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### ANTIMICROBIAL ACTIVITY OF *Capsicum* EXTRACTS AGAINST SOME PATHOGENIC BACTERIA

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Food-borne diseases are important worldwide, since the increase of international trade of commodities and food products has raised the risk of dispersion of pathogenic bacteria, from production sites to far away places of consumption.

On the other hand, consumers demand safer foods, containing less chemicals, which make us consider the study of natural antimicrobials (Beuchat and Golden, 1989). Serruti and Alzamora in 1996 showed that vanillin (one of the capsaicin analogues) inhibits the growth of yeast. López-Malo et al. (1998) observed the same effect when using moulds. Also Kim and Ryeon (1979) reported antibacterial effects of capsaicin from Korean hot peppers on *Bacillus subtilis*, *Bacillus cereus*, and *Sarcina lutea*. Given the above, the purpose of the present work was to know whether three *Capsicum* extracts had an inhibitory effect on four pathogenic bacteria, and then to validate such effects on an actual food, in this case the food being minced beef meat.

Extracts from habanero, serrano, and pimiento peppers were prepared. 20 µL of each extract were placed on sterile filter paper disks and set on plates containing the bacteria, then incubated at 37°C for 48 h.

The *Capsicum* extracts inhibited the growth of *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella typhimurium* and *Bacillus cereus*.

The pimiento extract showed a stronger antibacterial activity than the serrano extract, and this one in turn was stronger than the habanero.

Challenge tests were performed for *Salmonella's* growth in minced beef meat added with pimiento extract. Stain of *S. typhimurium* (ATCC 14028) was obtained from the culture collection of the Microbiology Department, ENCB-IPN, Mexico City and maintained at 4°C in Mueller-Hinton agar. The identity of the strain was confirmed. Post-rigor lean beef muscles were obtained from a local market in Mexico City. Each piece was immersed in boiling water for 3 min in order to reduce the microorganisms attached to the surface of the muscle. The bacterium was inoculated into the meat previously mixed with different concentrations of the extract. The concentrations were: 0.02, 0.06, 0.1, 0.3, 0.5, 0.75, 1, 1.25, 1.5, and 2.5 mL/100g of beef. Then the samples were stored at 7°C for 7 days. The combined effect of the *Capsicum* extract and sodium chloride on the bacterial growth was also evaluated. The minimum inhibitory concentration of the extract required to prevent the growth of *S. typhimurium* in minced beef was 1.5 mL/100 g of meat. The addition of 1, 2, 3 and 4% w/w of sodium chloride did not have any additional inhibitory effect on *Salmonella*.

The results obtained from this study support the idea of proposing the use of *Capsicum annum* extract as a natural antibacterial agent in a food such as raw beef, which is often contaminated.

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### CONTROL OF THE DISEASE INCIDENCE OF PLANT ROOT ON RED CHILE, BY PRACTICES OF DRIP IRRIGATION

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**INTRODUCTION.** Zacatecas Mexico is the leading state in the production of red chile, where the rain is infrequent during the growing season and the farmers use mainly furrows and few drip irrigation systems. Most of the production is carried out with pumping irrigation, and the mean production is about 1.6 ton/ha. In the last 5 years the loss of crops were increased by the root rot, either furrows and drip irrigation; caused by a group of fungal pathogens. Only in 1999, the loss ones ascended to 400 million pesos. The principal causal agent of chile root rot disease has not been well defined. Rincon (1999) found, under field condition, greater activity of *Phytophthora* spp in red chile plants, but he found the presence of *Rhizoctonia* spp and *Fusarium* spp, too. In general, high frequency irrigation with drip and furrow irrigation systems enhanced root rot in bell pepper (Ristiano 1991, and Cafe-Filho and Duniway 1996). Moreover, long periods of saturated soil are well known to increase the incidence and severity of plant chile diseases (Bowers and Mitchell 1990). Besides yield of green peppers from plots given alternate-row irrigation was significantly higher than plots given every-row irrigation, besides the disease progress of root rot appears to correlate with the first rains of the growing season (Biles et al 1992). And it is likely that the placement of drip emitter near plant stems allow more numerous infections at vital part of the root; consequently control of the disease in the field is achieved, at least partially, by the maintenance of low soil moisture (Cafe-Filho and Duniway 1996). Stolzy et al. (1985) suggested that the zoospores of *Phytophthora* spp. require water-filled pores to move through soil. While soil moisture is certainly important, there have been no attempts to examine, under field condition, the effects of different soil moisture levels and the incidence of root rot. The main objective of this study was to compare soil moisture content in regard to disease incidence of root rot and yield of red chile, under field condition. And generate some practice for drip irrigation.

**MATERIALS AND METHODS.** In this study the symptoms of disease incidence of plant root of red chile are similar to *Phytophthora* root rot. A field experiment was conducted out in the CEZAC-INIFAP in the 2000, in a soil with antecedents of



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contamination for fungal pathogens responsible of the disease of red chile plants. And a Criollo plant of red chile (Ancho Zacatecas) was used which is very susceptible to the root rot disease. It was planted April 27. Experimental design was randomized complete block with three replicates and four treatments. Each treatment had 72 plants of red chile. The amount of water to each drip treatment was varying from 60, 80, 100 y 120 % of the pan evaporation, obtained from a climatologic station. Drip irrigation was used with a emitter spacing of 30 cm and a rate of 3.0 l/h/m. The amount of water applied was measured by a flow meter. Soil water status was measured with TDR (Model ) weekly. Fertilization was as followed 220 kg/ha (N), 100 kg/ha (P<sub>2</sub>O<sub>5</sub>) and 150 kg/ha (K<sub>2</sub>O<sub>5</sub>). The numbers of diseased plants per plot were counted weekly and yields of red chile were measured at harvest.

**RESULTS.** The red chile plants were infested by the fungal pathogens, as a result of the high regimens of residual soil moisture during the growing season . For instance, the plots given 120% pan evaporation the residual soil moisture on July 28 was higher than field capacity point 21 cm<sup>3</sup>/cm<sup>3</sup> (Figure 1) . At the end of the season, red chile plots given 60 y 80% of the pan evaporation had significantly fewer plant diseases than plots given 100 and 120% (Table 1). The incidence of the disease in plots given 60% was 26.7, in 80% 27.2, in 100% 28.2 and in 120% 37.5 (Table 1). Yield of dry red chile was highest in plots given 80 and 100% of pan evaporation than plots given 60 and 120% (Table 1). On the other hand, eleven times were collected the number of plant diseases, during the season, of each treatment, in Figure 2 are shown the number of plant diseases and the 60, 80, 100 and 120% of pan evaporation. In this figure the time variable was replaced for the Heat Degree Days, which was calculated as followed:

$$GDC = \sum_{n=1}^n \left( \frac{T_{max} - T_{min}}{2} \right) \cdot T_b$$

where Tmax is maximum temperature, Tmin is minimum temperature and Tb is base temperature in this case 5°C. Disease incidence was compared statistically using an analysis of variance procedure. Repeated analysis of variance was used for data collected sequentially. Significant statistical differences were found for 8 data collected for comparing the effect of four rates of pan evaporation on disease incidences, whereas insignificant differences on disease incidences were found for the rest of data collected.

**DISCUSSION.** The data of the effects of the soil moisture conditions on the disease incidence are very clear. The number of plant diseases was influenced by the different rates of pan evaporation. Others investigators also have shown an increase in disease with high frequent irrigation (Bowers and Mitchell 1990, Cafe-Filho and Duniway 1996 and Biles et al 1992). For instance, plots given 120% of the pan evaporation the residual soil moisture was very high (saturation point) during the growing season, evidently in these plots the soil pores were full (Figure 1) of water, allowed the pathogens to promote root infection as high as 52% of sick plants at the end of the season. As Stolsy et al (1965) was observed, more over Bowers and Mitchell (1990) showed that periodic saturation of soil increased disease incidence of bell pepper. On the other hand, the plots that received less water during the season reduced their numbers of sick plants. This is very important, for example if we consider the density of plants of a hectare (45,000 plants) and if we apply the irrigation with the approach of 120% of the pan evaporation, we will have that a decreased of unproductive plants of 23,850. This data suggest that low soil moisture levels, applying 60 o 80% of pan evaporation, may be effective technique for management of root rot of red chile.

It is observed in this figure 2 that the biggest incidence of plant diseases were registered between 400 to 900 units of heat (Figure 2) (40 to 50 days after planting), this is due to the conditions of soil moisture, like it was discussed up and also to the precipitation conditions that prevailed mainly at the beginning of the season. During this same period of time were registered 152.4 mm of precipitation of a total of 239.7. These two conditions caused that the levels of residual soil moisture registered were very superior to the value of the field capacity (Figure 1). It is in this period where the biggest disease incidence of plants is presented, with a total of 17, for the treatment 120% pan evaporation, that is to say 42% of the total of sick plants.

The results found in this work, support the asseveration that high soil moisture increases plant disease incidence. Also and very important, the speed of the incidence of plant diseases is seriously increased with the presence of precipitations. The number of plant diseases can be diminished applying only volumes of water to restore to soil field capacity. In soils contaminated by these fungals, with drip irrigation it is recommended to apply volumes of water with t60% of pan evaporation.

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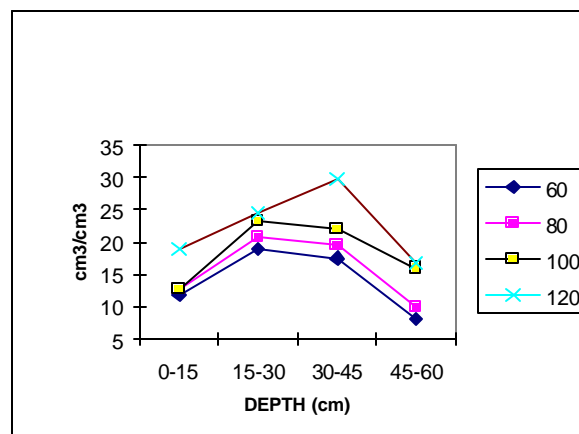


Figure 1. Soil moisture content cm<sup>3</sup>/cm<sup>3</sup>, measured with TDR, on July 28 for the different treatments.



Table 1. Means of disease incidence of root rot on red chile and yield, in the field.

Treatment (%)	Number of plant diseases	Yield (T/ha)
60	26.7 a	2.030 a
80	27.2 a	2.443 b
100	28.2 a	2.671 b
120	37.5 ab	2.164 a

Numbers in the same column followed by the same letter are not significantly different ( $p=0.05$ ) according to the Duncan test.

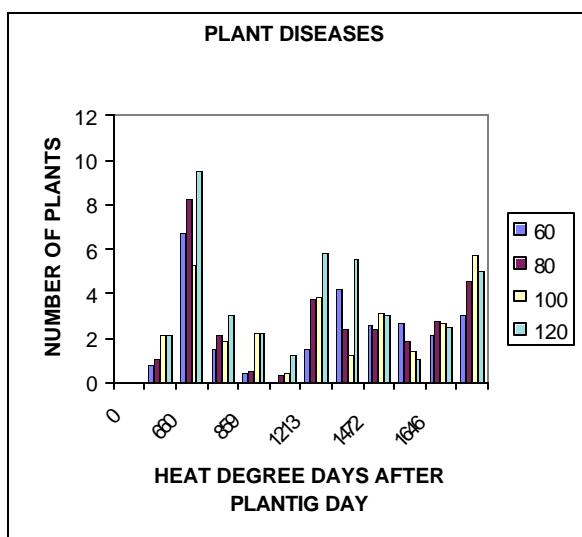


Figure 2. Disease incidence of root rot of red chile in rates of pan evaporation.

#### BACTERIAL SPOT RESISTANCE, YIELD, AND QUALITY OF BELL AND SPECIALTY PEPPERS

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After completing a three-year study of bell pepper cultivars under induced bacterial spot (*Xanthomonas campestris* pv. *vesicatoria* or *Xcv*) and bacterial spot-free environments, we began a new series of trials in 2000 to compare new cultivars with previously recommended cultivars that were either highly resistant ('Boynton Bell') and/or that had very attractive fruits ('X3R Wizard'). We also screened a large number of hot and specialty peppers, some of which also carry the *Bs2* gene for bacterial spot resistance. Near-duplicate trials were planted in Lexington (LEX), Kentucky and at an isolated location in eastern Kentucky (QSND). Plots at both locations consisted of 16 plants in double rows with four replications in a randomized complete block design for bells and in single plots for hot and specialty peppers. All were planted on raised beds with black plastic mulch and drip irrigation. No

preventive fungicide treatments were applied at QSND in order to encourage the development of a natural bacterial spot epidemic. Hot and specialty peppers included a group of 13 jalapeño cultivars of which two had the *Bs2* resistance gene (>X3R Ixtapa= and >El Rey=) and others claiming multiple virus resistance. These were compared with >Milla=. Other pepper types included three serrano cultivars, six anaheim cultivars, seven poblano/ancho cultivars (entry SVR 35-4845-7 had the *Bs2* gene), four Italian/cubanelle cultivars, four hot banana/wax cultivars (X3R Hot Spot and SVR 35-4846-7 with *Bs2* gene), six sweet banana/wax cultivars (>Pageant=, >Sweet Spot=, and PX 35-4360-7 with *Bs2* gene), two fresno cultivars, and two pepperoncini cultivars.

Although yields were somewhat lower than in 2000, most of the bell pepper cultivars were high yielding (20-25 tons/acre) at LEX with 9 that were not significantly different from the top yielding cultivar >X3R Aristotle=. >Aristotle=, >King Arthur= (bacterial spot susceptible), >4 Star=, >Boynton Bell=, and >Lexington= were also in this category in the 2000 LEX trial. There were no statistically significant differences among cultivars for total marketable yields or gross incomes at QSND. Marketable yields ranged from 13 to 18 tons per acre. Some of the highest yielding cultivars at QSND were also in the highest yielding group of varieties tested at LEX: >4 Star=, >X3R Aristotle=, >X3R Red Knight=. Yields at this location appeared to have been affected by the early bacterial spot epidemic.

Most jalapeño cultivars had high marketable yields at LEX ranging from 14 to 27 tons per acre with three cultivars exceeding >Milla=. Among these >Coyame=, >Summer Heat 6000', and RPP 7042-VP had the most attractive fruits. Marketable yields for the three serrano cultivars at LEX ranged from 15 to 22 tons per acre with >Tuxtlas= and >Serrano del Sol= having the highest yields and most attractive fruits. >Tuxtlas= was also the highest yielding and most attractive serrano in 2000.

Yields of the six anaheim cultivars ranged from 15 to 31 tons per acre at LEX; >Navojoa= was the highest yielding while PX-35-4606-7 and >Anaheim TMR 23' had the most attractive fruits. >Navojoa= was also highest yielding at QSND in spite of severe BLS symptoms early in the season. Yields among the seven poblano cultivars at LEX ranged from 4 to 21 tons per acre. >Ancho Villa= was again (as in 2000) the highest yielding with the largest fruit size. The only entry with the *Bs2* gene for resistance to bacterial spot (SVR 35-4845-7) was high yielding and had the highest appearance rating at LEX. Most poblano/ancho cultivars are quite susceptible to bacterial spot and yields at QSND may have been affected by the early epidemic at this location. >Mulato Isleno= had very low yields at both locations. Yields for the four Italian/cubanelle or frying peppers ranged from 17 to 28 tons per acre at LEX. >Aruba= had the highest yield and largest fruit size followed by >ACX 500'. As in 2000, >Corno di Toro= was considered to have the most attractive fruits although they were light to medium green in color instead of the typical light green or pale yellow. >Key West=, a new cultivar with the resistance to bacterial spot, appeared to be unaffected by the early epidemic at QSND. Two hot banana cultivars and >Santa Fe Grande= were tested. >X3R Hot Spot= (with the *Bs2* gene) had the highest marketable yield and good appearance ratings at LEX (26 tons/acre). Both >Inferno= and >Santa Fe Grande= had severe symptoms of bacterial spot associated with the early epidemic at QSND. The six sweet banana or sweet wax cultivars included two with the *Bs2* gene (>Pageant= and PX 35-4360-7); yields at LEX ranged from 21 to 32 tons per acre. PX 35-4360-7 was the highest yielding entry at both locations and had the most attractive fruits.



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Most cultivars had many >C=- or apostrophe-shaped fruits. >Market Sweet= was high yielding at LEX but exhibited severe BLS symptoms during the brief epidemic at QSND.

had declined. Only a few very small clusters of 1-3 ECB eggs were found in the pepper plots towards the end of the growing season. Larger replicated trials are being conducted in 2002.

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## BIOLOGICAL CONTROL OF EUROPEAN CORN BORER IN PEPPERS

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Integrated pest management practices have aided farmers in reducing the amount of pesticides used for insect control. Biological control is an often overlooked component of IPM that is growing in importance and popularity, especially among organic growers. An egg parasite originating from China, *Trichogramma ostriniae*, has proven to be an effective aid in the control of European corn borer (ECB, *Ostrinia nubilalis*) in trials with sweet corn in the Northeastern United States. ECB is the most serious insect pest of peppers in Kentucky and several other southeastern states. Pepper crops are often damaged by second generation ECB larvae in the middle to later part of the growing season. Nothing is known about *T. ostriniae*'s effectiveness in controlling ECB in peppers, and to our knowledge this species has not been previously evaluated for its effectiveness in peppers. In a preliminary trial, we released *T. ostriniae* in small, unreplicated plots in 2001 in order to get an idea of its potential for ECB control in bell peppers and to learn scouting and other procedures in order to better plan replicated trials in 2002.

Two bacterial spot-resistant bell pepper cultivars, >Early Sunation= and >Defiance=, were planted in each of two 50 ft by 50 ft plots which were prepared at separate sites at the University of Kentucky Horticultural Research Farm in Lexington, Kentucky. One of these identical plots was designated the release plot and the other the control plot (no *T. ostriniae* released). The plots were approximately 300 yards apart, with fields of other crops, a gravel parking lot, and a building between them. The release plot was located downwind from the control plot. Thirty thousand *T. ostriniae*-parasitized *Ephesia* eggs (glued inside two paper cups, each with 15,000 parasitized eggs) were obtained from Cornell University and placed in the release plot on July 11. This was the date of the first flight of second generation ECB moths for Lexington as predicted by the University of Kentucky ECB degree day model. That flight turned out to be very light and we decided to obtain and release a second batch of 30,000 *T. o.* on 18 July in the same field. Paper cups containing *T. o.* had been folded and stapled shut in order to protect against predators and exposure; numerous pinholes had been made in the cups to allow the parasites to emerge. Releases were simply a matter of hanging the two cups under the leaf canopy of a plant in the center of the plot. Plots were scouted twice weekly and the number of parasitized and unparasitized ECB egg masses recorded. Once eggs were located and their status recorded, leaves with eggs were flagged with a plastic marking ribbon and given a number. These egg masses were visited twice weekly and their condition recorded until hatching or their disappearance.

More than double the number and weight of ECB-damaged fruits occurred in the control plot than in the release plot. The release plot yields were slightly higher than control plot yields. Many *T. ostriniae*-parasitized ECB egg masses were also found in an adjacent sweet corn trial after ECB egg numbers in the pepper plot

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## THE EFFECT OF SOME HERBICIDES ON PEPPER HOST-VIRUS RELATIONS

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Plant viruses make up about 15-30% out of the whole plant diseases (plant physiological, genetical, caused by microorganisms). Chemical protection against viruses is unsuccessful *in vivo* or causes not only the death of viruses but also the death of the host. In spite of this, several natural and artificial substances are known to inhibit the spreading and replication of chemical plant protection is the major part of the integrated plant protection and that 50% of the pesticide sales is made up by herbicides, from practical point of view it is important to know the side effect of herbicides, including also the effect on host-virus relation. The best known in this respect is the antiviral activity of some triazines, carbamide, dinitroaniline and auxine-type herbicides. Virus diseases are one of the major limiting factors in successful pepper cultivation. The extent of infection varies between 20 and 60%, and 5-40% yield losses may occur due to virus infection. Pepper is known as natural host of ten economically important viruses in Hungary.

Greenhouse experiments were carried out to examine the effect of some commonly used herbicides in pepper (STOMP 330, DEVRINOL 45F, FUSILADE S) on host [(*Capsicum annuum* L.)(Csipke, Belecskai, Szentesi piacok, Macskapiros cultivars), *Solanum nigrum*] – tomato mosaic tobamovirus (ToMV-Ob) relations. DEVRINOL 45F and STOMP 330 were applied as preplant treatments one week before planting. FUSILADES and STOMP 330 were used also as postemergent ones mixed to the tissue sap of *Nicotiana tabacum* 'Samsun' at the time of virus infection. It has been concluded that the inhibitory effect of herbicides on ToMV-Ob greatly depends on host (species, varieties), type of herbicide, mode and dosage of application. Our results pay attention to the fact that certain herbicides may play important role not only against weeds, but also have inhibitory effect on economically important viruses, occurring on cultivated plants and also on weeds in agricultural ecosystems.

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