POSTER SESSION 5A

NEW COMPOUNDS, NEW CONCEPTS AND NEW USES

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Posters Papers: 5A-1 to 5A-3

New fungicide benthiavalicarb-isopropyl + mancozeb for foliar use in potatoes in Europe

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ABSTRACT

The new fungicide benthiavalicarb-isopropyl offers strong protectant and some curative activity on potato late blight (*Phytophthora infestans*). At the low dose rate of 28 g a.i./ha, benthiavalicarb-isopropyl, in combination with mancozeb, is a new powerful tool during periods of heavy disease pressure. It offers relatively long residual protection against foliar infection of late blight. Early blight (*Alternaria solani*) is also controlled well with this product.

INTRODUCTION

Benthiavalicarb-isopropyl was first discovered by Kumiai Chemical Industry Co, Ltd Japan. The code number during its development phase was KIF-230. The product belongs to a new chemical class with the proposed name of amino acid amide carbamates. This chemistry has excellent activity against Oomycete fungi, although it has no activity against *Pythium* spp. Benthiavalicarb-isopropyl will be introduced in various European and other countries in mixture with contact fungicides from 2004 onwards in crops like potatoes, vines and tomatoes. This paper will only discuss the performance of benthiavalicarb-isopropyl in combination with mancozeb for use in potatoes.

Biological properties

Benthiavalicarb-isopropyl belongs to the new chemical group of amino acid amide carbamates. However, the mode of action has not yet been fully identified. Nevertheless, the product is not affected by any existing resistance problems in potatoes. Kumiai has observed that for *Phytophthora infestans*, benthiavalicarb-isopropyl has very high activity on mycelial growth, sporulation, and sporangia and zoospore germination. The product has no strong activity on zoospore motility and zoospore release (indirect germination) (Miyake, *et al.*, 2003).

Benthiavalicarb-isopropyl can penetrate easily into treated leaves of various crops, where it shows its curative properties. Under practical circumstances, benthiavalicarb-isopropyl will be applied at relatively low rates per ha, which make use of its excellent protectant activity. At such rates, a locally systemic activity can be observed as well, but no systemic transportation

through the plant takes place. The rapid penetration of the active ingredient into foliage means that it also has a good rainfastness. The activity of benthiavalicarb-isopropyl is not dependent on ambient temperature, so it will be effective in both cool and warm climatic conditions.

Properties of benthiavalicarb-isopropyl

Phytotoxicity from benthiavalicarb-isopropyl or its combination product with mancozeb has never been observed in any of a wide range of crops tested, even at high dose rates. Toxicity to mammals, birds and aquatic life is also extremely low, so no restrictions are expected that could affect the use with respect to operator and worker exposure, nor spray free zones alongside surface water.

Benthiavalicarb-isopropyl will only be commercialised in combinations with multi-site inhibitors, as a resistance management strategy. Some other molecules with low to moderate resistance risk (cymoxanil and dimethomorph) have been widely used in combination with mancozeb in potatoes. This has not led to field observed resistance of late blight in potatoes.

Since benthiavalicarb-isopropyl is a strong protectant itself, it allows relatively low dose rates of the partner products in these combinations. This by itself can be considered as a benefit with respect to reduction of the total amount of active ingredient brought into the environment.

The combination of benthiavalicarb-isopropyl with mancozeb will be formulated as a WG (water dispersible granule) in order to minimise operator exposure, and maximise ease of use. The leading formulation, will contain 17.5 g benthiavalicarb-isopropyl + 700 g mancozeb/kg (recommended use rate 1.6 kg/ha). In some countries, a WG formulation containing 12.5 g benthiavalicarb-isopropyl + 700 g mancozeb/kg will be introduced (recommended use rate 2 kg/ha).

MATERIALS AND METHODS

From 1996 to 2002, large numbers of trials were conducted in Western Europe with benthiavalicarb-isopropyl + mancozeb. A representative selection of trials is used in this paper to illustrate the properties of benthiavalicarb-isopropyl. All trials were conducted according to Good Agricultural Practice and used standard protocols required for European registrations. All trials used a fully randomised block design with four replicates. If late blight infections did not occur naturally in the course of the growing season, untreated rows of potatoes bordering the trials were infested with a mixture of *Phytophthora infestans* strains originating from different locations.

RESULTS

Field performance on foliar blight in Germany, Belgium, Netherlands and UK

In field trials in Germany (F00WEK01 and F00WIK01) with continuous high disease pressure, the combination of benthiavalicarb-isopropyl + mancozeb used at 1.6 kg/ha (28 + 1120 g ai/ha) gave consistently good protection against late blight, equal to standards. Figure 1 shows the means of 2 trials conducted in 2000. After 9 applications in a spray schedule of 6 – 9 day intervals, no further applications were made, while the disease pressure remained high. Last applications were made August 10 and 11 2000, respectively. It was observed that the benthiavalicarb-isopropyl combination product showed a longer residual activity than fluazinam (200 g ai/ha) and mancozeb (1440 g ai/ha). Higher dose rates did not give further performance improvements, while lower dose rates gave control equal to reference products fluazinam and mancozeb.

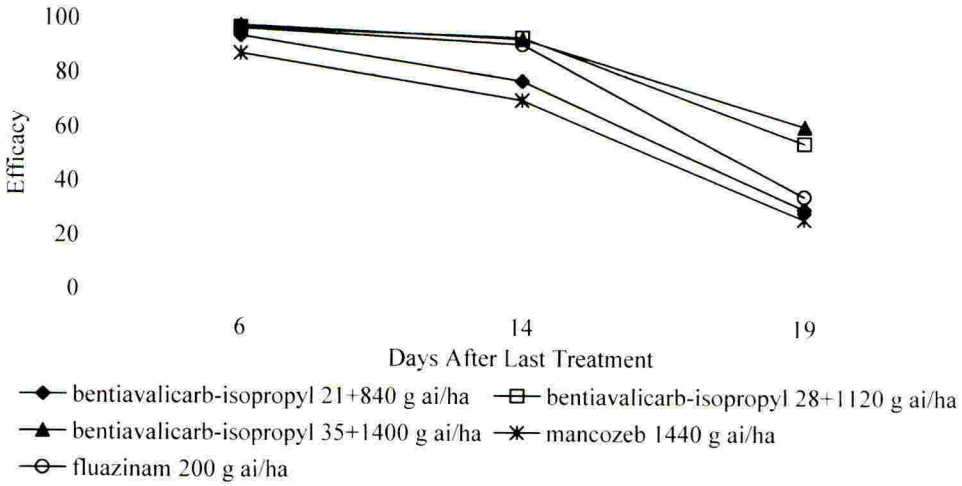

Figure 1. Residual activity of benthiavalicarb-isopropyl + mancozeb against late blight at various days after last application (DALA) in Germany.

C.R.A. Gembloux (Belgium) conducted two trials with applications made in a sequence of 7 – 9 days interval according to a late blight warning system during 2000. The last application was made 12 days prior to the last assessment date. Benthiavalicarb-isopropyl, when used alone at high dose rates (75 g ai/ha), gave excellent control of late blight in potatoes (Figure 2). At 12 days after the last application, very good efficacy was observed, comparable to or better than standards, while disease pressure remained high in these trials. The combination of benthiavalicarb-isopropyl with mancozeb allowed relatively low dose rates to be used. The additive strength of the combination product is clearly demonstrated by the results where the product is at least as effective as a combination of 2250 g ai/ha mancozeb + 200 g ai/ha fentinhydroxide.

During 2000, trials conducted by Agrisearch (Horti 303/pots/00/b) in the UK demonstrated again that 28 g benthiavalicarb-isopropyl/ha has a very strong additive activity on mancozeb used at 1120 g ai/ha (Figure 3). At these rates, it also performs at least as well as another

penetrant product dimethomorph + mancozeb (150 + 1334 g ai/ha). Also, it is much stronger than fluazinam at its registered use rate in the UK.

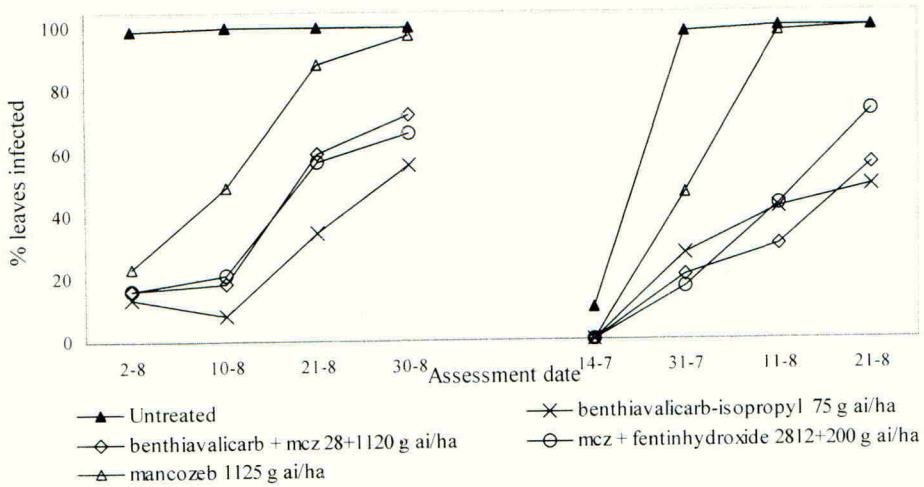

Figure 2. Performance of benthiavalicarb-isopropyl in Belgium.

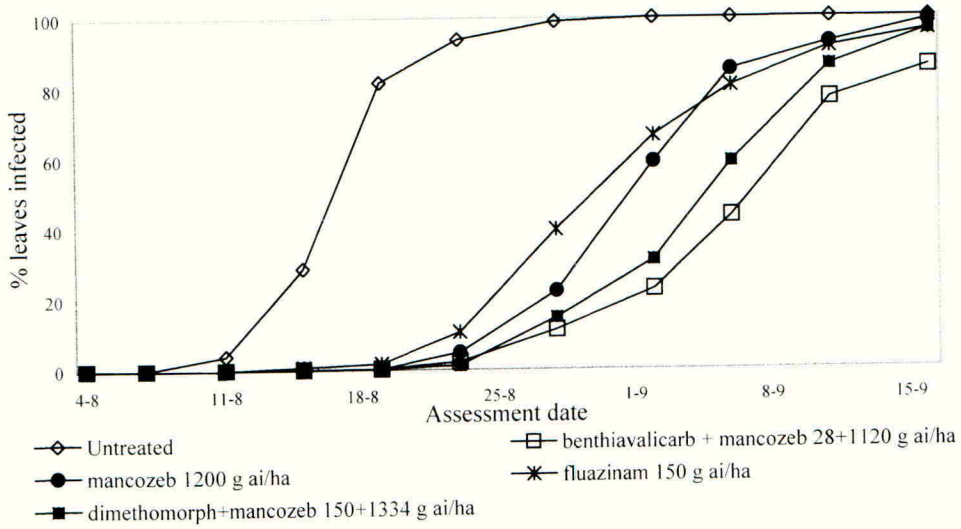

Figure 3. Field performance of benthiavalicarb-isopropyl + mancozeb in comparison UK standards.

Figure 4 shows results of foliar protection under extreme disease pressure in Netherlands with different ratios of benthiavalicarb-isopropyl and mancozeb in 2000 (trial F00.538.ARS2). Assessments are made according to the Dutch PD-scale. Here it can be concluded that the slightly higher dose of mancozeb (1400 g ai/ha) in combination with 25 g benthiavalicarb-isopropyl/ha gives better protection than the combination of 28 + 1120 g benthiavalicarb-isopropyl + mancozeb/ha. Under such disease pressure and climatic conditions, the

performance of dimethomorph + mancozeb, cymoxanil + mancozeb and to a minor extent benthiavalicarb-isopropyl + mancozeb at 28 + 1120 g ai/ha offer less effective disease control than benthiavalicarb-isopropyl + mancozeb at 25 + 1400 g ai/ha.

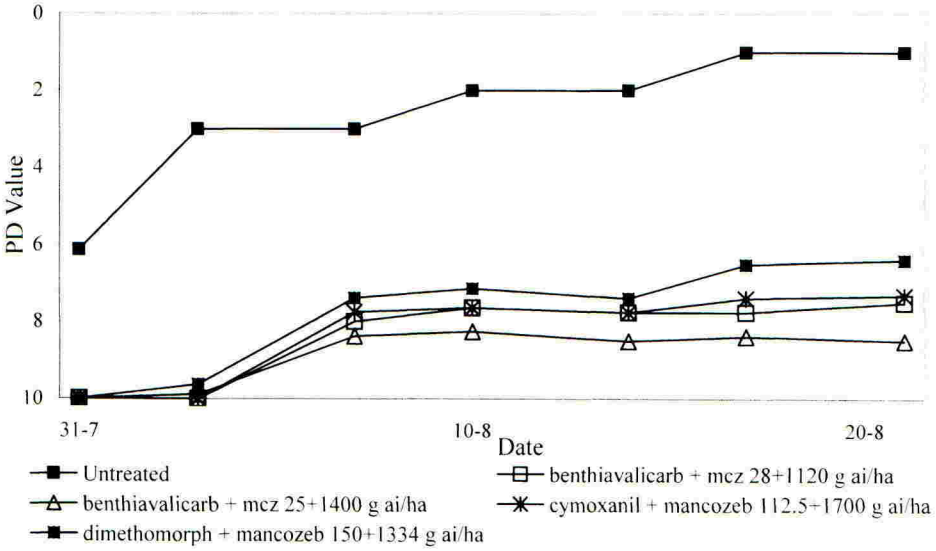

Figure 4. Comparison of 2 different ratios of benthiavalicarb-isopropyl + mancozeb with standard products in Netherlands under continuous extreme disease pressure.

Yield effects

In all the trials, effects on yield were associated with late blight disease severity in the foliage. Under heavy disease pressure, benthiavalicarb-isopropyl + mancozeb was usually the most effective and consistent product for foliar protection. Therefore, the resulting yields were also among the highest in these trials. In trials with no or very little disease pressure, yields of plots treated with benthiavalicarb-isopropyl + mancozeb were at similar levels to other standards with no significant differences.

Tuber protection

Tuber protection of potatoes by *Phytophthora infestans* is dependent on a number of factors: Sporulation of late blight on the crop, migration of spores to the tubers (active through swimming in moisture on plant, or passive with rain or irrigation water) and cracks in the soil surface to reach the tubers. Many potatoes are grown in soil types where chances for tuber infection are relatively low, since the number of cracks that could allow spores to infect tubers is relatively small. In most of our field trials, there were, therefore, no significant differences in tuber infection between benthiavalicarb-isopropyl + mancozeb and reference products (such as fluazinam or organo-tin combinations). However, in those trials conducted in soils where tuber infections are common (e.g. in Dutch trial sites on clay soils), it was observed that benthiavalicarb-isopropyl gave less effective control of tuber infection than fluazinam. In decreasing order of efficacy, it could be concluded that fluazinam gave the highest protection followed by dimethomorph + mancozeb, benthiavalicarb-isopropyl + mancozeb, propamocarb + chlorothalonil and, least effective, cymoxanil + mancozeb. This sequence can be explained

by the fact that benthiavalicarb-isopropyl has no effect on motility of zoospores. However, the fact that it has high activity preventing sporulation and mycelial growth, ensures that the effects are better than that of some commonly used other commercial products.

Control of *Alternaria* early blight

Table 1. Control of *Alternaria solani* in two trials in Netherlands during 2000.

Treatment	Rate (g a.i./ha)	Waardhuizen Bintje	Werkhoven Bintje
benthiavalicarb-isopropyl + mancozeb	25 + 1400	9.3	8.1
benthiavalicarb-isopropyl + mancozeb	28 + 1120	9.6	7.5
dimethomorph + mancozeb	150 + 1334	9.6	7.9
cymoxanil + mancozeb	112.5 + 1700	9.8	9.0
fluazinam	200	8.1	5.3

In several trials, infections of early blight (*Alternaria solani*) did occur, which were most significant towards the end of the season. Table 1 shows the assessments made during 2000, a year with relatively high disease incidence. Assessments were made 1 week prior to desiccation at the end of season. 1 = no control, fully diseased crop. 10 = healthy crop without any symptoms.

Early blight is not controlled by benthiavalicarb-isopropyl but only by the mancozeb component in the mixture product. Both products with benthiavalicarb-isopropyl and mancozeb give adequate and reliable levels of control of early blight, whereas fluazinam gives insufficient control.

CONCLUSIONS

At low rates (25 - 28 g ai/ha), benthiavalicarb-isopropyl is a highly active molecule with protectant and curative properties in potatoes. The combination with a relatively low rate of mancozeb (1120 g ai/ha), makes the product a very robust, consistent and reliable tool to protect potatoes from attack by late blight (*Phytophthora infestans*) and early blight (*Alternaria solani*) even under extreme disease pressure. The uptake of the product into the leaves gives it excellent rainfastness making it a valuable tool in modern potato production.

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Control of *Fusarium oxysporum* and *Meloidogyne* spp. with *Pseudomonas oryzihabitans*

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ABSTRACT

Pseudomonas oryzihabitans, a symbiont of the entomopathogenic nematode *Steinernema abbasi* acts as a biopesticide against the soil-borne pathogens *Fusarium oxysporum* and *Meloidogyne* spp. when applied in the absence of the nematode vector. The inhibition of the fungal growth in cultures and beneficial effects on tomato plants infected with the fungus and nematodes is demonstrated.

INTRODUCTION

Vagelas *et al.* (2002) showed that the bacterium *Pseudomonas oryzihabitans*, originally isolated from the entomopathogenic nematode *Steinernema abbasi*, produces antifungal metabolites (AFMs) *in vitro*. They demonstrated a) bacterial motility and chemotaxis towards fungal mycelia; b) when bacteria or the vector (entomopathogenic nematodes) were applied to soil, a significant number of cells were isolated; c) bacteria were detected in the rhizosphere up to 65 days after application; d) bacterial cells isolated from the soil or the rhizosphere were able to kill *Galleria mellonella* larvae producing the same symptoms as the entomopathogenic nematode *S. abbasi*. Finally, it was shown that applications of 10^4 cells/ml significantly reduced numbers of *Fusarium oxysporum* propagules in the soil. Work by Samaliev *et al.* (2000) has demonstrated the nematicidal efficacy of such bacteria. More evidence is provided to demonstrate *P. oryzihabitans* bio-activity against the soil-borne pathogens *F. oxysporum* and root knot nematodes (*Meloidogyne* spp.). Beneficial effects of bacterial cells on seeds and plants are also discussed.

MATERIALS AND METHODS

Microbial cultures

The pathogens used in this study were an isolate of *F. oxysporum* f. sp. *lycopersici* and a mixed culture of *Meloidogyne* spp. The bacterium *P. oryzihabitans*, a symbiont of the entomopathogenic nematode *S. abbasi*, was isolated from the haemolymph of infected wax moth larvae (*Galleria mellonella*), (Vagelas *et al.*, 2002). Pure colonies were multiplied in 3% nutrient broth No2, the suspension was centrifuged and the bacterial pellets were diluted with sterile tap water. Bacterial concentrations were determined using a spectrophotometer adjusted to the 600nm wavelength.

Bioassay

Petri dish tests

Inhibition of *F. oxysporum* f. sp. *lycopersici* mycelia *by P. oryzihabitans* was assayed *in vitro* on nutrient agar (NA) plates. Dual cultures were set up in 85 mm diameter Petri dishes by placing mycelial plugs, 5 mm in diameter, on one side of the dish and at the opposite side, parallel streaks of *P. oryzihabitans*. Zones of colony growth inhibition were recorded after 120 h and were estimated using the formula: 100 x (R1-R2) / R2, where R1 = fungus radius growth towards bacterial streak and R2 = fungus radius growth away from bacterial streak. Ten replicates per treatment were used.

Culture filtrate tests

The effect on mycelium growth and spore germination of *F. oxysporum* f. sp. *lycopersici* were tested in the presence of bacterial culture filtrates. Fungus was grown on solid malt extract medium (MEA) for seven days at 25 °C in the dark. Three 3-mm agar blocks were then placed in 250 ml conical flasks containing 100 ml Czapek-Dox Broth. Two concentrations (1 and 10%) of culture filtrates of the bacteria were selected and diluted in sterile distilled water (SDW). Treatments and controls were placed in a shaking incubator (150 revs/min) and incubated at 28 °C in the dark. After 6 days, fresh and dry weights of mycelium were recorded. Each treatment was replicated 4 times and the experiment was repeated twice. In addition, a 10 µl drop of treated and untreated fungus spores was placed onto 2% water agar used as germination medium. After 6 hours incubation at 25 °C, fungistatic properties of the bacterial filtrates were assayed by examining the drops under a microscope (x 200). There were 5 replicates per treatment and each plate was repeated 4 times.

Plants toxicity tests to tomato seeds

Two bacterial concentrations 10^4 and 10^6 cells/ml were tested (1 ml of cell suspension was used in each treatment). Fifty-tomato seeds cv. Craigella were added to 100 ml bottles containing 50 ml of the appropriate mixture plus SDW. Bottles only with SDW or amended with spores of *F. oxysporum* f. sp. *lycopersici* (2 ml 2 x 10^6 spores/ml) were used as controls. Treatments were shaken for 2 days and the length of radicle was recorded. Germinating and non-germinating seeds were planted in sand for further studies.

Association of *P. oryzihabitans* and *F. oxysporum* f.sp. *lycopersici* on tomato roots

Tomato (cv Craigella) seeds were germinated on 2% MEA in the dark at 25 °C. The seedlings were planted in 7-cm plant pots containing an autoclaved mixture of loam:sand 3:1 (v/v) and placed in the glasshouse at 25-30 °C. Each pot was inoculated with 10 ml of a 10^4 cells/ml suspension of *P. oryzihabitans* and with 20 ml mycelia (4%) of *F. oxysporum* f. sp. *lycopersici* plus the bacteria. After 25 days, the plants were harvested, their root systems washed free of all but the most closely adhering soil and the colonization of the tomato root system by bacteria was estimated using dilution plating techniques (Vagelas 2002) and by calculating the colony-forming units (cfu) per g fresh root weight. Each treatment was replicated 8 times and repeated twice.

The mode of action of the bacteria cells isolated from roots was further examined by applying them to NA plates and challenging with fungal plugs in dual culture tests.

Suppression of Fusarium wilt diseases in pot assays

Pots (3 litre), containing a mixture of soil loam-peat:sand 4:1 (v/v), were placed in a glasshouse at 25-30 °C and inoculated with 60ml of a microconidial suspension of *F. oxysporum* f. sp. *lycopersici* in Czapek-Dox broth (giving a final concentration of 10^6 spores/ml substrate). Pots were left in the glasshouse for 30 days in order to obtain chlamydospores. Seeds of tomato cv. Craigella were planted in an autoclaved mixture of loam:sand 3:1 (v/v) in Jiffypots. Jiffypots including seedlings (2 true leaf stages) were placed in the pots. Before transplanting, half the plants were inoculated with 10 ml of a 10^4 cells/ml suspension of *P. oryzihabitans*. After 32 days, plant growth was assessed. Each treatment was replicated 10 times and the experiment was done twice.

Impact of *P. oryzihabitans* to *Meloidogyne* spp. in soil

Tomato plants cv. Tiny Tim were grown in 9 cm diameter plastic pots in soil 3:1 loam/sand mixture (free of pathogens). Plants at the 2-leaf stage were infected with 1000 juveniles per pot. At the same time, 20 ml of bacterial cell suspensions, prepared in sterile water at concentration 10^4 and 0 cells/ml were applied to the soil surface. There were 12 replicates for each treatment.

Statistical analysis

Analyses were performed employing the SPSS 10.1 statistical programme. ANOVA and multiple range tests (Tukey's multiple comparisons) were applied to assess differences between treatments and identify statistical differences between means, respectively.

RESULTS

Bioassay

Petri dish tests

The results showed that bacteria cells produce freely diffusible compounds that are able to inhibit fungal growth (Table 1).

Table 1. Effect of bioactive compounds produced by *P. oryzihabitans* on mycelial growth (cm) of *F. oxysporum*

Treatment	Zones of inhibition	
(Fungus challenged with bacteria)	R1 (radius growth towards bacteria streak)	R2 (Control)
F. oxysporum f. sp. *lycopersici*	0.98 ± 0.042 **	2.8 ± 0.094 **

** Standard Deviation

Culture filtrate tests

Mycelial fresh and dry weights, hypha extension and sporulation of *F. oxysporum* f. sp. *lycopersici* were significantly reduced in all bacteria cell-free filtrate treatments (Table 2).

Table 2. *Pseudomonas oryzihabitans* culture filtrates fungistatic activity

Treatment	Mycelial fresh weight (mg)	Mycelial dry weight (mg)	Sporulation Index[1]
F. oxysporum (Untreated)	1148b	290b	5
F. oxysporum + 1% filtrate	459a*	96.2a	3
F. oxysporum + 10% filtrate	318a	95.7a	2
LSD $_{0.05}$	94	24	

[1] Sporulation index 0-5; 0 = no sporulation, 1 =1 -20% germination; 2 = 20-50% germination; 3 = 50-70% germination; 4 = 70-100% complete germination; 5 = 100% complete germination with abundant mycelium.
* Values within a column followed by the same letter do not differ significantly (P=0.05) according to Tukey's multiple comparisons test.

Plants toxicity tests to tomato seeds

Bacteria at the high concentration 10^6 cells/ml were phytotoxic to tomato seedlings (Table 3). Tomato phytotoxicity was recorded in *P. oryzihabitans* sand treatments in high applied concentration (10^6 cells/ml). Growth of tomato seedlings was less in the *F. oxysporum* treatment (Table 3) confirming the pathogenic nature of the fungus.

Table 3. Toxicity of bacterial cells and cell-free culture filtrates to tomato seeds in sterile distilled water (SDW) and sand

Treatment	Seed radicle length (mm) in SDW	Shoot fresh weight (mg) in sand
P. oryzihabitans 10^6 cells/ml	0.06 a*	14.2 a
P. oryzihabitans 10^4 cells/ml	1.30 bc	317.5 b
F. oxysporum f.sp *lycopersici*	1.27 b	71.7 a
SDW	1.26 b	338.3 b
LSD $_{0.05}$	0.092	56

* Values within a column followed by the same letter do not differ significantly (P=0.05) according to Tukey's multiple comparisons test.

Association of *P. oryzihabitans* and *F. oxysporum* f.sp. *lycopersici* on tomato roots

There was a significant difference in the colonization of *P. oryzihabitans* on tomato roots grown in the presence and absence of *F. oxysporum* (Table 4). This indicates a direct and active interaction between *P. oryzihabitans*, root and the fungus.

Table 4. Bacterial density in the rhizosphere challenged with or without fungus mycelia

Treatment	Bacterial cells density log 10 cfu/g fresh root
P. oryzihabitans 10^4 cells/ml (applied alone)	3.52 ± 0.349 **
P. oryzihabitans 10^4 cells/ml (challenged with *F. oxysporum*)	4.82 ± 0.032 **

** Standard Deviation

Pseudomonas oryzihabitans isolated from roots produced (in dual cultures) fungistatic effects when challenged with *F. oxysporum* plugs.

Suppression of Fusarium wilt diseases in pot assays

Bacterial cells applied to soil significantly increased tomato shoot and root dry weights compared with the pathogen treatment (Table 5). The data suggested that the applied bacterial dose caused no phytotoxicity to the plants compared with the untreated controls (Table 5).

Table 5. The effect of application of *P. oryzihabitans* to soil infested with *F. oxysporum* f. sp. *lycopersici* on the growth of tomato.

Treatment	Shoot dry weight (mg)	Root dry weight (mg)
Untreated (C)	7960 b*	730 ab
(C) with *P. oryzihabitans*	7590 b	720 ab
F. oxysporum f. sp *lycopersici* (Fol)	1600 a	420 a
(Fol) with *P. oryzihabitans*	6830 b	1010 b
$LSD_{0.05}$	553	52

* Values within a column followed by the same letter do not differ significantly (P=0.05) according to Tukey's multiple comparisons test.

Impact of *Pseudomonas oryzihabitans* to *Meloidogyne* spp. in soil

The results show that the nematode's parasitic phase (females) and the non-parasitic phase (egg masses) were affected by the bacterium *P. oryzihabitans* (Table 6). However, there is no effect of the bacterium on egg production by *Meloidogyne* spp. (Table 6).

Table 6. *Meloidogyne* spp developmental stages in roots, after inoculation with or without *P. oryzihabitans* cells

	Nematodes in the roots	Nematodes on the roots	
Inoculum level	Females	Egg masses	Eggs per egg mass
No Bacteria applied	214 b*	121 b	245
P. oryzihabitans 10^4 cells/ml	68 a	62 a	238
$LSD_{0.05}$	59.8	47.4	Not significant

* Values within a column followed by the same letter do not differ significantly (P=0.05) according to Tukey's multiple comparisons test.

CONCLUSION

In this paper, we demonstrate *P. oryzihabitans* bio-activity against soil-borne pathogens and consequent beneficial effects on tomato plants. Soil-borne bacteria such as *Pseudomonas* spp. or *Bacillus* spp. have received particular scientific attention concerning their activity as bio-pesticides or plant growth promoters. Detailed studies on the activity of the bacteria associated with entomopathogenic nematodes in the absence of the nematode vector have not been previously conducted. These results provide evidence that bacteria isolated from entomopathogenic nematodes could have a broad-spectrum bio-activity in soil.

We believe that it is important to characterize the bio-products of *P. oryzihabitans* and other bacterial associates of entomopathogenic nematodes. Studies of their biosynthesis are necessary to understand further how such compounds might be exploited. Probably such research will be important in the discovery of new biopesticides.

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Potential of *Bacillus thuringiensis* subsp. *kurstaki* (3a 3b) on young larval instars of rice stem borer, *Chilo suppressalis*

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ABSTRACT

Bacillus thuringiensis is a biological control agent which is used for a wide range of crop pests in the world. *B. thuringiensis* subsp. *kurstaki* (3a 3b) is more effective than other serotypes in controlling lepidopteran pests. Rice stem borer is one of the most destructive pests in the north of Iran. In order to reduce insecticide application, *B. thuringiensis* was used in 1998-99 in the western Mazandaran rice fields. In this study, experimental design was a split plot in randomized complete blocks with 3 replications. For this purpose, five concentrations of *Bt* were used (0, 1500, 2000, 2500 and 3000 ppm) and assessments time were made 12, 24, 48, 72 and 96 h after spraying. Then in each experimental unit, 20 larvae (L_1 and L_2 instars) were randomly selected and effects of treatments on the mortality of stem borer evaluated. The analysis of variance showed that there was a significant differences between the different concentrations and the times. Least significant differences (LSD) showed that the concentration of 2000 ppm was the best and the time 48 h after spraying was the most effective time for bacterial influence, respectively.

INTRODUCTION

Rice, a valuable graminaceous plant and food resource for millions of people in the world, is the host for 100 pest species, most of international importance (Jafari,1980). Replacement of a great number of native rice varieties with a few modified varieties in north of Iran led to a reduction in genetic diversity in this crop and increased damage by harmful factors. Rice stem borer, *Chilo suppressalis*, is the key pest in north of Iran (Moussavi, 1979).

This pest was first reported in 1972 in Ramsar and then its prevalance in Amol and Gorgan cities and even other regions in Iran was reported (Ebert,1972). To control this pest, in addition to extensive applications of chemicals, other methods like biological control with *Bt* bacteria were investigated during 2000-2001 and their results were satisfactory (Olomi, et al., 1977).

Some attempts have recently been done to transfer *Bt* genes into rice plant (Karimi, 1999). This study was carried out along with reduction in insecticide usage. We hope that with the use of this study results, we can reduce the application of pesticides.

MATERIALS AND METHODS

Investigations related to study of efficiency of *Bt* subsp. *kurstaki* (3a 3b) to control first larval instars of rice stem borer, *Chilo suppressalis*, were carried out between 1998-99 in the research fields of Faculty of Agricultural Sciences of Shahed Uiversity located in the west of Mazandaran province of Iran. For this purpose, one experimental plot of about two thousands square meters which was infested with rice stem borer larvae, was choosen and experimental plot was designed.

The experimental design was a split plot in randomized complete blocks with 3 replicates (R_1, R_2 and R_3), each replicate included 5 plots (100 m^2). Native rice varities in the region (Dilamani) were used for transplanting. On the basis of a previous study of pest biology in the region, 50% egg hatch was selected as the optimum timing for bacterial spraying. Rice stem borer has three generations in the region, with the second generation coinciding with the sensitive period of the rice plant (ear period) (Jafari, 1980). The application of the bacterium was used against young larval instars (L_1 and L_2 instars) before they enter the stem and the formulation used was Bactospeine. In this experiment, 5 concentrations of bacterium were used (0, 1500, 2000, 2500 and 3000 ppm) covering the maximum and minimum recommended rates and assessments were made 12, 24, 48, 72 and 96 h after spraying.

Then from each experimental plot and each replicate, 20 larvae were choosen at random and the effects of *Bt* on their mortality was studied. A quadrat sampling method was used (100 x 100 cm^2) randomly was used in four places in each plot. After spraying, infected larvae were transferred to the laboratory. Diseased larvae showed symptoms including color change, but, for better identification, the electron microscope was used to study the effect *Bt* on the epithelial cells of larval midgut.

RESULTS

Main damage of rice stem borer is related to different larval instars 3, 4 and 5, which enter the stem and feed from the internal contents of the stem and cut off the plant sap, destroying the plant. The first larval instars, until the end of second larval instar, live outside the stem, because their small size and mouthparts cannot cause sufficient damage to rice plant.

Table 1. The study of *B.t.* effect on the basis of concentration and assessment time on young larval instars (L_1 and L_2 instars) of stem borer

Concentration (ppm)	Number of larvae	assessment time (h) (mortality after *Bt* spraying)					mortality (%)
		12	24	48	72	96	
0	60	2	1	2	12	2	2.6
1500	60	11	14	17	1	28	30.1
2000	60	19	28	49	52	56	68.06
2500	60	21	29	53	57	54	70.1
3000	60	24	31	53	57	58	71.3

The analysis variance showed that there was a significant difference between different concentrations and assessment times and also in the interaction effect of concentration and time (Table1 and 2). Therefore, concentration factors and assessment timings were not independent and do interact on each other. In this respect, the average concentration and assessment time on basis of LSD method were compared (Table 2, 3 and 4).

Table 2. Analysis variance of obtained data of *Bt* concenteration and assessment time effects on young larval instars of rice stem borer.

Sources of variation	DF	MS
Replication	2	2.191 NS
Concenteration (A)	4	607.755*
Main error	8	0.334
Time (B)	4	143.176*
Sub main error	32	0.441
AB	16	15.548*
Total error	74	3279.106

*: Significant at 0.01 level NS: Not significant

Table 3. Comparison between average of different concentrations of bacterium against young larval instars of rice stem borer LSD method.

Treatment (concentration, ppm)	Mortality (%) (No. of larvae per 60)
0	2.6 c
1500	32.12 b
2000	79.6 a
2500	80.01 a
3000	81.3 a

Table 4. Comparison between assessment time average after *Bt* spraying against young larval instar of rice stem borer LSD method (at concentration 2000 ppm)

Treatment (assessment time)	Mortality (%) (No. of larvae per 60)
12	1.36 c
24	25.95 b
48	79.02 a
72	79.85 a
96	81.15 a

These results (Table 2 and 3) showed that there is no significant difference between concentrations 3, 4 and 5, but the difference was significant between the control and other concentrations. The results also showed that there is a significant difference between concentrations 3, 4 and 5 and the control and concentration 2, but no significant difference between concentrations 3, 4 and 5. The results (Table 2, 4 and Figure 1) showed that there is no significant difference between assessment time 3, 4 and 5, but between times 1 and 2 and times 3, 4 and 5, the difference was significant. Therefore, with regard to the same effect of time 3, 4 and 5, we can say that the best time for sampling to monitor the maximum effect of *Bt* spraying is time 3 (48 h after spraying) and after this time the maximum rate of mortality in the first larval instar population had occurred and there is no need for more time (Table 4 and Figure 1).

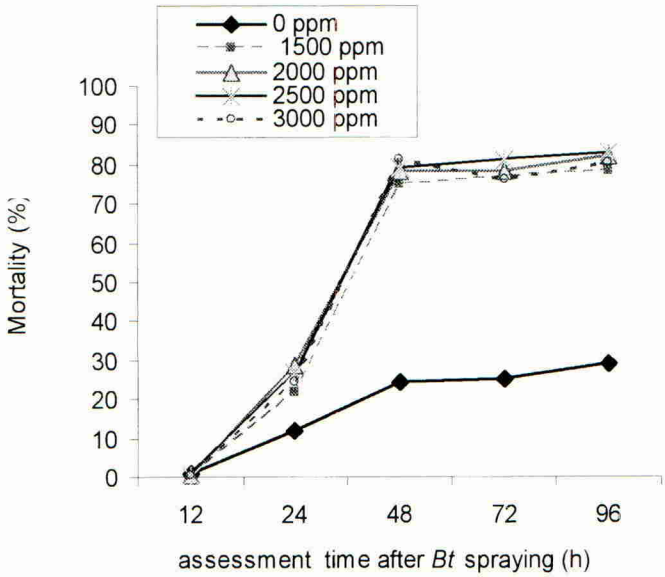

Figure 1. Effect of different concentrations and assessment time on the basis of mortality percent of rice stem borer larvae.

DISCUSSION

Previous studies have shown that the second generation of rice stem borer, *Chilo suppressalis* occurs at the sensitive period of rice (ear stage) and it can cause severe damage if control methods are not taken (Ebert, 1972). In this study, in order to reduce insecticide usage, *Bt* subsp. *kurstaki* bacterium, which is specifically active on lepidopteran larvae was used. *Bt* bacterium has numerous strains of which subsp. *kurstaki* (3a 3b), is specific for lepidopterans (Burges, 1992). After spraying the bacterium on the foliage, the rice borer larvae will consume these contaminated leaves and the protoxin will

enter the larval body and penetrate into the digestive organ. After peneterating the epithelial cells of the larval midgut (pH >9), the protoxin is activated and the insecticidal delta-endotoxin is released. From electron microscope photography of epithelial cells of rice stem borer larval midgut, we have observed that this bacterium is effective in binding to and destroying the epithelial cells in the midgut of rice stem borer larvae. The results showed that *Bt* bacterium can be used against young larval instars of rice stem borer. Larger rice stem borer larvae at the end of second instar, however, will enter the stem, thereby limiting the effectiveness of the *Bt* bacterium to the first and second larval instars. In this respect, exact information about rice stem borer biology in the period for bacterium spraying is necessary. To ensure effective use of the bacterial sprays, it is essential to use a monitoring method (sampling adult numbers with a light trap) and to apply the spray when 50% of the insect eggs have hatched.

If the control method is used at exactly the correct time and on the basis of integrated pest management (IPM) principals, such that control is targeted against first larval instar which do no damage to the crop, the control of first larval instars is more effective. First instar larvae, compared to last instar larvae are more sensitive to the bacterium and so a lower dose and less time is needed for larval control (Moazami, 1989). With respect to the above information the use of the bacterium can be recommended against first and second rice stem borer larval stages. In this study, results showed that concentration 3 (2000 ppm) was the best concentration and the assessment time 3 (48 h after spraying) was the best time to monitor the effect of the bacterium on the rice stem borer larvae. Also the result showed that, if the proper application is used, about of 80% first instar larvae can be controlled with *Bt* bacterium. With this regard, bacterium application in comparision with other insecticides has low risk on the environment and natural enemies, but gives the same level of control.

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