Effective Microorganisms for Control Of Xanthomonas Campestris pv Vesicatoria in Sweet Pepper

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Abstract

A field study was conducted to determine the potential use of Effective Microorganisms (EM) for control of *Xanthomonas campestris* pv *vesicatoria*, a bacterial pathogen of sweet pepper (*Capsicum annum* cv *margareth*). Sweet pepper seedlings were transplanted into field plots where soil had been treated with EM, a biofertilizer (Vairo), or left untreated as controls. After 60 days, a suspension of 10^8 CFU ml^{-1} of *Xanthomonas campestris* pv *vesicatoria* was sprayed over the plants. Disease incidence and the number of bacteria were evaluated. The results indicate that both EM and Vairo (a biofertilizer product derived from anaerobic fermentation of cow manure) may have potential for the control of *Xanthomonas campestris* pv *vesicatoria* in sweet pepper.

Introduction

Xanthomonas campestris, a phyiopathogenic bacteria, can cause severe leaf shedding and decreased yields in sweet pepper crops. Control of this organism was observed during the growth period following the application of Vairo, a biofertilizer derived from anaerobic fermentation of cow manure (Santos, 1992) and also with Effective Microorganisms (EM), a microbial inoculant comprised of actinomycetes, photosynthetic bacteria, lactic acid bacteria and yeasts (Higa, 1994).

Materials and Methods

Pepper seedlings (*Capsicum annum* cv *ma,gareth*) were started in a greenhouse and then transplanted into field plots. Four treatments were included in the study:

1. *EM Treatment.*
   Green manure (grass) was applied to the soil with 100 g m^{-2} of bokashi (a fermented mixture of rice bran, soybean cake, oil cake and fish meal inoculated with EM). The green manure was plowed into the soil and sprayed with a 1:300 dilution of EM 4 at 1 liter m^{-2}. After transplanting, two weekly sprayings of EM 4 at 1:1000 dilution and a 20 g side-dressing of bokashi were applied every 30 days.
2. *Biofertilizer Treatment.*
   Undiluted biofertilizer (Vairo) at 1 liter m^{-2} was added to the green manure and incorporated by plowing. After transplanting, pepper plants were sprayed weekly with a 1:5 dilution of the biofertilizer; each month, a 1:2 dilution of the biofertilizer was applied.
3. *Fungicide Treatment.*
   Chemical fertilizer and water were applied along with copper oxychloride plus the fungicide Maneb (Peprosam B) at 200 g 100 $Iiter^{-1}$ of water
4. *Fertilizer Control.*
   Chemical fertilizer (N:P:K at a ratio of 10:10:10) was applied to control plants during soil preparation at 100 g m^{-2} along with 107 g of urea per plot based on the soil chemical analysis. Water was sprayed weekly on the leaves as in the other treatments.

Each plot was 2 x 8 m; 30 seedlings were planted with 50 cm between them in a strip experimental design without replication because of the difficulty in controlling inoculum dispersion and interference between the plots. Sixty days after transplanting, the plants were inoculated with 10^8 CFU ml^{-1} of a pure culture of *X. campestris* pv *vesicatoria*; some leaves of each plant were mechanically injured. Inoculation was followed by spray irrigation for 48 hours. Bacterial quantification in the leaves was determined on a weekly basis beginning one month after inoculation. Control plants were examined to determine which leaf had the most uniform symptoms in all plants, and then a corresponding leaf was collected from plants in the remaining treatments.

Three 1 cm^2 cork-borer samples were collected from each leaf for a total of 24 per treatment, shaken for 15 minutes in 20 ml of 8.5 percent salt solution and centrifuged at 7500 x gravity for 10 minutes. After discarding the supernatant, the residue was suspended in 2 ml of the salt solution, and serially diluted to 10^{-8}. From each dilution, 0.1 ml was added to petri dishes with DYGS culture medium and incubated at 30°C in a BOD chamber. Colony forming units (CFU) were counted after 48 hours.

For a quantitative disease evaluation, two samples were examined 5 months after seedlings were transplanted into the field. For the first, the number of healthy and diseased leaves was counted on the 10 most vegetatively-developed plants; minimal leaf size was 5 cm. For the second, two plants were chosen for a disease index evaluation from among the 10 previously selected plants (Wheeler, 1969) according to the following ratings:

1. healthy leaf;
2. one bacterial pustule per leaf and/or micro-pustules;
3. up to 5 bacterial pustules, and/or micro-pustules;
4. from 5 to 10 bacterial pustules per leaf;
5. more than 10 bacterial pustules per leaf, leaves with burned edges, deformed leaves.

Disease index was calculated according to the formula:

DI = [(number of diseased leaves x ratings) x 100] + [total number of diseased leaves x 4].

Results and Discussion

Colony forming units (CFU) per cm^2 of diseased leaf in each treatment were: EM, 0.5 x 10^8; biofertilizer, 0.4 x 10^8; fungicide, 0.2 x 10^8. control, 3.5 x 10^8. Similar results were also observed in *in vitro* tests with other phytopathogens.

The total number of leaves per plant was lower for the control treatment and was significantly different from the other treatments; this indicated an interaction with the degree of bacterial infection (Table 1). However, the calculated disease index was not different (DI = 30.9) for the control plants since the shed, heavily diseased leaves were not counted. Early leaf shedding is a major concern since it affects the number, size and quality of the fruit, and may make the fruit unsuitable for market.

Table 1. Effect of Various Treatments on the Mean Number of Leaves Per Plant and Disease Index of Pepper With Bacterial Pustules Following the Inoculation of Plants with *Xanthomonas campestris* pv *vesicatoria.*

Treatment	Leaves per Plant (No.)	Disease Index (%)
EM	214.8a	24.9
Biofertilizer	229.5a	33
Fungicide	231.9a	28.8
Control	158.1b	30.9

Mean values with the same letter are not significantly different at the 5% level of probability.

The EM treatment had the fewest leaves at the highest disease level and the lowest disease index. For the biofertilizer treatment, more of the infected leaves had the highest level of infection; however, those leaves remained on the plant.

There were no statistically significant differences in the number of leaves among the EM, biofertilizer and the chemical fertilizer treatments; also there were no significant differences in the disease index. However, the EM treatment consistently had the fewest leaves at the highest level of infection. The cupric fungicide treatment resulted in a large number of leaves with micro-pustules. With increasing humidity, micro-pustules coalesce and rapidly reach the highest level of disease; however, the number of bacteria was not different from those found with the EM and biofertilizer treatments. Only the control had a bacterial population of 20 times greater than the cupric fungicide treatment.

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