipur.

[73] Buckerfield, J.C., Flavel, T.C., Lee, K.E. and Webster, K.A. (1999) Vermicompost in solid and liquid forms as a plant—growth promoter. *Pedobiologia*, **43(6)**, 753-759.

[74] Giraddi, R.S. (2003) Method of extraction of earthworm wash: A plant promoter substance. *VII$^{th}$ National Symposium on Soil Biology and Ecology*, Bangalore.

[75] Thangavel, P., Balagurunathan, R., Divakaran, J. and Prabhakaran, J. (2003) effect of vermiwash and vermicast extract on soil nutrient status, growth and yield of paddy. *Advances of Plant Sciences*, **16(1)**, 187-190.

[76] Saumaya, G., Giraddi, R.S. and Patil, R.H. (2007) Utility of vermiwash for the management of thrips and mites on chilli (capiscum annum) amended with soil organics. *Karnataka Journal of Agriculture Sciences*, **20(3)**, 657-659.

[77] Suthar, S. (2010) Evidence of plant hormone like substances in vermiwash: An ecologically safe option of synthetic chemicals for sustainable farming. *Journal of Ecological Engineering*, **36(8)**, 1089-1092.

[78] CRIDA (2009) *Vermicompost from Waste*; Pub. Of Central Research Institute for Dryland Agriculture; Hyderabad, (Unit of Indian Council for Agricultural Research).

[79] Lotzof, M. (2000) Vermiculture: An Australian Technology Success Story. *Waste Management Magazine*.

[80] NCSU (1997) Large Scale Vermi-composting Operations—Data from Vermicycle Organics, Inc., North Carolina State University, Raleigh.

[81] White, S. (1997) A Vermi-adventure in India. *Journal of Worm Digest*, **15(1)**, 27-30.

[82] Bogdanov, P. (2004) The single largest producer of vermicompost in world. In: Bogdanov, P. Ed., *Casting Call*, **9(3)**. http://www.vermico.com

[83] Sinha, R.K. (1998) Embarking on the second green revolution for sustainable agriculture in India: A judicious mix of traditional wisdom and modern knowledge in ecological farming. *Journal of Agricultural & Environmental Ethics*, Kluwer Acad. Pub., Dordrecht, 183-197.

[84] Sinha, R.K. (2004) Sustainable Agriculture. Surabhee Publication, Jaipur, 370.

[85] Grappelli, A., Tomati, V., Galli, E. and Vergari, B. (1985) Earthworm Casting in Plant Propagation. *Journal of Horticultural Science*, **20(5)**, 874-876.

[86] Appelhof, M. (2003) Notable Bits. *WormEzine*, **2(5)**, http://www.wormwoman.com

*Agricultural Sciences*, **2010, 1, 51-94**
Published Online August 2010 in SciRes (http://www.SciRP.org/journal/as/).

# TABLE OF CONTENTS

**Volume 1 Number 2** **August 2010**