J. Korean Agric. Chem. Soc.
한국농화학회지 32(4), 393—400(1989)

Bioavailability of Soil-aged Residues of the Herbicide Bentazon to Rice Plants

Jae-Koo Lee, Kee-Sung Kyung and F. Führ*
Department of Agricultural Chemistry, Chung Buk National University
Cheong Ju 360-763, Korea
*Institute of Radioagronomy, Nuclear Research Center Jülich GmbH
P.O.B. 1913, D-5170 Jülich, West Germany

土壤中 新生 및 熟成 Bentazon 殘留物의 벼에 依한 吸收

李載球 · 慶箕性 · F. Führ*
忠北大學校 農科大學 農化學科, *西獨原子力廳 放射線 農學研究所

초 록

토양 중에서 3개월과 6개월간 ^{14}C-bentazon을 숙성시키는 동안 방출된 $^{14}CO_2$의 량은 처리 방사능의 각각 6.1과 14.8%이었다. 토양중의 지렁이는 벼의 뿌리에 의한 ^{14}C-bentazon의 흡수를 증가시켰다. 토양중의 ^{14}C-bentazon으로부터 방출되는 $^{14}CO_2$량은 벼를 재배하거나 지렁이가 존재할 때 증가하였다. 벼에 의한 ^{14}C-bentazon 잔류물의 흡수는 토양중 지렁이의 유무에 관계없이 숙성기간이 증가함에 따라 현저히 감소하였다. 벼의 경우에는 옥수수를 이용한 연구와 비교해 볼 때 ^{14}C-표지화합물의 훨씬 많은 량이 지상부로 이행되었다. 추출 불가능한 흡착잔류물의 량은 3개월까지는 숙성기간이 증가할수록 현저히 증가하였다. 토양으로부터 추출된 화합물의 극성은 숙성기간과 벼의 생육에 따라 증가하였으며 이는 극성 대사 산물의 형성을 시사해 준다.

Introduction

Bentazon, 3-isopropyl-2, 1, 3-benzothiadiazin-4-one-2, 2-dioxide, has been in wide use as a post-emergence herbicide in such crops as soybean, cereals and rice. Hence, the bioavailability of its residues in soil to crops would be of utmost importance in terms of the environmental safety and human health.

However, few investigations have been reported on this subject as yet, even if the bioavailability of bound residues of some other pesticides in soil has been reported1~6). Müller and Sanad7) reported the distribution and behaviour of various combinations of herbicides containing bentazon in winter wheat under field conditions. The degradation of bentazon in plants and soils was investigated by Otto et al.8). Lee et al.9) reported the formation and bioavailability of bentazon residues in two soils to maize plant.

In the present investigation, the uptake of the freshly added and soil-aged residues of bentazon by rice plants was investigated in a specially devised micro-ecosystem containing ^{14}C-bentazon both in the absence and presence of earthworms.

Materials and Methods

Bentazon

Received October 25
Corresponding author : J.K. Lee
"This paper was supported by NON DIRECTED RESEARCH FUND, Korea Research Foundation, 1988."

Bentazon (>99.5% purity) and uniformly ring-labelled ^{14}C-bentazon (specific activity: 1616.86 KBq/mg) were provided by the BASF Corporation, Limburgerhof, West Germany. The purity of the ^{14}C-bentazon was confirmed by autoradiography and HPLC prior to use.

Soil used

A fresh soil sample taken from a rice paddy in Kakyung-dong, Cheong Ju, Korea was used. The physico-chemical properties are as follows; pH (KCl, 1 : 5), 5.4; organic matter, 1.3%; C.E.C. (me/100g soil), 10.2; sand, 38.1%; silt, 37.6%; clay, 24.3%; texture, loam.

Autoradiography

In order to confirm the radiochemical purity of the ^{14}C-bentazon and to characterize the possible degradation products of bentazon in soil, autoradiography was performed using the Fuji X-Ray Film, Medical(Fuji Photo Film Co., Ltd, Japan, 20.3×25.4cm) and the developer X-DOL (X-ray film developer, Poohung Photochemical Co., Ltd, Korea). As the fixer, X-Fix (Poohung Photo-chemical Co., Ltd, Korea) was used. For TLC, the pre-coated aluminum plates were used. The developing solvent was a mixture of $CHCl_3$-CH_3OH(7 : 3, v/v) and the spots were visualized under a UV lamp (254 nm, Mineral Light Model UV GL-58).

Formation of soil-aged bentazon residues

Based on the degradation rate of the ^{14}C-bentazon in soil which had been obtained from a preliminary experiment, the soils treated with a mixture of labelled and non-labelled bentazon were aged at 23±1°C for 3 and 6 months(Table 1). The finaldradioactivity and concentration after the aging were intended to be 185 KBq/1.5 kg soil and 5 mg/kg soil, respectively. Throughout the aging, the moisture contents were kept at 50% of the maximum water-holding capacity of the soil. The $^{14}CO_2$ evolved during the aging was absorbed in 1N-NaOH and measured at an interval of one week with a Liquid Scintillation Counter (PW 4700, Philips).

Table 1. Treatment levels of ^{14}C-bentazon in the aging in soil (6.5 kg of soil)

Aging period (month)	Radioactivity* (KBq/kg soil)	Total bentazon* concentration (mg/kg soil)
3	134	5.4
6	145	5.9

*Based on the preliminary experiments, the radioactivity of ^{14}C-bentazon and the total bentazon(labelled+unlabelled) for rice plant growing were adjusted to be:
185 KBq/1.5 kg soil
5mg/kg soil

Growing of rice plants

After the aging periods of 3 and 6 months, respectively, the soils were air-dried and the radioactivities of them were measured by combustion with a Biological Oxidizer(R.J. Harvey Instrument Corporation, U.S.A.). For the growing of rice plants, the sample soils were fertilized with N-P-K at the ratio of 15-9-11 kg/10 a, respectively. Three variants of soil samples containing bentazon residues which were freshly applied, 3-month aged and 6-month aged, respectively, were put into specially devised pots made of stainless steel (I.D. 17 cm× H 10 cm) and the 50-day grown seedlings of rice plants were transplanted. In each pot 8 seedlings were grown with 2 seedlings per hill. Rice plants were grown in a vinyl house with good ventillation for 42 days. Four days after transplanting, 10g of earthworms collected from upland soils near Chung Buk National University were added to each pot of 1.5 kg soil. The loss of moisture by transpiration was supplied daily. Table 2 represents the design for the rice plant experiment. For the cultivation of rice plants in soils containing bentazon residues, basically three treatments were adopted; freshly added (T-1), 3-month aging (T-2), and 6-month aging (T-3). For comparison, control plots(T-0) were set up in duplicate and to each treatment the earthworms were

Table 2. The design for the growing of rice plants in the fresh, 3-month-aged, and 6-month-aged soils. 1.5 kg of soil and 8 rice plants per pot

Treatment	Description				Replicate
	Aging	Earthworm	^{14}C-bentazon in soil		
			KBq/kg	mg/kg	
T-0 (Control)	0	0	0	0	2
T-1-1	Freshly treated	0	123	5.0	〃
T-1-2	〃	+	〃	〃	〃
T-2-1	3-month-aged	0	125	5.1*	〃
T-2-2	〃	+	〃	〃	〃
T-3-1	6-month-aged	0	122	4.9*	〃
T-3-2	〃	+	〃	〃	〃

* Bentazon equivalents calculated on the basis of the specific ^{14}C-activity of the ^{14}C-bentazon applied for the aging

added with an intention to get some preliminary information on the possible effect of the presence of earthworms on the uptake of bentazon residues by rice plants. Each treatment involved two replicates. The pots were placed randomly.

Measurement of radioactivities of rice plants, earthworms, and soil samples

Air-dried soil samples were evenly ground in a mortar. Rice plants divided into roots and shoots, and earthworms were freeze-dried and pulverized. 0.3 g of each sample was combusted with a Biological Oxidizer. The $^{14}CO_2$ evolved was absorbed in ^{14}C-cocktail solutions (For Harvey Biological Oxidizer, R.J. Harvey Instrument Corporation, New Jersey, U.S.A.) and the radioactivities were measured with a Liquid Scintillation Counter.

Extraction of soil samples treated with ^{14}C-bentazon

Since the previous research⁹⁾ showed that distilled water turned out to be the best solvent to extract ^{14}C-bentazon residues from soils, each soil sample(15g) of all treatments was extracted with distilled water (50 ml) on a shaker for 4 hrs and centrifuged at 13,000 rpm for 10 min. The extraction was repeated until the radioactivity of the extract showed the background level.

Partition of the radioactivity of soil extracts between aqueous phase and organic phase

The pH of each soil extract was adjusted to be 2 with HCl¹⁰⁾. 5 ml of this extract were taken in a screw-capped tube and 5 ml of ethyl acetate were added to it. After being shaken vigorously and kept undisturbed for some time, 2 ml from aqueous and organic layers were taken separately into scintillation vials and Aquasol and toluene cocktail were added, respectively. The radioactivities were measured with the Liquid Scintillation Counter.

Analysis of non-extractable soil-bound residues of ^{14}C-bentazon

Two grams of soil samples which had been exhaustively extracted with distilled water down to the background level were exhaustively extracted again with 5 ml of 0.1M-sodium pyrophosphate. Centrifugation at 10,000 rpm for 10 min separated the insoluble material, humin, and acidification of the supernatant with conc. HCl caused flocculation of the humic acid fraction. The fulvic acid fraction remained in solution. After fulvic acid was separated from humic acid by centrifugation, the humic acid precipitate was redissolved with 1N-NaOH and the radioactivity was measured. Radioactivity in the humin fraction was measured by combustion.

Results and Discussion

Mineralization of ^{14}C-bentazon to $^{14}CO_2$ during the aging period

As can be seen in Fig. 1, the total amounts of $^{14}CO_2$ evolved from ^{14}C-bentazon aged in soil for 3 and 6 months were 6.1 and 14.8% of the applied radioactivity, respectively. This result indicates that the mineralization rate of bentazon per week ranges from 0.51 to 0.62% in this soil.

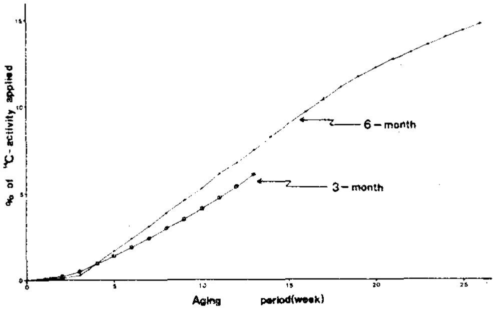

Fig. 1. $^{14}CO_2$ evolution from ^{14}C-bentazon in soil during the 3- and 6-month aging.
Applied ^{14}C-activity=100%

Mineralization to $^{14}CO_2$ during the growing of rice plants

The mineralization of ^{14}C-bentazon to $^{14}CO_2$ during 42 days of rice plant growing is presented in Table 3. The presence of earthworms tends to increase $^{14}CO_2$ evolution with the exception of T-2-2. The plausible explanation for this result could be the fact that a lot of earthworms were dead due to the possibly unfavorable environment in the pots of T-2-2 during the experiment. The possible toxicity of bentazon and/or other effects could be ruled out, since in T-1-2 where bentazon had been freshly applied, there were no remarkable effects. The $^{14}CO_2$ evolution during this period ranges from 2.5 to 5.0% on average, showing no pronounced differences among treatments. The much higher amounts of $^{14}CO_2$ evolved during the growing of rice plants than those during the same period of mere aging could have been due to the rhizosphere effect. The roots of plants release various substances into the rhizosphere soil. The kinds and quantity of them are different depending on the species and developmental stage of plants, various soil physical stress factors, plant nutrition, mechanical or disease injury, microbial activities, and foliar-applied chemicals[11]. In the case of rice plants, it is reported that various kinds of sugars, amino acids, and organic acids are accumulated in the rhizosphere[12]. Miskovic et al.[13] reported that a higher activity of dehydrogenase was observed in the rhizosphere of corn than in root-free soil. Generally, the rhizosphere is a zone of intense microbiological activity due to its higher concentration of carbohydrates, amino acids, vitamins, and other growth promoting substances[14]. So in general, a high microbial activity can be expected which is responsible for the intensive turnover of bentazon and its metabolites leading to $^{14}CO_2$.

Table 3. Mineralization of ^{14}C-bentazon in soil to $^{14}CO_2$ during 42 days of rice plant growing

Treatment	Description			Mineralization (%)*
	KBq/1.5 kg soil	mg/1.5 kg soil	Earthworm	
T-1-1	185	7.5	0	3.14±0.21
T-1-2	〃	〃	+	3.89±0.08
T-2-1	188	7.7	0	4.66±1.22
T-2-2	〃	〃	+	4.33±0.46**
T-3-1	183	7.4	0	2.45±0.85
T-3-2	〃	〃	+	5.00±0.50

*All data represent averages of 2 replicates in each treatment. Each replicate involves 3 samples.
**A lot of earthworms were dead.

Uptake and translocation of ^{14}C-bentazon by rice plants

Table 4 and 5 show the uptake of ^{14}C-benta-

Table 4. Plant uptake of radiocarbon from soil containing ^{14}C-bentazon or bentazon residues during the growth period of 42 days. ^{14}C-Activity in soil at day 0=100%

Treatment	Description		Uptake(%)[a,b]		
	Aging	Earthworm	Root	Shoot	Total
T-1-1	Freshly treated	0	25.80A	12.52A	38.32A
T-1-2	〃	+	29.36A	12.67A	42.03A
T-2-1	3-month-aged	0	2.54B	1.55B	4.08B
T-2-2	〃	+	4.30B	1.36B	5.66B
T-3-1	6-month-aged	0	1.34B	1.15B	2.48B
T-3-2	〃	+	1.90B	1.14B	3.04B

a) All data represent averages of 2 replicates in each treatment. Each replicate involves 3 samples.

b) Numbers followed by the same letter within a column are not significantly different at the 5% levels using Duncan's multiple range test.

Table 5. The relative ^{14}C-activity present in roots and shoots of rice plants (per g dry weight) as influenced by ^{14}C-bentazon aging in soil. ^{14}C-uptake by rice plants grown in soil containing ^{14}C-bentazon residues without earthworms (T-3-1) after 6 months of aging=1

Treatment	Uptake ratio	
	Root	Shoot
T-1-1	16.7	12.2
T-1-2	16.7	10.5
T-2-1	1.4	1.3
T-2-2	2.5	1.1
T-3-1	1.0	1.0
T-3-2	1.3	1.0

zon and its metabolites from the soil by rice plants during the growth period of 42 days. As can be seen in Table 4, in T-1-1 38.3% of the applied radioactivity was absorbed by rice plants, whereas in the variants T-2-1 and T-3-1 which contained bentazon residues aged for 3 and 6 months, respectively, 4.1 and 2.5% of the radioactivities in the soil at the start of the plant experiment were absorbed. These results strongly indicate that mere aging leads to soil-bound bentazon residues and their availability to plant roots was greatly reduced. The presence of earthworms in soil seem to enhance the bioavailability and hence the uptake of the residues of bentazon and its metabolites via rice roots, even if it was not statistically significant. The mechanism involved can not be elucidated and needs further investigation. The radioactivity of the shoots is an indication that bentazon and its metabolites were translocated to the upper parts of the plant. The distribution of the radioactivity between roots and shoots was a little different from that observed earlier in maize plants[9]. In the case of rice plant, much more radioactivity was translocated to the shoots, whereas in the maize plant, small amounts were moved (Table 5). This seems to demonstrate that the translocation of bentazon and its metabolites in plants can vary between species.

Recovery of radioactivity in the rice plant experiment

The overall behaviour of ^{14}C-bentazon applied to soil during the 42 days, growing period of rice plants is presented in Table 6. In T-1-1, where ^{14}C-bentazon was freshly added, the radioactivity remaining in soil at the end of the experiment ranges from 49 to 63%, whereas in the case of aged residues (Treatments T-2-1 and T-3-1), the radioactivities remaining in soil amount to 91~94%. These results prove that due to degradation as well as adsorption processes during the preceding aging period bentazon residues were apparently more strongly fixed and hence less bioavailable for plant roots.

Table 6. Recovery of radioactivity in the rice experiment after soil application of ^{14}C-bentazon. ^{14}C-activity applied per pot=100% and duration of the experiment=42 days

Treatment	^{14}C-bentazon in soil KBq/kg	mg/kg	$^{14}CO_2$ evolution during rice planting(%)	^{14}C-bentazon(%) In roots	In shoots	Remaining in soil	Absorbed by earthworms	Recovery(%)
T-1-1	123	5.0	3.3	21.2	12.1	62.5		99.1
〃	〃	〃	2.9	30.4	12.9	51.7		97.9
T-1-2	〃	〃	4.0	33.8	13.5	48.5	0.05	99.8
〃	〃	〃	3.8	24.9	11.8	59.6	—*	100.1
T-2-1	125	5.1	3.4	2.2	1.5	91.2		98.3
〃	〃	〃	5.9	2.8	1.6	90.5		100.8
T-2-2	〃	〃	3.9	4.9	1.4	91.7	<0.01	101.9
〃	〃	〃	4.8	3.7	1.3	91.2	<0.01	101.0
T-3-1	122	4.9	3.3	1.3	1.2	93.3		99.1
〃	〃	〃	1.6	1.4	1.1	91.3		95.4
T-3-2	〃	〃	4.5	1.9	1.1	93.8	0.10	101.4
〃	〃	〃	5.5	1.9	1.2	93.2	0.10	101.9

* Earthworms were dead and the radioactivity could not be measured.

It is of interest, however, that no remarkable differences were observed between T-2-1 and T-3-1 in terms of the residue fixation onto soil. This means that the major aging processes leading to a reduced bioavailability already take place predominantly during the first 3 months after bentazon application. The table also shows the uptake of radioactivity representing ^{14}C-bentazon or its metabolites by earthworms from soil during their survival period of 38 days. The earthworms were totally dead in one pot of T-1-2, probably due to the unfavorable environment for their life. In T-2-2, a lot of earthworms were also dead, resulting in smaller radioactivities of earthworms than expected. Accordingly, the uptake amounts were less than 0.01% of the originally applied radioactivity, showing no consistent differences among the treatments. The recoveries ranging from 95 to 102% are good indications that the experiments were performed quite accurately.

Non-extractable soil-bound residues of bentazon

Table 7 shows the radioactivity remaining in soil following the exhaustive extraction of the

Table 7. Comparison of the formation of non-extractable bound residues of bentazon in soil as a function of aging and the growing of rice plants

Treatment	Rice planting	Mineralization to $^{14}CO_2$(%)	Plant uptake(%)	Water extractable(%)	Non-extractable(%)	Recovery (%)
T-1-1	Before	—	—	97.3	3.3	100.6
	After	3.2	38.3	18.6	39.9a)	100
T-2-1	Before	—	—	31.8	71.2	103
	After	4.7	4.0	19.4	71.9a)	100
T-3-1	Before	—	—	28.2	75.3	103.5
	After	2.4	2.5	20.7	74.4a)	100

a) 100%-[Mineralization(%)+Plant uptake(%)+Water-extractable(%)]

soils with distilled water. From T-1-1, the soil containing bentazon freshly added immediately before the start of the plant experiment, only 3.3% of the applied ^{14}C could not be extracted. After cultivating rice plants for 42 days, the soil-bound residues increased up to 39.9%. Meanwhile, in T-2-1 and T-3-1, the bound residues did not change after growing rice plants during the same period. Based on this result, it is clear that the increase in non-extractable bound residue in T-1-1 after the growing of rice plants is mainly due to the aging effect of 42 days rather than the effect of rice plants.

Distribution of ^{14}C-radioactivity of soil extracts between aqueous phase and organic phase

In order to examine the change in polarity of the labelled compounds extracted from soils which were either freshly treated with bentazon, aged with bentazon for 3 months, or 6 months, in the absence and presence of rice plants, the radioactivity of the soil extracts was partitioned between distilled water and ethyl acetate. As can be seen in Fig. 2, it is obvious that the polarity of the extracted compounds increases with the aging period. Especially, as seen in T-1-1 where bentazon had been freshly applied to soil just before transplanting rice plants, the polarity increased remarkably by growing rice plants for 42 days.

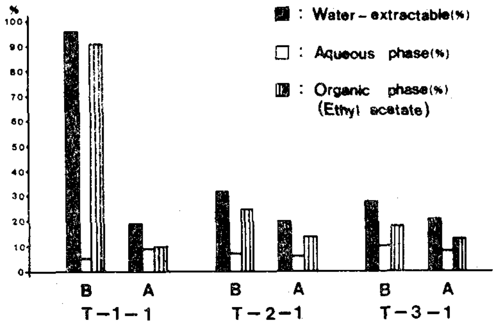

Fig. 2. Change in the water-extractable ^{14}C from soil samples treated with ^{14}C-bentazon in the absence and presence of rice plants.

B : Before rice planting (^{14}C-activity in soil before planting=100%)

A : After rice planting (^{14}C-activity in soil after the plant experiment=100%)

Change in the non-extractable ^{14}C

Fig. 3 shows the radioactivities distributed in fulvic acid, humic acid, and humin of the non-extractable bound residues formed in the three treatments. As can be seen, in general, the ^{14}C-activity associated with the fulvic acid and humin fractions is greater than that with humic acid. When bentazon was freshly added, the growing of rice plants increased greatly the amounts of bound residues in fulvic acid, humic acid, and humin. This will be obviously due to the aging effect of the growing of rice plants for 42 days rather than the effect of rhizosphere.

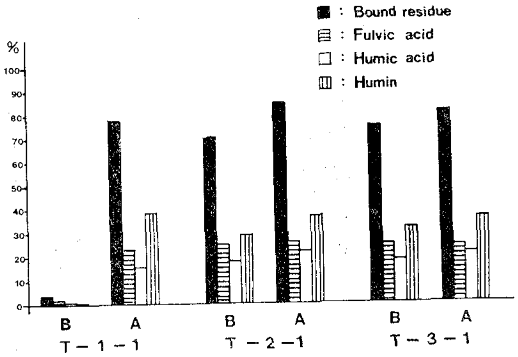

Fig. 3. Change in the non-extractable ^{14}C after applied to soil samples as ^{14}C-bentazon in the absence and presence of rice plants.

B : Before rice planting (^{14}C-activity in soil before planting=100%)

A : After rice planting(^{14}C-activity in soil after the plant experiment=100%)

Abstract

The amounts of $^{14}CO_2$ evolved during the ^{14}C-bentazon aging in soil for 3 and 6 months were 6.1 and 14.8% of the original radioactivity, respectively. The presence of earthworms in soil tended to increase the uptake of ^{14}C-bentazon by the roots of rice plants, even if it was not statistically significant. The evolution of $^{14}CO_2$ from ^{14}C-bentazon in soil increased in the presence of rice plants and earthworms comp-

ared with in the absence of them. The uptake of ^{14}C-bentazon residues by rice plants decreased remarkably with increasing the aging period within the limit of 3 months both in the absence and presence of earthworms, but there is not much difference between 3-month-aging and 6-month-aging. Much larger amounts of ^{14}C-labelled compounds were translocated to the shoots, compared with the data from a previous investigation using maize plants. The amount of non-extractable bound residue increased remarkably with the aging period up to 3 months. The polarity of the compounds extracted from soil increased with the aging and the growing of rice plants, indicating the formation of some polar metabolites.

Acknowledgment

The authors acknowledge The Korea Research Foundation for the research grant and express their thanks to BASF, West Germany, for supplying ^{14}C-bentazon.

References

1. Fuhremann, T.W. and Lichtenstein, E.P.: J. Agr. Food Chem., 26 : 605(1978)
2. Helling, C.S. and Krivonak, A.E.: J. Agr. Food Chem., 26 : 1164(1978)
3. Führ, F. and Mittelstaedt, W.: J. Agr. Food Chem., 28 : 122(1980)
4. Khan, S.U.: J. Agr. Food Chem., 28 : 1096 (1980)
5. Roberts, T.R. and Standen, M.E.: Pest. Sci., 12 : 285(1981)
6. Kloskowski, R. and Führ, F.: FAO/IAEA, Research Coordination Meeting, München-Neuherberg 11-15, July, Wien, 133(1984)
7. Müller, F. and Sanad, A.: Med. Fac. Landbouww. Rijks Univ. Gent, 43/2, 1167(1978)
8. Otto, S., Beutel, P., Drescher, N., and Huber, R.: IUPAC Advances in Pesticide Science(Zürich, 1978), Part 3, ed. by H. Geisbuhler, Pergamon Press, Oxford and New York, 551(1979)
9. Lee, J.K., Führ, F., and Mittelstaedt, W.: Chemosphere, 17-2 : 441(1988)
10. Booth, G.M., Yu, C.C. and Hansen, D.J.: J. Environ. Quality, 2(3) : 408(1973)
11. Hale, M.G. and Moore, L.D.: Adv. Agron., 23 : 89(1979)
12. Kimura, M., Wade, H., and Takai, Y.: Jpn. J. Soil Sci. Plant Nutr., 48(11,12) : 540 (1977)
13. Miskovic, K., Rasovic, B., Starcevic, L., and Milosevic, N.: Mikrobiologiya, 14 : 105 (1977)
14. Nicholas, D.J.D., Baker, K.F., and Snyder, W.C. (eds): Ecology of soil-borne Plant Pathogens, Univ. Calif. Press, Berkeley, 210 (1965)