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Microbial characterization of cow pat pit and biodynamic preparations used in biodynamic agriculture

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ABSTRACT

As of today, biodynamic agriculture is practised across 60 countries of the world. Cow pat pit (CPP) and biodynamic preparations (BD) are the key components of biodynamic agriculture. However, there a is dearth of scientific explanation on account of their mode of action. CPP and BD preparations are very effective in compost production, soil health management and eventually, enhancing the yield and quality of crop produce. Efficacies of these preparations were evaluated with isolation and characterization of beneficial microbes. Study revealed that CPP contained maximum gram positive and gram negative bacteria (184 ± 14 × 10^5cfu/g), (225 ± 9 × 10^5cfu/g) and Rhizobium (310 ± 24 × 10^7cfu/g), while BD-507 contained the highest number of actinomycetes (792 ± 194 × 10^6cfu/g) and *Azotobacter* (201 ± 14 × 10^5cfu/g) among the all preparations. Actinomycetes isolated from CPP and BD-507, showed ammonia, indole acetic acid (IAA), siderophore and HCN producing activities. Out of total isolated microbes from CPP and BD preparations, 9 isolates showed high ammonia, 23 IAA, 18 siderophore and 12 HCN producing activities. Based on the study, it may be suggested that CPP and BD preparations may be used as bio-inoculants and combined with compost in organic production of various crops.

Key words: Actinomycetes, *Azotobacter*, *Azospirillum*, BD-500, Cow pat pit, Plant growth promoting activities

Rudolf Steiner initially developed the biodynamic agriculture in the year 1920 wherein all the inputs required for crop production were produced in the farm itself. Steiner (1997) defined that biodynamic agriculture is a system of "systematic and synergistic harnessing of energies from Cosmos, Earth, Plants and Cow for sustainable production". According to recent data, biodynamic agriculture is being practised in 161,074 ha land in across 60 countries of the world. Germany accounts for 45% of the global biodynamic agriculture; the remainder average is 1750 ha per country (Shankaraswamy *et al.* 2017). A few BD preparations are used in minute quantities but show remarkable effects on plant growth, yield and quality (Reganold *et al.* 1993, Droogers and Bouma, 1996). BD-500, BD-501, CPP and BD-502-507 are used for improving soil fertility, biotic, abiotic stress management and compost production. These BD preparations are produced with cow dung and other herbal plants and fermented for a specific period. Deffune and Scolfield (1995) found that humic acids extracted from BD-500 and other BD preparations (505 and 507) caused positive growth response in wheat seedlings relative to the control. However, scientific explanations are lacking. Reganold (1995) also reported improvement in soil quality and profitability after use of BD preparations. It is, therefore, imperative to study these preparations from microbiological point of view.

MATERIALS AND METHODS

Cow pat pit, a field preparation, is also called as 'soil shampoo'. It is prepared with fresh cow dung collected from lactating and pasture going cows and fermented along with crushed egg shells powder and basalt/bentonite (clay) dust duly mixed and placed in a pit sized of 3' × 2' × 1.5' in shed. Two sets of BD-502-507 are incorporated for catalyzing the composting process. Compost gets ready in 90-120 days at 30-40°C atmospheric temperature and 60-70% humidity. Ready compost was stored with 50-60% moisture in earthen pot. BD-500 was produced in cleaned cow horns filled with fresh cow dung like CPP and buried at 30 cm depth in the soil in root free zone during descending period of Moon in the months of October-November. After 6 months of incubation, horns were taken out during descending period of Moon in the months of March-April. Properly decomposed compost was stored at cool place with 50-60% moisture in earthen pot. After taking out of BD-500, same horns were thoroughly cleaned with water, filled with silica powder paste and buried in same pit

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where cow horns were buried for the preparation of BD-500 during the ascending period of Moon in the months of March-April. After 6 months of incubation, horns were taken out in October-November during ascending period of Moon. Light yellowish silica powder was taken out from the horn and stored in glass jars (Pathak and Ram, 2003). This preparation is called as BD-501. BD preparation set (BD- 502-507) was purchased from M/S Supa Biotech (P) Ltd, Nainital (Organic certified), Uttarakhand, India and were used for compost, CPP, biodynamic liquid pesticides production. Main ingredients of BD preparations are as follows: BD-502 contains fermented flowers of yarrow grass (*Achillea millefolium*), BD-503- fermented chamomile flowers (*Matricaria recutita*), BD-504- air dried leaves of stinging nettle (*Urtica dioca*) fermented in the soil, BD-505 - fermented oak bark (*Ouercus* sp), BD-506 - fermented flower of dandelion (*Taraxacum officinalis*) and BD-507- valerian plant (*Valeriana officinalis*) extract. one g compost of each and 10 ml of BD-507 were added in compost heap, cow pat pit and biodynamic liquid pesticides to catalyse the fermentation process (Koepf *et al.* 1990, Steiner 1993).

Enumeration of different beneficial microbial populations, viz. bacteria, fungi, actinomycetes, *Pseudomonas*, gram positive and negative bacteria, p-solubilizing bacteria, *Rhizobium*, *Azotobacter* and *Azospirillum* were carried out by using dilution plate count method using selective media, viz. Nutrient agar, Rose Bengal Chloramphenicol Agar (RBCA), actinomycetes isolation agar, King's B (King *et al.* 1954), methyl red agar (Hagedorn and Holt 1954), crystal violate agar (Goud *et al.* 1985), Pikovskaya's agar (Pikovskaya 1948), yeast extract mannitol agar with congo red (CRYEMA, Fred *et al.*, 1932), modified Jenson's agar (Jensen 1954, Norris and Chapman 1968) and N-free malate medium (Okon *et al.* 1977), respectively.

The compositions of the selective media were:- Nutrient agar (Hi-Media): Beef extract-3.0 g, peptone-5.0 g, NaCl-5.0 g, agar-15.0 g, distilled water-1 l., pH-7.2; RBCA (Hi-Media): mycological peptone-5.0 g, dextrose-10.0 g, monopotassium phosphate-1.0 g, $MgSO_4$-0.5 g, rose bengal-0.05 g, chloramphenicol -0.1 g, agar-15.5 g, final pH (at 25°C) 7.2 ± 0.2; actinomycetes isolation agar (Hi-Media): Sodium caseinate-2.0 g, L-Asparagine-0.1 g, sodium propionate,-4.0 g, dipotassium phosphate-0.5 g, magnesium sulphate-0.1 g, ferrous sulphate-0.001 g, agar 15.000, distilled water-1 l. pH-8.1 ± 0.2; King's B: protease peptone-20 g, glycerol-10.0 g, K_2HPO_4-1.5 g, $MgSO_4$, 7 H_2O-1.5 g, agar-15.0g, distilled water-1 l, pH-7.2; methyl red agar: beef extract-3.0 g, peptone-5.0 g, methyl red-0.15 g, agar-15.0 g, distilled water-1 l, pH-7.0; crystal violate agar: beef extract-3.0 g, peptone-5.0 g, crystal violet-4 ml (stock solution of crystal violet 0.05 w/v), agar-15.0 g, distilled water-1 l, pH-7.0; Pikovskaya's agar (Hi-Media): yeast extract-0.5 g, dextrose-10.0 g, calcium phosphate-5.0 g, ammonium sulphate-0.5 g, KCl-0.2 g, $MgSO_4$-0.1 g, manganese sulphate 0.0001 g, ferrous sulphate 0.0001 g, agar 15.0 g, distilled water-1 l., pH-7.5; CRYEMA (Hi-Media): yeast extract 1.0 g, mannitol 10.0 g, dipotassium phosphate 0.5 g, magnesium sulphate 0.2

Table 1 Different microbial populations in cow pat pit and biodynamic preparations

Type of microbe	Multiplication factor	Microbial population (cfu/g) (Mean±sd)								
		Cow Pat Pit	Biodynamic preparations							
			500	501	502	503	504	505	506	507
Bacteria	10^8	16.7 ± 0.91	3.80 ± 0.67	1.80 ± 0.31	2.40 ± 0.46	85.50 ± 7.45	8.50 ± 1.62	110.3 ± 8.92	12.20 ± 1.49	2.60 ± 0.27
Fungi	10^5	8.30 ± 0.96	6.90 ± 1.00	11.30 ±1.55	11.97 ± 1.50	28.33 ± 7.55	33.53 ± 3.18	13.27 ± 2.00	35.07 ± 5.61	5.10 ± 0.60
Actinomycetes	10^6	12.7 ± 3.2	15.9 ± 3.7	3.1 ± 1.5	24.9 ± 5.5	14.8 ± 4.3	68.0 ± 12.7	20.3 ± 4.8	207.2 ± 71.3	792.0 ± 194.2
Gram positive bacteria	10^8	184.1 ± 14.21	0.55 ± 0.21	0.10 ± 0.05	0.89 ± 0.35	0	6.57 ± 0.85	2.85 ±0.21	12.30 ± 2.91	1.47 ± 0.35
Gram negative bacteria	10^7	225.1 ± 9.75	0.02 ± 0.01	0.02 ± 0.01	1.58 ± 0.21	0.03 ± 0.01	15.63 ± 2.15	0.63 ±0.15	11.23 ± 1.05	15.43 ± 2.76
Pseudomonas	10^6	6.47 ± 0.67	1.38 ± 0.04	4.73 ± 0.61	0.65 ± 0.25	1.85 ± 0.50	21.73 ± 7.29	2.78 ±0.43	7.03 ± 0.59	12.17 ± 3.21
P-solubilizing microbes	10^5	8.30 ± 0.56	3.93 ± 0.67	25.43 ± 3.95	39.63 ± 3.71	24.17 ± 1.10	0.69 ± 0.17	95.13 ±7.20	21.70 ± 7.33	10.00 ± 2.41
Azotobacter	10^5	28.37 ± 2.85	23.57 ± 2.42	46.5 ± 11.53	34.30 ± 0.95	77.43 ± 7.03	28.60 ± 3.85	26.89 ±3.03	53.07 ± 12.83	201.4 ± 14.91
Azospirillum	10^5	224.3 ± 30.01	9.80 ± 1.28	0.67 ± 0.15	54.40 ± 6.62	96.10 ± 9.22	76.47 ± 8.27	0.53 ±0.23	528.8 ± 77.52	830.3 ± 94.38
Rhizobium	10^7	310.8 ± 24.85	6.00 ± 1.51	4.80 ± 0.95	6.17 ± 0.83	10.83 ± 2.61	18.10 ± 2.95	2.10 ±0.53	6.07 ± 0.50	0.02 ± 0.01

g, sodium chloride 0.1 g, congo red 0.025 g, agar 20.0 g, distilled water-1 l., final pH (at 25°C) 6.8±0.2; modified Jenson's agar: sucrose-20.0 g, K_2HPO_4-1.0 g, $MgSO_4$-0.5 g, Na_2MoO_4-0.001 g, FeSO4, $7H_2O$-0.01 g, $CaCO_3$-2.0 g, agar-18.0 g, distilled water-1 l., pH-7.2.; N-free malate medium: malic acid-5.0 g, K_2HPO_4-0.5 g, KOH-4.0 g, MgSO4-0.1 g, NaCl-0.02 g, $CaCl_2$-0.01 g, $FeSO_4$-0.05 g, Na_2MoO_4-0.002 g, $MnSO_4$-0.01 g, bromothymol blue-0.002 g, agar-18.0 g, pH-9.6-7.3, distilled water-1 l. Petri dishes were prepared by pouring each specific solid medium. Then 10 ml of each preparation sample was diluted with 90 ml sterile water and that was considered being 10^{-1} dilution factor. Transferring of 1 ml of 10^{-1} dilution to 9 ml sterilized water with the help of a sterilized pipettes yielded 10^{-2} dilution. In this way, a series of up to 10^{-8} dilutions were prepared under aseptic condition. Point one ml (0.1ml) of the suspension from required dilution (e.g. 10^{-8}) was taken and poured into the respective agar media on petri dish and spread with L-spreader with the help of Plate Master (Hi-Media). Then plates were incubated at 28±2°C for 3-5 days. The number of visible colonies were counted. The total count was obtained by multiplying number of visible colonies on the plate by the dilution factor. The individual selected colonies were streaked on a new perti dish with respective solid medium for two consecutive times to purify the microbial cultures. The purified cultures were stored on agar slants in a refrigerator for further use. Observations were statistically analysed for mean and standard deviation and presented in the tables (Panse and Sukhatme 1976). This study was undertaken during 2016-17.

Table 2 Plant growth promoting activity of bacterial isolates from cow pat pit

PGPR Isolate	HCN Production	Ammonia production	Siderophore production	IAA production
CISH-PGPR 69	-	+	-	+
CISH-PGPR 70	-	++	-	+
CISH-PGPR 71	-	+	-	+
CISH-PGPR 72	-	+	+	+
CISH-PGPR 73	+	-	-	-
CISH-PGPR 74	+	+	+	-
CISH-PGPR 86	+	+	-	-
CISH-PGPR 87	-	-	-	-
CISH-PGPR 88	-	+	-	+

(+): Test positive, (-): Test negative; (+): Low, (++): High activity.

Purified microbial isolates were evaluated for different plant growth promoting attributes, viz. ammonia, IAA, siderophore and HCN producing activities. Ammonia, indole acetic acid (Bric *et al.* 1991) and siderophore producing activity was determined by growth in chrome azurol S (CAS) medium after 48–72 hr growth at 28°C. HCN producing activity was estimated by change in the colour of filter paper from yellow to brown (Bakker and Schippers 1987). The test results were denoted as (+): positive, (-): negative; (+): Low and (++): High.

RESULTS AND DISCUSSION

Microbial population dynamics in CPP and BD preparations (BD -500-507)

Microbial population, viz. bacteria, fungi, actinomycetes, gram positive and gram negative bacteria, *Pseudomonas*, P-solubilizing microbes, *Azotobacter*, *Azospirillum* and *Rhizobium* in cow pat pit and BD-502-07 were enumerated. Maximum total bacteria ($110.3 \pm 8.92 \times 10^8$cfu/g) were counted in BD- 505 and minimum ($1.80 \pm 0.31 \times 10^8$cfu/g) in BD-501. This might be due to oak bark a good source of tannins which are bioactive, while BD-506 contained maximum fungi population ($35.07 \pm 5.61 \times 10^5$cfu/g) and BD-500 ($6.90 \pm 1.00 \times 10^5$ cfu/g) contained minimum. Maximum actinomycetes ($792 \pm 194 \times 10^6$ cfu/g) were isolated in BD-507 and minimum ($3.1 \pm 1.5 \times 10^5$ cfu/g) in BD-501. It might be due to phosphorus utilizing microbes as BD-507 is a good source of phosphorus. Highest gram positive and negative bacteria ($184 \pm 14 \times 10^5$ cfu/g), ($225 \pm 9 \times 10^5$ cfu/g) were isolated from CPP, respectively. Increase in bacteria might be due to inoculation of BD-502-507 during CPP preparation. Maximum *Pseudomonas* population ($21.73 \pm 7.29 \times 10^6$cfu/g) was recorded in BD-504 and p-solubilizing microbes ($39.63 \pm 3.73 \times 10^5$cfu/g) in BD-502, while highest population of *Azotobacter* ($201 \pm 14 \times 10^5$ cfu/g) and *Azospirillum* ($830 \pm 94 \times 10^5$ cfu/g) were found in BD-507. Microbial studies on this issue are scanty. Findings of this study therefore, can't be supported with sufficient number of references. CPP contained maximum

Table 3 Plant growth promoting activity of actinomycetes isolates from cow pat pit

PGPR strains	HCN production	Ammonia production	Siderophore production	IAA production
CISH-PGPA 13	++	-	+	-
CISH-PGPA 14	-	-	-	-
CISH-PGPA 15	-	+	-	+

(+): Test positive, (-): Test negative; (+): Low, (++): High activity.

Table 4 Multifarious plant growth promoting traits of bacterial cultures isolated from biodynamic preparations

Culture No.	HCN production	Ammonia production	Siderophore production	IAA production
CISH-PGPR-BD-500 -1	-	++	+	+
CISH-PGPR-BD -500 -2	++	++	+	+
CISH-PGPR-BD -500 -3	+	+	-	+
CISH-PGPR-BD -500 -4	++	++	+	+
CISH-PGPR-BD -500 -C	++	++	+	+
CISH-PGPR-BD -501 -I	-	+	-	-
CISH-PGPR-BD -501 -II	-	++	-	-
CISH-PGPR-BD -501 C I	-	-	-	+
CISH-PGPR-BD -501 CII	-	+	-	-
CISH-PGPR-BD -501 C III	-	++	+	+
CISH-PGPR-BD -501 C IV	-	++	-	-
CISH-PGPR-BD -502 I	-	++	+	+
CISH-PGPR-BD -502 II	-	++	-	-
CISH-PGPR-BD -503 1MR	-	++	-	-
CISH-PGPR-BD -503 2MR	-	++	-	-
CISH-PGPR-BD -503 3MR	-	++	-	-
CISH-PGPR-BD -503 4MR	-	++	-	-
CISH-PGPR-BD -503 5MR	-	++	-	-
CISH-PGPR-BD -503 6MR	-	++	-	-
CISH-PGPR-BD -503 6	-	++	-	+
CISH-PGPR-BD -503 N7	-	++	-	-
CISH-PGPR-BD -503 C8	-	++	-	-
CISH-PGPR-BD -503 8	-	++		+
CISH-PGPR-BD -503 9	-	++	-	+
CISH-PGPR-BD -503 10	-	++	-	+
CISH-PGPR-BD -503 11	-	++	-	+
CISH-PGPR-BD -504 C1	-	++	-	-
CISH-PGPR-BD -506 I	-	++	-	+
CISH-PGPR-BD -506 MRI	+	+	+	-
CISH-PGPR-BD -506 MRII	-	+	+	-
CISH-PGPR-BD -506 MRIII	-	+	+	-
CISH-PGPR-BD -506 MRIV	-	-	+	+
CISH-PGPR-BD -507 CRI	-	++	-	-
CISH-PGPR-BD -507 CR2	-	+	+	-
CISH-PGPR-BD -507 CR3	-	++	-	-
CISH-PGPR-BD -507 CR4	-	++	+	-
CISH-PGPR-BD -507 CR5	-	+	+	-
CISH-PGPR-BD -507 CR 6	-	+	-	-
CISH-PGPR-BD -507 NI	-	++	+	+
CISH-PGPR-BD -507 NII	-	++	+	+
CISH-PGPR-BD -507 MEI	+	+	-	-
CISH-PGPR-BD -507 MEII	-	-	-	-
CISH-PGPR-BD -501 MRI	+	+	-	-

(+): Test positive, (-): Test negative; (+): Low, (++): High activity

population of *Rhizobium* (310 ± 24 x 10^7cfu/g) (Table 1). This might be due to added natural calcium and cow dung and bentonite powder. Stalin *et al.* (2014) have also enumerated microorganisms in organic and biodynamic manures, and reported that cow pat pit contained highest bacterial load (4.8 x 10^6 cfu/g); in which *Bacillus subtilis* was predominant.

Growth promoting activities of microbial isolates

Fifty five microbial isolates (52 bacteria, 3 actinomycetes) were isolated and evaluated for different plant growth promoting attributes, viz. ammonia, indole acetic acid, siderophore and HCN producing activities (Table 2, 3, 4). Among them, 9 bacterial isolates and 3 actinomycetes isolates were from CPP and rest 43 bacterial isolates from other BD preparations. Actinomycetes isolated from CPP showed siderophore and HCN producing activity (CISH-PGPA 13). Similarly, CISH-PGPA 15, an actinomycete isolated from CPP produced both ammonia and IAA in the test medium. Radha and Rao (2014) have also reported presence of actinomycetes in CPP due to addition of calcium during preparation. The bacterial isolates from CPP also tested for ammonia, IAA, HCN and siderophore producing activities. Perumal *et al. (*2006) reported plant growth hormones such as Indole Acetic Acid IAA (28.6 mg/kg), kinetin (7.6 mg/kg) and gibberellic acid (23.6 mg/kg) in CPP. Possibly for this reason, CPP stimulates plant growth by providing nutrients, plant hormones and protects plant's root zone against fungal diseases with bacteria and actinomycetes produces HCN and siderophores. Fifty two bacterial isolates from BD preparations have shown multifarious plant growth promoting activities. Nine of them showed high ammonia and 23 IAA producing activity. Eighteen of them showed siderophore and 12 HCN producing activity together which revealed their bio-inoculation potential for either making compost or spraying directly to the crop plant or soil.

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REFERENCES

Bakker P A H M, Bakker A W, Marugg J D, Weisbeek P J and Schippers B. 1987. Bioassay for studying the role of siderophores in potato growth stimulation by *Pseudomonas* sp in short potato rotations. *Soil Biology & Biochemistry* **19**: 443–9.

Bric J M, Bostock R M and Silver Stone S E. 1991. Rapid *in situ* assay for indole acetic acid production by bacteria immobilized on a nitrocellulose membrane. *Applied Environmental Microbiology* **57** (2): 535–8.

Deffune G and A M Scolfied. 1995. Effects of humic acids and three bio-dynamic preparations on the growth of wheat seedlings. (*In*) *Proc. 3rd ESA Congress,* Abano-Podova, Paper Ref No. 3–56.

Droogers P and Bouma J. 1996. Biodynamic vs. conventional farming effects on soil structure expressed by simulated potential productivity. *Soil Science Society of America Journal* **60**: 1552–8.

Fred E B, Baldwin I L and McCoy F. 1932. Root nodule bacteria and leguminous plants with supplement. University of Wisconsin study in Science 5 WI, 343 p.

Goud W D, Hagedorn C, Bardinelli T R and Zablotowiez R M. 1985. New selective medium for enumeration and recovery for fluorescent pseudomonads from various habitats. *Applied Environmental Microbiolology* **49**: 28–32.

Hagedorn C and Holt J G.1954. Ecology of soil arthrobactrers in Clarion-Websters toposequence of Iowa. *Applied Microbiology* **29**: 211–8.

Jensen H L. 1954. The Azotobacteriaceae. *Bacterial Review* **18**: 195–214.

Koepf H H, Pettersson B D and Schaumann W. 1990. *Bio-Dynamic Agriculture: Practical Applications of the Bio-Dynamic Method*. Anthroposophic Press, Hudson, New York.

Norris J R and Chapman H M. 1968. Classification of *Azotobacter*. (*In): Identification Methods for Microbiologists,* pp 19–27. Gibbs B M and Shapton D A(Eds), Academic Press, London and New York.

Okon Y, Alberecht S L and Burris R H. 1977. Methods for growing *Spirillum lipoferum* and for counting it in pure culture and in association with plants. *Applied Environmental Microbiology* **33**: 85–7.

Panse V G and Sukhatme P V. 1976. *Statistical Methods for Agricultural Works.* ICAR, New Delhi.

Pathak R K and Ram R A. 2003. Role of cow in agriculture. (*In*) *National Seminar on Cow in Agriculture and Human Health*, Organized by Asian Agri-History Foundation, p 59.

Perumal K V, Varadarajan S, Murugappa A. 2006. Production of plant growth hormones and subtilin from organic biodynamic manures organic farming - Advantages and disadvantages for soils, water quality and sustainability. *(In) 18th World Congress of Soil Science*, July, 9-15, 2006 held at Philipadaia, USA **162**: 4. 1A.

Pikovskaya R E. 1948. Mobilization of phosphorous in soil in connection with vital activity of some microbial species. *Mikrobiology* **17**: 363–70.

Pathak R K and Ram R A. 2003. Role of cow in agriculture. *(In) National Seminar on Cow in Agriculture and human health*, Organized by Asian Agri-History Foundation, p 59.

Radha T K and Rao D L N. 2014. Plant Growth promoting bacteria from cow dung based biodynamic preparations. *Indian Journal of Microbiology* **54** (4): 413–8.

Reganold J P, Palmer A S, Lockhart J C, and MacGregor A N. 1993. Soil quality and financial performance of biodynamic and conventional farms in New Zealand. *Science* **260**: 344–9, 24.

Shankaraswamy J, Arakeri K B and Mantena S V. 2017. Bio dynamic farming: A future agriculture farming. *Adv Plants Agric Res* **6**(2): 00209. DOI: 10.15406/apar.2017.06.00209.

Stalin V, Perumal K, Stanley Abraham L and Kalaichelvan P T. 2010. Screening and production of subtilin from *Bacillus subtilis* isolated from nutrient-rich organic and biodynamic manures. *IUP Journal Life Science* **4**: 34–44.

Steiner R. 1997. Theosophy. An *Introduction to the Supersensible Knowledge of the World and the Destination of Man*. Anthrosophic Press, Hudson, New York.

Steiner R. 1993. *Agriculture: Spiritual Foundations for the Renewal of Agriculture*. Anthroposophic Press, New York.