CURRENT STATUS OF BIO-FERTILIZERS AND BIO-PESTICIDES DEVELOPMENT, FARMER'S ACCEPTANCE AND THEIR UTILIZATION IN KOREA

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ABSTRACT

To reduce input of chemical fertilizers and pesticides yet sustain the productivity of the agro-ecosystem, bio-fertilizers and bio-pesticides have become ideal substitutes for environment-friendly agriculture in Korea. At present, a total of 138 companies produce hundreds of commercial products, and 23 bio-pesticides are now registered in the country. Among the registered bio-pesticides, 12 are fungicides and the others are insecticides. The microbial agents used for bio-pesticides include Paenibacillus polymyxa, Bacillus subtilis, Bacillus amyloliquifacians, Paecilomyes fumosoroseus, Streptomyces goshikiensis, Bacillus thuringiensis var. kurstaki, Bacillus thuringiensis subsp. aizawai, etc. Among the commercial bio-fertilizers, EXTN-1 developed by the author's research group is the most widely accepted and dependable bio-fertilizer in Korea. EXTN-1 which was identified as Bacillus vallismortis is a PGPR and ISR agent that originated from pepper roots. Soil drenching or seed priming of EXTN-1 stimulated seed germination and growth of about 20 crops without any harmful effects. Furthermore, it showed a broad controlling spectrum to various viral, bacterial, and fungal plant diseases such as CMV, TMV, PVY, and bacterial wilt, anthracnose, rice blast and Fusarium wilt. Besides bio-fertilizers and bio-pesticides, egg yolk and cooking oil (EYCO) mixture developed by the author is widely adopted by Korean farmers for the control of various pests and increase in plant health. Among various cooking oils, sunflower and rape seed oil (canola) at 0.3-0.5% emulsified with 0.1% egg yolk was the most effective showing over 90% control value of powdery mildew and mites. It shows relatively high protective but low curative effects. Because of its simple preparation at home, cheap price of about 1/4 of chemical pesticide, and safety for organic and environment friendly agriculture, EYCO is now widely used by Korean farmers.

Key words: bio-fertilizers, bio-pesticides, environment-friendly agriculture, microbial agents, EXTN-1, egg yolk and cooking oil (EYCO)

INTRODUCTION

Intensive agriculture is inevitable in Korea because of the limited arable land and the high population. Hence, agricultural researches and technologies aimed to increase high quality yields until the 1980s. However, the intensive and high-input agriculture generated various negative side effects on the agro-ecosystem and the environment such as pollution and soil degradation. Improvements in living standards during the last decades are associated with an ever-increasing demand for a year-round and diverse supply of food in the country. It was recently reported that Korean consumers are concerned most about food safety for their health, as well as their lifestyle's sustainability.

Since mid-1990s, environment-friendly agriculture has been a new trend to ensure sustainable agriculture and sound environment in Korea. The agricultural policy of the government (Ministry of Agriculture and Forestry, www.maf.go.kr) has driven the 'Environment-friendly Agriculture Supporting Law' since 1997. The policy aims to sustain productivity and conserve environmental quality of soil and water, reduce pollution and other environmentally harmful effects, recycle organic resources, and produce safe foods. Substantially, the MAF aimed to reduce 40%

of chemical fertilizers and pesticides produced in 2003 until 2013. Inputs of fertilizers are decreasing slowly from 382 kg in 2000 to 257 kg in 2006. However, the use of pesticides to control various pests and weeds has not been reduced much yet, applying ca. 12.5 kg/ha/year. Nevertheless, it is expected that the input of chemical fertilizers and pesticides will decrease because the certified environment-friendly agricultural acreage has rapidly increased from 2,000 ha in 2000 to 74,995 ha in 2006.

BIO-FERTILIZERS

To reduce input of chemical fertilizers yet sustain productivity and maintain the agro-ecosystem, bio-fertilizers have become an ideal substitute for fertilization and conditioning of soil in Korean. At present a total of 138 companies are registered as bio-fertilizer manufacturers and hundreds of commercial products comprise ca. $30 million in the agricultural material market. The most commonly used microbial agents are *Bacillus* spp. *Rhizobium* spp., *Pseudomonas* spp., *Bradyrhizobium* sp., and *Azospirrilum* sp. (Table 1). The purposes of the bio-fertilizers are soil fertilization and conditioning, and increment in plant growth and health. Various research projects to develop bio-fertilizers and bio-bed soils have been actively pursued by using plant growth promotion rhizobacteria (PGPR), phosphate solubilization microbes, nitrogen fixation microbes, etc..

EXTN-1

Among the commercial bio-fertilizers, EXTN-1 developed by the author's research group is the most widely accepted and dependable bio-fertilizer in Korea. EXTN-1 identified as *Bacillus vallismortis* is a PGPR and an induced systemic resistance (ISR) agent which originated from pepper roots. Soil drenching or seed priming of EXTN-1 stimulated seed germination and growth of about 20 crops without any harmful effects. Furthermore, it showed a broad controlling spectrum to various viral, bacterial, and fungal plant diseases such as CMV, TMV, PVY, and bacterial wilt, anthracnose, rice blast and *Fusarium* wilt. As a rhizobacterium, population of EXTN-1 increased or stabilized on lettuce roots in hydroponics up to 3-4 weeks after

Table 1. Patent microbes used for bio-fertilizer production in Korea

Microbial agent	Use and target crop	Patent
Bacillus vallismortis EXTN-1	PGPR, ISR, pepper, tomato, rice, etc.	'03, Korea
Bacillus stearothermophilus	Decomposition of OM High temp. resistance	'02, Korea
B. megaterium	Barley, wheat, perilla, cabbage, etc.	'04, Korea
Bacillus sp. KR083	Barley, potato, pepper, tomato, cucumber, etc	'04, Korea '05, International
Rhizobium sp. KR181	Barley, oat, pea, lupine	'04, Korea
Pseudomonas sp. RRj228	Rice, barley, pepper	'04, Korea
P. putida	Soil detoxication, PGPR	'90, Korea
Bacillus polymyxa	Pepper blight, PGPR Soil detoxication	'92, Korea
Bradyrhizobium japonicum	Soybean	'94, Korea
Rhizobium sp. YCK15	Peanut	'94, Korea
Rhizobium meliloti YCK	Alfalfa	'94, Korea

treatment. In the case of cucumber, soil drenching after seed coating showed best plant growth promotion and disease protection in soils as well as in hydroponics.

EXTN-1 consistently promoted growth and quality of lettuce grown during cool season and inhibited bacterial wilt on tomato caused by *Ralstonia solanacearum* for 4 weeks after treatment. In hydroponics, yield, fruit color, and quality of paprika were improved. In the field, *Fusarium* wilt of pepper was also effectively suppressed by seed treatment or drenching. For mass production and commercialization, the EXTN-1 powder with a concentration of 10^{11} cfu per gram was developed. EXTN-1 increased H_2O_2 in the early stage and induced the expression of resistance genes, PR-1a, HMGR, and PAL. Pre-treatment of EXTN-1 reduced germination and appressorium formation of conidia of *Colletotrichum orbiculare* on the cucumber leaf (Tables 2-5).

Table 2. Plants showing growth promotion by EXTN-1 treatment depending on soil type

Soil type	Plant growth: Promoted	Plant growth: Normal	Plant growth: Inhibited
Sandy loam	Perilla, buckwheat, corn	Wheat, barley, rice, pepper, pea, perilla, tomato, lettuce,	
Clay	Welsh onion, pepper, pea perilla, kidney bean, lettuce, corn	Tomato, perilla, wheat, barley, lettuce, cowpea millet, chard, red-bean	Rice

Table 3. Effect of soil drenching of EXTN-1 on growth of Chinese cabbage and lettuce

Treatment	Plant height (cm)		Fresh wt. (g)	
	Lettuce	C. cabbage	Lettuce	C. cabbage
EXTN-1	18.3±2.0	22.1±1.4	62.4±0.2	35.1±3.2
Control	11.2±1.0	19.1±1.1	21.8±3.9	24.9±3.5

Table 4. Effect of EXTN-1 on control of tomato bacterial wilt caused by *Ralstonia solanacearum* in hydroponics

Treatment	Disease incidence (%) after treatment			
	10d	20d	30d	40d
EXTN-1	0.0b	0.0c	15.0c	65.0c
Commercial	0.0b	5.0b	25.0b	80.0b
Control	15.0a	55.0a	85.0a	95.0a

Table 5. Effect of EXTN-1 on suppression of *Fusarium* wilt and yield of hot pepper in the field

Treatment	Disease incidence (%)		Total yield (kg)
	Aug. 16	Sep. 17	
EXTN-1	2.5	10.0	29.5
Commercial	22.5	37.5	20.4
Control	42.5	42.5	22.6

BIO-PESTICIDES

A total of 23 bio-pesticides are registered in Korea. Among them, 12 are fungicides and the rest are insecticides. The microbial agents include *Paenibacillus polymyxa, Bacillus subtilis, Bacillus amyloliquifacians, Paecilomyes fumosoroseus, Streptomyces goshikiensis, Bacillus thuringiensis* var. *kurstaki, Bacillus thuringiensis* subsp. *Aizawai*, etc. (Table 6). Target diseases of the bio-fungicides are powdery mildew, gray mold, *Pythium* and *Phytophthora* blight, *Rhizoctonia* patch, and sheath blight. Nine out of the 11 bio-insecticides are *Bacillus thringiensis* products for the control of various moths attacking vegetables like Chinese cabbage (Table 7). The bio-insecticides formulated with *Beauveria bassiana* and *Paecilomyces fumosoroseus* targeted mite and white fly which are troublesome pests in greenhouse cultivation. In spite of their many advantages, bio-pesticides occupy only 2.8% share in the pesticide market comprising $35 million in Korea. However, it is expected that the bio-pesticide market will steadily increase to reach $80 million until the year 2010.

EGG YOLK AND COOKING OIL (EYCO) MIXTURE

Besides bio-fertilizers and bio-pesticides, egg yolk and cooking oil (EYCO) mixture developed by the author is widely adopted by Korean farmers for the control of various pests and increase in plant health. The EYCO is simply made at home using cooking oil, egg yolk, and motor-mixer. Cooking oils showed direct or indirect effect in controlling plant pathogens

Table 6. List of registered bio-fungicides in Korea in 2007

Microbial agent	Crop	Target disease
1. *Bacillus subtilis* Y1336	Strawberry, pepper, cucumber	Powdery mildew
2. *B. subtilis* QST 713	Strawberry, cucumber, peach	Gray mold Powdery mildew Bacterial leaf spot
3. *B. subtilis* QST 713	Strawberry, cucumber, tomato	Gray mold Powdery mildew
4. *B. subtilis* JKK 238	Strawberry	Powdery mildew
5. *B. subtilis* GB-0365	Turf grass, fig	*Phytophthora* blight *Pythium* blight
6. *B. subtilis* GB-0365	Tomato, fig	*Phytophthora* blight Gray mold
7. *B. subtilis* KBC 1010	Cucumber	Gray mold
8. *Streptomyces colombiensis* WYE 20	Strawberry, turf grass, cucumber	Gray mold Brown leaf blight Powdery mildew
9. *S. goshikiensis* WYE 325	Rice, turf grass	Sheath blight Large patch
10. *Ampelomyces quisqualis* AG 94013	Strawberry, cucumber	Powdery mildew
11. *Peanibacillus polymyxa* AC-1	Pepper, cucumber	*Phytophthora* blight Powdery mildew
12. *B. subtilis* DB 1501	Turf grass	Brown leaf blight

Table 7. List of registered bio-insecticides in Korea in 2007

Microbial agent	Crop	Target disease
1. *Beauveria bassiana* 1	Strawberry, tomato	Spider mite, white fly
2. *Bacillus thringiensis* subsp. *aizawai* GB 413	Chinese cabbage	Diamond-back moth
3. *Bacillus thringiensis* subsp. *aizawai* GB 413	C. cabbage, cucumber, leek etc. (10 vegetables)	Cabbage armyworm, diamond-back moth, cutworm, armyworm, common cabbage worm, etc.
4. *B. thringiensis* var. *kurstaki*	C. cabbage, pine	Diamond-back moth common Chinese worm
5. *B. thringiensis* var. *kurstaki*	C. cabbage	Diamond-back moth
6. *B. thringiensis* var. *kurstaki*	Perilla, C. cabbage, wild vegetable	Diamond-back moth armyworm *Pyrausta panopealis*
7. *Bacillus thringiensis* subsp. *aizawai* GB 413	Pepper, perilla, C. cabbage, cucumber, rice	Tobacco budworm tobacco cutworm diamond-back moth rice leaf roller, etc.
8. *Bacillus thringiensis* subsp. *aizawai* GB 413	C. cabbage	Diamond-back moth
9. *Bacillus thringiensis* subsp. *aizawai* GB 413	Perilla, C. cabbage, rice	Tobacco cutworm diamond-back moth armyworm, rice leaf roller
10. *Bacillus thringiensis*	Pumpkin, pear, C. cabbage, apple, leek, pine, onion, kale	Cabbage armyworm, diamond-back moth, cutworm, armyworm common cabbage worm etc.
11. *Paecilomyces fumosoroseus* DBB 2032	Cucumber, strawberry	Mite, white fly

and insects, and the egg yolk was used as a natural emulsifier and bio-fertilizer. Among various cooking oils, sunflower and rape seed oil (canola) at 0.3-0.5% emulsified with 0.1% egg yolk was the most effective showing over 95% control value of powdery mildew in cucumber and lettuce in a greenhouse. To emulsify the cooking oil with egg yolk effectively, the mixture should be vigorously homogenized for more than five minutes using a high-speed motor-mixer (Tables 8-13, Fig. 1).

When the EYCO was applied, the lettuce seedling stand increased for more than 70% and showed 89.6% to 96.3% control value of powdery mildew that was comparable to a fungicide, Azoxystrobin. Moreover, lettuce yield increased about two times compared to the conventional cultivation. In organic Chinese cabbage production, incidence of downy mildew and *Alternaria* leaf spot in farms where the EYCO and Bt (*Bacillus thringiensis*) mixture was applied at 7-day intervals was lower than 1% in two other farms. The degree of insect damage in the farms was negligible (0.3-0.5%). The other two farms that did not apply the technology showed severe damages caused by diseases and insect pests. EYCO and neem mixture effectively controlled trips on lettuce showing 82.2% control value.

Fungal mycelia and conidia were severely distorted or shrunken when sprayed with EYCO. Microbial population on the treated cucumber leaf decreased significantly over a period of seven (7) days. Consequently, it was

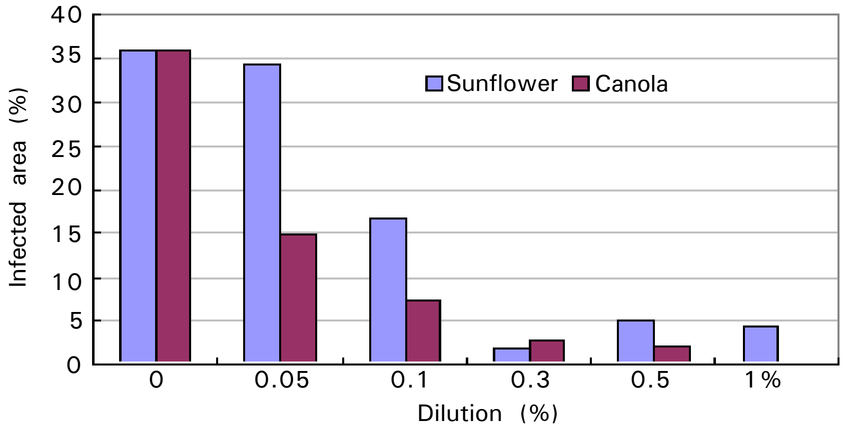

Fig. 1. Effective concentration of EYCO on control of powdery mildew in cucumber in the greenhouse.

Table 8. Effect of EYCO on control of powdery mildew and downy in cucumber in a farmer's greenhouse

Treatment	Powdery mildew(%)		Downy mildew(%)	
	Infected leaf area	Control value	Infected leaf area	Control value
Sunflower EYCO	0.1	98.9	0.4	96.3
CA23+Caster EYCO	3.3	63.7	1.2	88.8
CA23+wood vinegar	2.5	72.5	2.5	76.6
Commercial product	2.8	69.2	3.6	66.4
Untreated control	9.1	-	10.7	-

Table 9. Effect of EYCO and air circulation fan (ACF) on lettuce yield in a greenhouse

Treatment	Total yield (kg)	Fresh wt. (g/leaf)	Dry wt. (g/leaf)
ACF+Canola EYCO	25.88	6.1	0.43
ACF+Sunflower EYCO	23.32	5.8	0.37
ACF+Sunflower EYCO+Ca	26.52	6.7	0.48
ACF	23.52	6.1	0.36
Untreated control	11.86	4.9	0.33

Table 10. Comparisons of yield of organic Chinese cabbage among treatments of environment-friendly materials

Treatment	Total no. of plants	Market rate (%)	Fresh wt./plant(kg)	
			Average	DMRT(0.5%)
EYCO	1,716	95.3	2.77±0.30	B
EYCO+pepper seed extract	1,586	94.8	2.89±0.45	AB
Bio-pesticides (Bt)	1,846	93.2	2.96±0.36	A
Neem oil	1,898	93.2	3.00±0.45	A
Non-treatment	880	88.4	2.21±0.49	C

Table 11. Effect of canola EYCO and Bt (*Bacillus thringiensis*) mixture on suppression of diseases and insect damage on organic Chinese cabbage in fields

Field	Treatment	Disease incidence (%)		Degree of insect damage[a]	Fresh wt.(kg) /plant
		Downy mildew	*Alternaria* leaf spot		
A	Natural enemy Commercial	3.3	2.5	1.5	2.3(2.0-2.5)
B	Non-treatment	95.0	57.5	2.2	2.2(1.8-2.5)
C	EYCO+Bt	<1.0	<1.0	0.5	2.8(2.2-3.4)
D	EYCO+Bt	<1.0	<1.0	0.3	2.9(2.2-3.4)

a)Degree of insect damage: 0=0%, 1=<2%, 2=2-10%, 3=11-40%, 4= over 40% leaf damaged area

Table 12. Effect of EYCO and other environment-friendly materials on control of thrip in lettuce

Treatment[a/]	Damaged leaf (%)[b/]			Control value (%)
	1st (8.4)	2nd (8.14)	Average	
EYCO	10.5	27.4	18.9 ab	38.8
EYCO+PSE	6.2	14.6	10.4 ab	66.3
EYCO+neem	3.2	7.7	5.4 b	82.5
Neem	3.6	11.1	7.3 b	76.4
Non-treatment	20.5	41.3	30.9 a	0

a/EYCO: egg yolk and cooking oil mixture, PSE: pepper seed extract
b/Non-marketable leaves caused by thrip damage

Table 13. Comparison of lettuce yield among treatments

Treatment	Leaf		Total	Average/	Yield
	No.	Fresh wt.(g)	(kg)	harvest (kg)	Increase (%)
EYCO	3.98	6.35	89.5	22.38 ab	129.5
EYCO+PSE	4.13	6.76	84.5	21.13 ab	116.7
EYCO+neem	4.12	6.57	100.5	25.13 a	157.7
Neem	3.82	6.35	98.5	24.63 a	152.6
Non-treatment	2.80	4.88	39.0	9.75 b	-

assumed that the EYCO acts directly against fungal pathogens rather than induces disease resistance on the plant.

FARMER'S ACCEPTANCE AND UTILIZATION

The EYCO can be applied to all kinds of crops to protect various diseases and insect pests. However, it shows relatively high protective effects but low curative effects. Nevertheless, it is widely adopted by Korean farmers because of its simple preparation at home, cheaper price, which is about 1/4 of the price of chemical pesticide, and safety for organic and environment-friendly agriculture. In many field trials, the EYCO proved highly effective in controlling powdery mildews and small insects such as mites. However, application of the EYCO alone could not achieve sufficient control effects on thrips, bigger insects such as moths, and harsh pathogens such as *Colletotrichum*.

In that case, various fungicidal or insecticidal substances allowed for organic farming are mixed with EYCO to increase effectiveness. Substances allowed for organic agriculture in combination with EYCO are bio-pesticides such as B.t., neem oil (azadirachtin), extracts of plant materials such as hot pepper seed or gingko leaf, sulfur, and copper hydroxide. For example, on control of thrips in lettuce, the mixture of EYCO and 20 ppm of azadirachtin or hot-pepper seed extract increased control value to 66.3-82%, while EYCO alone has less than 40% control value. Depending on the target disease and insect, farmers need to select the appropriate additive to EYCO.

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