www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(2): 393-397

www.thepharmajournal.com
Received: 01-12-2018
Accepted: 05-01-2019

Mehakpreet Kaur
Department of Microbiology, Punjab Agricultural University, Ludhiana, Punjab, India

Sukhmandeep Kaur
Department of Microbiology, Punjab Agricultural University, Ludhiana, Punjab, India

Rajni Devi
Department of Microbiology, Punjab Agricultural University, Ludhiana, Punjab, India

S Kapoor
Department of Microbiology, Punjab Agricultural University, Ludhiana, Punjab, India

Correspondence
Mehakpreet Kaur
Department of Microbiology, Punjab Agricultural University, Ludhiana, Punjab, India

Wheat straw and maize stalks based compost for cultivation of *Agaricus bisporus*

Mehakpreet Kaur, Sukhmandeep Kaur, Rajni Devi and S Kapoor

Abstract
Compost is the result of decomposition process governed by a number of micro-organisms that produce important chemical and biological reactions thereby making it selective for *A. bisporus*. Wheat straw and maize stalks based compost was prepared in different ratios viz. 1:1 and 2:1 and compared with standard wheat straw based compost for the cultivation of *Agaricus bisporus*. The physiochemical characters of these compost formulations revealed their pH, temperature, moisture and C/N to be within the range of 7.0-7.9, 75˚C-42˚C, 74%-62% and 19:1- 20.33:1 respectively. Proximate analysis of compost at five mushroom cultivation stages indicated a statistical reduction in neutral detergent fiber (67.4%-33.31%), acid detergent fiber (53.1%-27.84%), ash content (25.3%-12.87%) and crude protein (15.24%-8.46%).The cellulose and hemicelluloses content also showed a gradual decline up to pinhead stage. There was no significant change in lignin content in all the compost. The yield data indicated that wheat straw + maize stalk 2:1 with 15.8 Kg/q compost was at par with the wheat straw based control yield indicating that maize stalk can be safely used with wheat straw at 2:1 ratio without affecting the mushroom yield potential.

Keywords: *Agaricus bisporus,* wheat straw, maize stalk, proximate analysis

Introduction
The world mushroom industry is growing at a rapid rate and mushroom production has increased more than 25-fold during the last three decades from a meager 1 million tons in 1978 to about 27 million tons in 2012 (Royse 2014) [12]. *Agaricus* (primarily *A. bisporus*) is the major genus, contributing about 30% of the total cultivated mushrooms in the world. *Pleurotus,* with 5 to 6 cultivated species, is a close 2nd and contributes about 27% of the world's output while *Lentinula edodes* (shiitake) contributes approximately 17%.
Quality compost production is one of the most important steps for achieving high yields during *A. bisporus* cultivation. Compost preparation also accounts for majority of costs in the *Agaricus* cultivation and has been suggested to account for about 50% of the costs of cultivation (Royse and Chalupa 2009, Royse 2010) [13, 15]. Mushroom compost is a highly complex substrate offering appropriate nutrition to the mushroom mycelium while discouraging growth of other microorganisms. The composting technique used to prepare this complex substrate is not only complicated but difficult to examine due to usage of large number of different types of raw materials used for its preparation. A great deal of research into compost ingredients, as well as composting process has led to better understanding of the process itself. Compost plays a comprehensive and important role in mushroom production like soil does in higher plants. This material is the result of decomposition process governed by a number of micro-organisms that produce important chemical and biological reactions thereby making it selective for *A. bisporus*.
Due to non availability of horse manure compost, formulation based on wheat straw plus other nutrients (organic and inorganic) has become the standard and is being used by commercial growers in India. Various formulations have been developed by different workers from time to time which are mainly based on locally available materials (Schisler 1964, Hayes and Shandilya 1977, Shandilya 1980) [17, 19]. To make mushroom cultivation more viable using alternative raw materials, one must consider the availability, transport, seasonality, and the costs of these alternative materials (Stamets 2005) [21]. Furthermore, based on the choice of materials, confirmed with the analysis of carbon and nitrogen concentrations, aiming to promote better development of the fungus, the compost is formulated with ideal carbon/nitrogen (C/N) ratio. According to Oei (2003) [11], there are more than 40 different vegetable wastes that could be exploited for mushroom cultivation, and amongst them, about

80% can be used directly as substrate, whereas 20% can be used as nutritive supplement.

The present investigation deals with the mixing of easily available, cheap maize stalks to wheat straw during composting and to test this combination for sustainable mushroom yield.

Material and Method

Long method composting used for preparation of wheat straw based compost as per the method of Khanna and Kapoor (2016) [8] was followed. The substrates i.e. wheat straw and maize stalks were chopped to 4-6” size and different compost formulations using wheat straw: maize stalks (1:1w/w and 2:1w/w) were prepared. Wheat straw and maize stalk mixture were spread on the cemented floor and wetted thoroughly for 48 h in the form of loose heap to achieve 70-75% moisture content. Wheat bran (dry) mixed with four chemical fertilizers was moistened with water and kept covered overnight to facilitate solubilization and absorption of chemical fertilizers onto the bran. The mixture was evenly broadcasted on wetted wheat straw, mixed thoroughly and stacked. The whole mixture was made to compact rectangular pile of 5′x 5′x 5′. Seven periodic turnings were given, first three turnings on every fourth day and other four turnings on every third day. A turning schedule of 0, 4, 8, 12, 15, 18, 21 and 24 day was followed. At every turning approximately 30 cm layer was separated from all the exposed surfaces of the pile, mixed well and moistened. The remaining pile was dismantled and mixed well. The material was restacked in such a way that outer portion of pile was placed in centre of the new pile. Molasses, gypsum, furadan and γ BHC were mixed at the time of first, third, fifth and seventh turning, respectively. Two days after the last turning, the compost pile was lowered. The pH of compost at each turning was observed by the potentiometric method (Sekhon *et al* 1986) [18] on the elico pH meter. The moisture percentage was measured by drying 100g sample in an oven at 70˚ C to a constant weight. The difference between the final weight and initial weight was used for calculating the moisture percentage in the substrates (AOAC, 2000) [1]. The daily pile temperatures were measured by using a digital thermometer with a two meter long probe .further, by calculating carbon and nitrogen content of each sample individually by Nelson and Sommers (1996) [10] and kjeldahl method (Kacar 1994) respectively, C: N ratio of was calculated. The fresh compost samples collected were also analyzed for viable population of bacteria and fungi by the standard serial dilution plate count method by plating (10^{-5}, 10^{-2}) ml volume of diluted suspension (Vlassak *et al.*, 1992) [22] at each turning using Media *viz.*, Nutrient Agar and Potato Dextrose Agar.

Agaricus bisporus Lange (Sing.) strain U-3 was procured from germ plasm collection bank of Mushroom Research Complex, Department of Microbiology, Punjab Agricultural University, Ludhiana .The culture was propagated on PDA at 25 ± 2°C for 15 days and stored at 4°C for 2 months before next subculture. Freshly prepared culture slants were used to prepare master culture and then generation spawnon wheat grains in empty bottles using the standard methodology of Garcha (1997) [5]. Mushroom growing was carried out in polythene bags (20”×24”). Five kg of ready compost was filled to a depth of 10-12” per bag which was spawned thoroughly at the rate of 0.6 per cent on fresh weight basis of compost Ten replicates for each of the three compost formulation were used for cultivation. Bags were left undisturbed until complete impregnation of compost with mycelium was achieved. Standard methods of AOAC (2000) [1] were followed for the determination of proximate composition of developed composts during different stages of growth (first day of composting, 28th day of composting, pinhead stage, after first flush and at crop termination) i.e. Neutral detergent fibre (NDF), Acid detergent fibre (ADF), Lignin, Cellulose, Hemicellulose, Crude protein, Total ash.

A standard casing mixture was prepared by mixing farmyard manure (1 year old) and sandy soil (4:1 v/v). This mixture was disinfected with 4% formalin Yield data for total number &total weight of fruiting bodies per bag was recorded upto a maximum period of 6 weeks after appearance of pin head and percentage yield was calculated as

$$\% \text{ yield} = \frac{\text{Kg of fresh fruiting}}{\text{100 Kg of compost}}$$

Results and Discussion

Characterization of compost formulations

The physicochemical characteristics of compost formulations using wheat straw and maize stalks were studied using wheat straw as control. Different parameters such as moisture, pH, temperature, C: N ratio and microbiological counts were recorded at each turning. The data has been tabulated in the table 1.

The moisture and pH level during the compost preparation was observed between 58.6 to 74.3% and 7.0 to 7.9, respectively. The maximum temperature in wheat straw + maize stalks (1:1) compost was recorded during 3rd (70.3˚C), 4th (73.2˚C) and 5th (67.6˚C) turnings and that of wheat straw + maize stalks (2:1) it was maximum during 3rd (69.7˚C) and 4th (74.1˚C) turning. The maximum temperature in control was observed during the 4th (74.4˚C) turning of compost preparation. The C: N ratio of the final grade compost was statistically non-significant in all the compost preparations. The C: N was recorded from 34.76:1 - 42.49:1 at the start of composting which gradually decreased to 19.99:1-20.33:1 in the final grade compost. Composting process consisted of three major phases *i.e.* mesophilic, thermophilic and cooling phase. During mesophilic phase there is vigorous microbial activity and a lot of heat is generated. Mesophilic phase is usually between 10- 13 days of composting, after which it enters into the thermophilic stage. During this, majority of mesophilic population is killed and there by mesophilic count is reduced. Finally, the mesophilic population stabilized during cooling and maturation phase. In the above mentioned composts, mesophilic count of bacteria and fungi was maximum during 2nd turning which was ranged 23-26 cfu and 6.7-7.9 cfu respectively. The thermophilic fungi in wheat straw + maize stalk (1:1) was maximum during 3rd (6.1 cfu) and 4th (6.9cfu) turning while in other compost preparations it was maximum in 4th turning.

The moisture content correlates well with the studies of Kaur and Khanna (2001) [7] which showed maximum moisture content of 72.1 percent for wheat straw based compost at the end of composting. Bels-konoing (1962) [2] reported that during compost formulation in the first few days, ammonia softens the straw, which subsequently absorbs more moisture thus becomes more suitable for microbial degradation. Royse *et al* (2008) [14] revealed that the acceptable range of pH at the time of spawning should be from 6.5 - 8.2. He also concluded that compost containing relatively high pH and no ammonia supports good mycelial growth. Fidanza and Beyer (2009) [4] considered an ideal pH value of 7.4 for final compost. Kaur and Khanna (2001) [7] reported maximum temperature during the course of composting to be 67˚-73˚ C among the four composts evaluated namely, wheat straw, Wheat straw + paddy straw (1:1w/w), Wheat straw + paddy straw (1:2w/w) and wheat straw alone. In wheat straw compost maximum temperature recorded was 69˚C. Burrow (1951) [3] reported the importance of C: N ratio in the compost. High nitrogen content tended to increase the rate of ammonia formation and a high C: N ratio enabled ammonia to be rapidly assimilated by compost micro flora. Zheng *et al* (1995) [24] reported that C: N ratio of 33:1 during fermentation and 18:1 during mycelial growth is required for best quality mushroom yield.

Proximate analysis of different compost formulations

Proximate analysis of compost formulation using Wheat straw & Maize stalks in 1:1 and 1:2 ratio was carried out and compared with the standard wheat straw based compost formulation as control at 5 different stages during the cultivation of *A. bisporus*. The data has been tabulated in the table 2 and 3.

The Neutral detergent fiber value statistically reduced during the process of compost formation. A gradual decrease at different stages was observe in control that was from 66.1% to 36.69%, followed by wheat straw + maize stalks (1:1) and Wheat straw+ Maize stalks (1:2).Similarly, Acid detergent fiber reduced significantly up to pinhead stage. The total ash content & crude protein also showed a similar pattern of reduction up to the pinhead stage in both the compost formulations and control.

Cellulose, hemicelluloses and lignin which constitute the major part of plant waste are known to have direct impact on the growth and development of mushroom fungi (Zadrazil, 1975) [23]. The cellulose and hemicelluloses content also showed a gradual decline up to the pinhead stage while the values were statistically at par after first flush till the crop termination. There was no statistically significant change in lignin content in all the compost The results from the present investigation are in consonance with Moorthy (1981) and Singh *et al* (1989) [9, 20]. They reported that cellulose, hemicelluloses and lignin are degraded during the growth period. Kaur (2000) [6] reported that the substrate composition different composts (Wheat straw, wheat straw + paddy straw (1:1), wheat straw + paddy straw (1:2) and paddy straw) depicted a progressive decrease of various constituents i.e. Neutral detergent fiber, Acid detergent fiber, cellulose, lignin and hemicelluloses from spawning to the end of third flush.

Yield potential of *Agaricus bisporus* U-3 strain on wheat straw and maize stalk based composts

The yield potential of both formulations wheat straw + maize stalk (1:1, 2:1) were compared with wheat straw based compost as control using standard long method of composting. The observations on spawn run, case run, pinning, yield, number of fruit bodies and average weight of fruit bodies was recorded. The data has been tabulated in the table 4.

Spawn run and case run took 15-20 days and 15-21 days respectively. Pinning appeared in 35 days of spawning in wheat straw + maize stalk 2:1 and wheat straw based compost. The yield data indicated that wheat straw + maize stalk 2:1 with 15.8 Kg/q compost which was at par with the wheat straw based control yield indicating that maize stalk can be safely used with wheat straw at 2:1 ratio without affecting the mushroom yield potential. However, in case of wheat straw and maize stalk (1:1), the yield declined to 10.3 Kg/q of compost. The average weight of mushroom fruit body harvested from all the compost ranged from 11.8-13.2. No disease or pest was observed during the 4 weeks of crop harvest.

Three different compost formulations based on increasing replacement of wheat straw by paddy straw during compost preparation were evaluated by Kaur and Khanna (2001) [7]. They reported that sustainable yields could be obtained with a wheat straw + paddy straw (1:2, w/w) combination. Maximum yield was recorded on wheat straw + paddy straw (1:2, w/w) based compost that ranged from 21.9-23.7 Kg/100Kg of compost in comparison to lower yield obtained in standardized wheat straw based compost mixtures that ranged from 14.3-14.9 Kg/100 Kg of compost, 17.9-20.7 Kg/100Kg of compost for wheat straw + paddy straw (1:1, w/w) and 15.3- 21.9 Kg/100 Kg of compost for paddy straw compost.

Conclusion

The growth of mushrooms fully depends upon the compost for their nutrition. The efficiency of the mushrooms to utilize the various constituents of compost depend upon the substrate used in composting which further depends upon many physiochemical factors responsible during the composting process and mushroom growth. The yield data indicated that wheat straw + maize stalk 2:1 with 15.8 Kg/q compost was at par with the wheat straw based control yield indicating that maize stalk can be safely used with wheat straw at 2:1 ratio without affecting the mushroom yield potential. Thus, from the above study, it was concluded that alternative lignocelluloses substrates such as maize stalks for compost preparation would serve as an alternate option for recycling of agricultural wastes.

Acknowledgement

The authors would like to thank the Department of Microbiology, Punjab Agricultural University for providing laboratory facilities.

Table 1: Physical, chemical and microbiological properties of different compost formulation

Compost	Turning (No.)	Physical Properties			Chemical Properties	Microbiological Assay			
		Moisture (%)	pH	Temperature (°C)	Carbon: Nitrogen (%)	Bacterial ($\times 10^5$cfu g^{-1} compost)		Fungal ($\times 10^2$cfu g^{-1} compost)	
						Mesophilic	Thermophilic	Mesophilic	Thermophilic
Wheat straw: Maize stalk (1:1)	1	74.3	7.6	64.4	34.76:1	17	81	4.5	3.1
	2	72.1	7.4	66.8	31.20:1	23	93	6.7	4.4
	3	66.4	7.1	70.3	29.35:1	14	115	4.3	6.1
	4	58.6	7.3	73.2	27.50:1	8.9	132	3.1	6.9
	5	61.0	7.4	67.6	23.91:1	6.0	112	2.6	2.1
	6	60.0	7.4	52.3	23.18:1	4.7	58	1.4	1.1
	7	62.0	7.5	43.1	19.99:1	4.2	33	1.1	0.9
CD (5%)		7.89	NS	6.54	NS	2.82	9.07	0.81	0.95

Wheat straw: Maize stalk (2:1)	1	73.2	7.9	63.5	42.49:1	24	96	4.7	3.3
	2	69.3	7.5	65.8	38.47:1	26	112	7.2	4.2
	3	62.4	7.2	69.7	35.44:1	15	135	4.5	6.6
	4	57.3	7.0	74.1	29.83:1	12	152	3.4	7.7
	5	63.0	7.1	66.4	23.37:1	18	103	2.1	3.1
	6	60.1	7.3	53.1	21.61:1	23	89	1.6	1.3
	7	62.0	7.4	42.7	20.33:1	26	79	1.2	0.8
CD (5%)		NS	NS	5.39	NS	6.78	20.98	0.72	0.88
Wheat straw (control)	1	65.6	7.9	64.4	35.53:1	18	87	5.2	3.6
	2	63.2	7.7	67.9	34.82:1	24	110	7.9	4.8
	3	62.3	7.2	69.7	28.42:1	14	132	4.8	6.4
	4	59.7	7.0	74.4	24.07:1	9.7	130	3.5	7.3
	5	61.3	7.2	65.1	20.32:1	6.9	92	1.9	2.8
	6	63.2	7.3	55.6	19.21:1	5.8	64	1.6	1.2
	7	62.1	7.3	42.3	19.00:1	4.7	39	1.3	0.8
CD (5%)		NS	NS	3.33	NS	4.40	16.41	0.87	0.716

Table 2: Proximate composition of compost

Compost	Intervals	Neutral Detergent Fiber (%)	Acid Detergent Fiber (%)	Total Ash (%)	Crude Protein (%)
Wheat	T1	67.40	51.70	21.40	15.24
straw:	T2	52.57	38.26	18.61	12.03
Maize	T3	44.16	33.28	15.63	10.47
stalk (1:1)	T4	36.21	29.62	14.70	9.11
	T5	33.31	27.85	14.11	8.56
	CD (5%)	6.78	1.43	1.88	1.16
Wheat	T1	63.20	52.30	25.30	15.20
straw:	T2	51.19	40.79	20.74	13.37
Maize	T3	45.04	33.45	18.04	11.63
stalk (2:1)	T4	36.94	29.77	17.68	9.19
	T5	33.98	27.98	16.62	8.73
	CD (5%)	2.93	1.85	2.43	0.711
Wheat	T1	66.10	53.10	22.70	14.87
Straw	T2	52.15	42.21	18.06	11.82
(control)	T3	42.97	36.90	14.90	9.75
	T4	38.54	32.80	13.34	8.53
	T5	36.69	31.55	12.87	8.46
	CD (5%)	3.73	NS	1.07	0.44

Table 3: Proximate composition of compost

Compost	Intervals	Cellulose (%)	Lignin (%)	Hemi cellulose (%)
Wheat straw:	T1	31.80	13.85	14.70
Maize stalk	T2	25.24	12.6	10.60
(1:1)	T3	22.11	12.1	9.40
	T4	18.22	11.13	8.42
	T5	17.35	10.35	8.01
	CD (5%)	1.41	NS	0.64
Wheat straw:	T1	36.40	12.45	17.59
Maize stalk	T2	27.22	11.33	15.39
(2:1)	T3	23.74	10.42	12.28
	T4	20.56	9.59	9.07
	T5	19.20	9.01	8.41
	CD (5%)	4.10	NS	0.83
Wheat straw	T1	32.5	9.97	13.8
(control)	T2	25.90	8.11	10.83
	T3	22.58	9.87	9.52
	T4	19.96	9.67	8.70
	T5	19.18	9.21	8.65
	CD (5%)	1.54	NS	0.552

Stages-
T1 - Fresh chopped straw (first day)
T2 - Final grade compost (28^{th} day)
T3 - Pinhead stage
T4 - After first flush
T5 - At crop termination

Table 4: Yield potential of *Agaricus bisporus* of U-3 strain on compost formulation with wheat straw and maize stalk

Compost	Spawn run (d)	Case run (d)	Pinning (d)	Yield (kg/q compost)	No. of fruit bodies (no./q compost)	Average Weight of a fruit body (g)	Disease/Pest
Wheat straw: Maize stalk (1:1)	17-20	21	42	10.3	851	12.1	negative
Wheat straw: Maize stalk (2:1)	15-19	17	35	15.8	1338	11.8	negative
Wheat straw (control)	15-17	15	35	16.2	1227	13.2	Negative
CD (5%)				1.69	30.91		

References

1. AOAC. Official methods of analysis of AOAC. International 17th edition; Gaithersburg, MD, USA Association of Analytical Communities, 2000.
2. Bels-Konoing HC. Preliminary note on the analysis of the composting process. Mushroom Sci. 1962; 5:30-38
3. Burrow S. The chemistry of mushroom compost (1) General introduction and methods of investigation (2) Nitrogen changes during composting and cropping process. J Sci Fd Agric. 1951; 9:395-410.
4. Fidanza MA, Beyer DM. Plant nutrients and fresh mushroom compost. Mush News. 2009; 12:78-83.
5. Garcha HS. Mushroom Growing. Punjab Agricultural University, Ludhiana, 1997, 60.
6. Kaur H. Evaluation of strains of *Agaricus bisporus* for yield and quality characteristics. M.Sc. Thesis Punjab Agricultural University, Ludhiana, 2000.
7. Kaur H, Khanna PK. Physicochemical and microbiological characteristics of paddy straw based compost for *Agaricus bisporus* production. Ind J Mush, 2001; 19:1.
8. Khanna PK, Kapoor S. Mushroom Growing Bulletin. Punjab Agricultural University, Ludhiana, 2016, 78.
9. Moorthy VK. Microbial and chemical studies on the cultivation of oyster mushroom (*Pleurotus sajar-caju*) in paddy straw. European J Appl Microbiol Biotechnol. 1981; 12: 58-61.
10. Nelson DW, Sommers. Total Carbon, organic carbon and organic matter. In: Page A. (ed.) Methods of Soil Analysis. Science Society of America and American Society of Agronomy, Madison, Wisconsin, 1996, 961-1010.
11. Oei P. Mushroom cultivation: Appropriate technology for mushroom growers CTA: Wageningen. 2003; 3:429.
12. Royse DJ. A global perspective on the high five: Agaricus, Pleurotus, Lentinula, Auricularia & Flammulina. Proceedings of the 8th International Conference on Mushroom Biology and Mushroom Products (ICMBMP8), 2014, 1-6.
13. Royse DJ, Chalupa W. Effects of spawn, supplement and phase II compost additions and time of re-casing second break compost on mushroom (*Agaricus bisporus*) yield and biological efficiency. Bioresour Technol, 2009; 100: 5277-82.
14. Royse DJ, Sanchez JE, Beelman RB, Davidson J. Re-supplementing and recasing mushroom (*Agaricus bisporus*) compost for a second crop. World J Microbial Biotechnol. 2008; 24:319-25.
15. Royse DJ. Effects of fragmentation, supplementation and the addition of phase II compost to 2nd break compost on mushroom (*Agaricus bisporus*) yield. Bioresour Technol, 2010; 101: 188–92.
16. Sánchez JE, Royse DJ. Adapting substrate formulas used for shiitake for production of brown Agaricus bisporus. Bioresour Technol. 2001; 77:65-69.
17. Schisler LC. Nutrient supplementation of compost during mushroom growth cycle. MGA Bull. 1964; 179:503-37.
18. Sekhon GS, Chibba IM, Arora CL. Introduction to soil fertility (Part II). In Soil Science Laboratory Manual. College of Agriculture, Punjab Agricultural University, Ludhiana, 1986.
19. Shandilya TR. Wheat straw and chicken manure mixture as high yielding substrate for *Agaricus bisporus. Taiwan Mushrooms*. 1980; 4:6-12.
20. Singh H. Mushrooms, the art of cultivation. Pub. Ghai, S.K. Sterling publishers Pvt. Ltd. L-10 Green Park Extension, New Delhi, 1991.
21. Stamets P. Mycelium running: how mushrooms can help save the world. Hong Kong: Ten Speed, 2005, 356.
22. Vlassak KL, Holm LV, Duchateau L. Isolation and characterization of fluorescent *Pseudomonas* associated with roots of rice and banana grown in Srilanka. Plant and Soil. 1992; 145:51-63
23. Zadrazil F. Die Zersetzeng des stroch- Zellulose-lignin-Komplexesmit *Pleurotus florida* and dessn Netzung. Z Pflan Zenernahs Bodenkd. 1975; 138: 263-78.
24. Zheng FQ, Yang RC, Liu RX. Effects of C/N ratios in compost on nutrient transformation and yield and quality of *Agaricus bisporus*. Acta Agric. 1995; 11:33-38.