

Past, Present, and Future Researches on Biological Control of Plant Diseases in Korea

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ABSTRACT : Biological control of plant diseases has been considered a potential control strategy for integrated pest management in recent years. This paper reviewed the progress of research on the biological control of plant diseases in Korea during the last two decades and adopts some future prospects. The crop diseases included, red pepper *Phytophthora* blight, ginseng root rots, cucumber wilt, sesame damping-off, strawberry wilt and tobacco bacterial wilt and mosaic. Biological control of plant diseases requires a multi-disciplinary approach involving input from plant pathologists, ecologists, mycologists and molecular biologists. The author proposed to organize a group "Committee for Biological Control" including researchers, industries, growers and administrators.

With increasing awareness of possible deleterious effects of environmental pollution on the ecosystem, including growing interest in pesticide-free agricultural products, the biological control of plant diseases has received considerable attention recently. Biological control offers many advantages for the growers and society in general and must be pursued as a component of integrated pest management. A more stable, sustainable and safe food supply with biological control can be produced at lower cost compared to conventional control measures when the cost to environmental impacts is computed properly.

The increased interest in biological control of plant diseases can be illustrated by the frequent symposia and the recent publication of a reference text developed entirely to this subject (2, 15, 33). In the last decade, papers on biological control presented at the "International Congress of Plant Pathology" increased to about five folds from '83 to '93, from 45 to 236. This is the second International Symposium on biological control of plant diseases organized by the Korean Society of Plant Pathology (KSPP). The first one was held with two foreign guests and three native speakers at the Agricultural Science Institute, in Suwon on August 29, 1988. According to the survey results conducted by KSPP in 1991, of the 46 members of KSPP, responded 13 favored "biological control" as the subject of the symposium and followed by 2~3 of "biotechnology", "recent research trends" and others.

Biological control of plant pathogens has been considered a potential control strategy for integrated pest management in recent years. However, finding a practical approach to the biological control of plant pathogens has been quite slow, since many biological control practices and most research efforts are directed at diseases caused by soil-borne pathogens.

Recently, there have been investigations concerning the effect of organisms on foliage in the phyllosphere (1,44).

My interest in biological control began in 1955 with my M. S. thesis entitled "Antagonistic effects of microflora of barley leaves on the pathogenicity of *Helminthosporium sativum*" under the supervision of advisor Dr. J. J. Christensen. There were only five citations on the control of leaf diseases by antagonists in the review article "Control of plant diseases by use of antagonistic organisms" by Wood and Tveit published in 1955 (44). The above thesis published in the Journal of Seoul National University in 1958 (5), is one of the early papers concerning the biological control of foliage diseases in Korea and worldwide. His associate, Chung (10) reported that an isolate of *Trichoderma harzianum* and *Bacillus subtilis* obtained from rice phylloplane was effective as benomyl for controlling seedling rice blast caused by *Pyricularia grisea*.

This paper reviews the progress of researches on biological control of crop diseases during the last two decades. The crop diseases included, red pepper *Phytophthora* blight, ginseng root rots, cucumber wilt, sesame damping off, strawberry wilt, and tobacco bacterial wilt and mosaic. Some portions of the similar paper have been published elsewhere (9).

***PHYTOPHTHORA* Blight of Red Pepper**

Red pepper (*Capsicum annum*) is one of the most valuable cash crops and occupies more than 10% of the total cultivated upland in Korea. *Phytophthora* blight caused by *P. capsici* has been causing severe epidemics due to continuous monocropping practices. The disease has been one of the greatest limiting factors for red pepper productions in Korea (18,41). According to extensive surveys made in 1986, more than one third of 60 fields observed in three major production area indicated 50~100% losses of the crop in late September. Most of the infected plants resulted from soil-borne primary inoculum, and further spread by splashing rains during the period from late June to late July (18).

Current control measures rely mostly on frequent fungicide spray. However, control efficacy of fungicide applications is low since the causal organism is soil-borne in nature. Recently, efforts have been made in controlling the disease through biological means. In most of the research, antagonistic agents were screened and identified and assessed their antagonistic activity *in vitro* or pot tests in the greenhouse.

Some investigators reported *Trichoderma* spp. (18, 34), *Pseudomonas cepacia* (41), *Ps. fluorescens* (41), *Bacillus* spp. (18), *Enterobacter agglomerans* (38), and 29 bacterial isolates (22) as the best potential biological agent against *Phytophthora* blight because of their ability of hyphal lysis, mycoparasitism, inhibition of growth and sporangial germination, and inhibitory metabolites. An isolate of avirulent *P. capsici* inhibited development of *Phytophthora* blight in the two cultivars (17). Park and colleagues (40) purified antibiotics from *Ps. cepacia* which significantly inhibited the growth of *P. capsici*.

Incidence of this blight was significantly suppressed by *T. harzianum* or *Ps. cepacia* amended

with wheat bran or arrowroot leaf tissues to the nursery soil infested with 30 sporangia/g dry soil (34). Attempts were made to develop biofungicides from both agents formulated into granule and/or powder forms. Application of *Ps. cepacia* granules with alginate formulation provided better suppression of the disease as compared to direct drenching of the suspension into the soil, whereas the case was not true for *T. harzianum* (39). Similarly, an addition of 2% sodium alginate with 3% rice bran as a nutrient source resulted in the highest viability of *Ps. cepacia* (16).

In a polyethylene filmhouse, yield and growth of red pepper grown on seedbed soil treated with both antagonists were at least similar or higher compared to those of untreated (28). In field trials, yields appeared higher in the plots treated with the above antagonists as compared to the untreated check plots, however, the differences in yield between treatments were not statistically significant (23). Kim and colleagues (24) reported that metalaxyl together with antagonistic *T. harzianum* or *Ps. cepacia* greatly enhanced the control efficacy of red pepper blight in the greenhouse.

Ginseng Root Rots

Korean ginseng (*Panax ginseng*) has been recognized in the Orient as a medicinal crop for more than 2,000 years, and its demand increases yearly. However, "soil sickness" causes not only poor quality and low yield but ginseng cannot be cultivated again in the same field for more than 10 years afterwards. The principal cause of "soil sickness" is known to be due to soil-borne root rot diseases, among which *Fusarium solani* and *Cylindrocarpon destructans* are the most common causal fungi. The losses in yield in 1977 due to various root rots at harvest were 43~59% at Yangji, and 20~49% at Kimpo. In 1965, a bacterial root rot associated probably with *F. solani* and/or *C. destructans* caused losses of 48% of the crop at Keumsan and as much as 80% of the crop at Buyo (6).

Since ginseng requires fertile soil, rich in humus, and continuous shade for six years until harvest, there are no practical control measures for the root rots. Fungicidal application to the soil is not feasible, because of residual problems and other reasons. Furthermore, cultivars resistant to the pathogens are not known even though alternative nonpolluting soil amendments and suppressive soils were attempted in order to improve the quantity and quality of ginseng production in Korea.

Kim & Ohh (25) reviewed biological control of ginseng root rots and damping-off, investigated by the Korea Tobacco & Ginseng Research Institute, thoroughly at the symposium held in 1985. Ginseng seeds treated with *Trichoderma koningii*, *T. viride*, *T. hamatum* and *Gliocladium virens* reduced the incidence of damping-off, especially when cellulose or rice hull powder was added to the antagonistic fungal suspensions. In the same experiment, bacterial antagonists, *Bacillus subtilis* or *Arthrobacter* sp. were less effective than those of the fungi. The effect could be increased by combining with solarization and/or fumigation to the nursery bed soil.

Incidence of ginseng root rot caused by *Fusarium solani* has been reduced by dressing the seedling roots or dipping them suspensions of the antagonists. When the seedling roots were treated rye straw plus antagonistic fungi, bacteria and Actinomycetes, root rot was greatly reduced in the pot soil naturally infested with *F. solani*. Rice straw amendments increased considerable amounts of cell wall lytic bacteria and antagonistic Actinomycetes against *F. solani*, though the amount of rot greatly increased in the third year regardless of the type of antagonists or combination of the antagonists tested. In other trials, antagonist with rice straw amendments resulted in effective to the third year after treatment (25).

Chung & Kim (6) reported that crab shell and cow bone amendments resulted in almost complete control of the seedling root rots in soil infested with *C. destructans* or *F. solani*. In amended soil, populations of various Actinomycetes increased in the range of 10~25 times over than that of non-amended soil, whereas those of *C. destructans* were reduced to about 50~70% as compared to the control (6). In 1982 (13) antagonistic *Streptomyces* species to *F. solani* were identified as *S. alboniger* and *S. roseolilacinus*. All 9 isolates of the two species produced chitinase lysing cells of *F. solani* and inhibited germination of the conidia. When the species of *Streptomyces* were grown simultaneously in the medium, chitinase activity increased during the incubation period, whereas mycelial volume of *F. solani* decreased (14).

Soil suppressive and conducive to ginseng root rot have been studied extensively in Korea. Population of antagonistic microorganisms to *F. solani* was much higher in suppressive soils than in conducive soils, whereas the numbers of *Fusarium* species were less in suppressive soils. Mycelial growth and chlamydospore formation of *F. solani* were inhibited in suppressive soils (11). The same authors reported that mycelial growth of *F. solani*, *Phytophthora cactorum*, and *Sclerotinia* sp., was severely restrained on the suppressive soil extract agar than that of conducive ones (12).

FUSARIUM Wilt of Cucumber

Cucumber (*Cucumis sativus*) has been cultivated extensively, either in polyethylene filmhouse and in the field, throughout the country. Since available land is limited in Korea, continuous monocropping practices resulted in frequent heavy crop losses of cucumber wilt caused by *Fusarium oxysporum* f. sp. *cucumerinum* (36). Cultivars resistant to the disease, and soil disinfection with chemicals or physical means are available, however efficacy of control is limited due to various reasons. Consequently, biological control of cucumber *Fusarium* wilt is generally accepted offering answers to many serious problems. Biological control is an essential component of sustainable cultivation capable of continuing without interruption of diminution.

Jee & Kim (20) isolated three antagonistic bacteria, *Pseudomonas fluorescens*, *Ps. putida* and *Serratia* sp. and three fungi, *Gliocladium* sp., *Trichoderma harzianum* and *T. viride* to *Fusarium oxysporum* f. sp. *cucumerinum* from rhizosphere soil by the modified triple layer agar techni-

que. In tests, the antagonists inhibited conidial germination and showed mycoparasitism, and lysis of *Fusarium oxysporum* f. sp. *cucumerinum* *in vitro*. The same authors (20) found that among the antagonists tested *Ps. fluorescens* and *T. harzianum* were the most effective for controlling cucumber wilt over 50% compared to non-treated pot tests. Furthermore, cucumber seedling growth was significantly promoted with antagonists being about 20~50% greater in natural field soil and sterilized soil. In another study (19), cucumber growth was significantly increased using seed treatment with conidia of *T. harzianum* T95 and *G. virens* G872 in potted natural field soil.

Park (36) found that among the selected bacteria, 9 isolates reduced incidence of cucumber seedling wilt more than 50% when the bacteria were introduced to germinating seeds prior to seeding in soil infested with an inoculum concentration of 400 cfu/g. A strain of *Ps. putida* and *Ps. fluorescens* significantly inhibited the disease incidence even at high inoculum concentration of up to 1600 cfu/g soil. In a similar study (3), *Gliocladium virens* and *T. harzianum* were also antagonistic to the wilt fungus and the former suppressed 52.3~59.7% of the disease when biological agent was treated with wheat bran in pot tests. They reported in 1993 that *Ps. gladioli* with organic amendments such as barley bran or wheat bran greatly suppressed incidence of wilt in a greenhouse (4). Growth of cucumber increased significantly by the seed treatment with conidia of *T. harzianum* T95 and *G. virens* G872 in potted natural field soil (19). The presence of the biocontrol agent *Ps. putida* Pf3 in a cucumber system was highly beneficial to the colonization of the other agent *G. virens* G872B at rhizosphere (21).

Sesame Damping-Off

Sesame (*Sesamum indicum*) is one of the most important oil crops in Korea and domestic demand is increasing yearly. However, production is limited by many diseases, including damping-off caused by *Rhizoctonia solani* or *Fusarium oxysporum* f. sp. *sesami*. The disease incidence was as high as 70% in many fields. Farmers frequently sow sesame seeds twice or even three times to obtain adequate stands.

Shin and colleagues (42) found that *Bacillus subtilis*, *B. polymyxa* and *Streptomyces*, isolated from rhizosphere of sesame plant lysed the cell wall of hyphae and conidia of *F. oxysporum* and reduced formation of macroconidia and chlamyospores of fungus *in vitro* tests. Highly antagonistic *Trichoderma viride* against *F. oxysporum* reduced incidence of sesame damping off when sesame seeds were sown in sterile soil treated with antagonist and the pathogen simultaneously. With high populations of antagonistic *T. viride*, sesame seed germination and seedling growth have been inhibited in a pot test.

Attempts were made to control sesame damping-off caused by *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *sesami* by coating seeds with three isolates of *Trichoderma viride* in pot and field trials using naturally infested soils (7,8). When sesame seeds were treated with Benlate T or conidia (10^7 /ml) of the antagonists, the emergence of seedlings was signifi-

cantly increased compared with an untreated control. Soil samples taken from the crown area of sesame seedlings, showed an increase in *Trichoderma* spp. population during the growing season, whereas those of *R. solani* and *F. oxysporum* decreased. Mycoparasitism between the antagonists and the pathogens was observed after mycelial contact in dual culture.

FUSARIUM Wilt of Strawberry

Strawberry (*Fragaria ananassa*) is one of the most important cash crops in Korea and cultivated land has increased yearly during the last two decades up to 8,281 ha in 1980. However, the cultivated land decreased to 4,851 ha in 1984 due mainly to continuous monocropping which created soilborne disease problems. Three major strawberry producing regions, Daeduk, Nonsan, and Samnangjin moved to neighbouring lands because wilt caused by *Fusarium oxysporum* f. sp. *fragariae* occurred over 80% of the crops (30). Attempts have been made to control *Fusarium* wilt of strawberry by biological means with antagonistic fungi (31) and bacterium (32) in Korea, recently.

An isolate of *Pseudomonas gladioli* showed the most effective inhibition against mycelial growth and conidial germination *in vitro* test. The incorporation of *Ps. gladioli* into field and sterile soil infested with the pathogen, decreased incidence of strawberry wilt significantly by 60% compared to the control without the antagonist (32). Moon and colleagues (30) reported that *Trichoderma harzianum* and *T. viride* parasitized both the hyphae and the conidia of *F. oxysporum* f. sp. *fragariae* and inhibited mycelial growth of the pathogen in the dual cultures. Factors affecting mycoparasitism of the above antagonists have been investigated in details on culture media (31). Maltose and $\text{Ca}(\text{NO}_3)_2$ were best for mycoparasitism of *T. harzianum* as a carbon and nitrogen source, respectively. Addition of organic materials such as corn, wheat bran, and rice straw powder increased the mycoparasitism, whereas clover leaf powder amendment annuled the efficiency.

Tobacco Bacterial Wilt and Mosaic

There are only a limited number of researches on biological control of bacterial or viral plant diseases at present. The severity of tobacco bacterial wilt was significantly reduced by dipping with suspensions of an avirulent isolate of *Pseudomonas solanacearum* in the early growing stage (46). Further studies showed that penetration of virulent bacteria into the root systems and their multiplication in tobacco stem were inhibited remarkably by preinoculation with avirulent one (47).

An antiviral protein, insularin purified from the leaves of *Phytolacca insularis* completely inhibited transmission of TMV at the contraction of 500 ng/ml (27).

Concluding Remarks and Future Prospects

It is well known that the application of biological agents controlling important diseases

needs more time and effort, but once it is successful, it will be a useful method in agricultural practices and environmental protection. Although certain diseases can be controlled completely or partially by using biological agents, the best method is through integrated pest management wherein a biological control component would be included together with the use of pesticides and resistant cultivars as well as other cultural methods. Biological control, no doubt, can overcome various problems created by the use of pesticides.

Biological control by microbial antagonism can expand, provided that effective formulations can be developed in the near future. Soil-borne pathogens have been a target for applying biological control and most research efforts for a long time. But a few foliar plant diseases may be amendable to biological control and future investigations will be promising. The double stranded RNA genetic elements associated with the phenomenon of "transmissible hypovirulence" is another possibility in the biological control of aerial diseases of plants (35).

Biological control of postharvest diseases appears to hold greater promise than other biological control methods for diseases of field crops (43). Exploration of natural products as fungicides in addition to antagonistic microorganisms is also a promising approach for control of postharvest diseases. The mechanisms of biological control have had resounding success by the recombinant DNA technology, and this will ultimately prove to be its lasting contribution. These tactics along with plant expressed antimicrobial defenses, including induced resistance, cross protection and a new types of "soft" pesticides, will be the leading players in the coming era of plant protection.

Biological control of plant diseases requires a multidisciplinary approach involving input from plant pathologists, ecologists, mycologists, and molecular biologists. I propose to organize a group "Committee for Biological Control", including researchers, industries, growers and administrators in order to feed the ever increasing population with environmentally sound and safe products. Mehrotra (29) said "We have to fight the plant pathogens on all fronts and with all the weapons at our command".

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