v. 4, n. 7, 2024

International Journal of Biological and Natural Sciences

Acceptance date: 16/10/2024

EFFICIENCY OF THE MICROTUNNEL AND BIOLOGICAL PRODUCTS IN THE PRODUCTION OF POTATO TUBERS (*Solanum tuberosum* L.) FOR SEED

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Abstract: Potato purple top (PMP) is one of the main phytosanitary problems of potato in Mexico and some other producing regions in the world. The main causal agents of the disease are considered to be phytoplasmas and the bacterium *Candidatus* Liberibacter solanacearum (CaLso), which are pathogens located in the phloem of the host. Leafhoppers are considered as the vectors of phytoplasmas and the psyllid *Bactericera cockerelli* as the vector of CaLso. Losses caused by PMP can reach 100% as it reduces yields as well as the quality of the tuber produced. Currently, the management and control of the disease is done by chemical control of the vector with massive applications of pesticides with a great adverse effect on the environment and human health. For this reason, it is considered necessary to generate alternatives to obtain a profitable and sustainable production for the producer, such as the use of resistant or tolerant varieties to the disease, as well as the use of biological products and cultural measures that greatly reduce the use of pesticide applications. The objective of this study was to evaluate the efficiency of the microtunnel and the use of biological products to produce seed-quality tubers. Five treatments were evaluated, two consisted of the use of microtunnels with and without the application of biological products for the control of soil pests and pathogens, two treatments were open field with and without the application of biological products and one treatment was open field without the application of any biological product. The treatments with the use of microtunnels were efficient in producing tubers with 100% sprouting. However, its use as seed for commercial sowing is not recommended if a small percentage of CaLso infection is detected, since it constitutes the primary inoculum for infection in the entire crop, but it is feasible to use it in the production of potatoes for self-consumption. The biological products used have no effect in reducing the percentage and intensity of tuber browning caused by CaLso.

Keywords: PMP, Microtunnel, *Trichoderma*, *Rhizophagus*

INTRODUCTION

Potato purple top (PMP) is one of the main phytosanitary problems affecting potato cultivation in Mexico (Rubio *et al*., 2006) and is also of great importance in several regions of New Zealand (Liefting *et al*., 2008), United States of America and Central America (Munyaneza *et al*., 2007; Munyaneza *et al*., 2008; Secor *et al*., 2009). The most affected regions in Mexico are Central Mexico (Edo. de México, Puebla, Tlaxcala, Hidalgo, Veracruz), Northeast (Coahuila and Nuevo León) and Bajío (Guanajuato and Michoacán). The Northwest region (Sonora, Sinaloa, Baja California, Chihuahua) and the area of Tapalpa, Jal. are affected to a lesser degree than the previous ones; however, the problem has been increasing (Rubio *et al*., 2011a). Phytoplasmas (Almeyda *et al*., 1999, Cadena *et al*., 2003) and *Candidatus* Liberibacter solanacearum (CaLso) (Caixedo *et al*., 2020), which are obligate pathogens located in the phloem of the plant and are transmitted by insect vectors, are reported as causal agents of PMP. In addition, the transmission of these pathogens also occurs through the use of infected tubers as seed (Caixedo *et al*., 2020; Cuesta *et al*., 2021). Phytoplasmas are associated with the presence of leafhoppers and leafhoppers, for the case of CaLso, *Bactericera cockerelli,* the potato psyllid, is reported as a vector (Cuesta *et al*., 2021). In a study conducted in Mexico (Rubio, *et al*., 2011b), a close association was obtained between the population of *B. cockerelli* and the incidence of PMP and it was also observed that 54% of tubers with disease symptoms were positive for *Ca. Liberibacter bacteria* and only 3.5% were positive to phytoplasmas, which supports the reports of other researchers (Hansen *et al*., 2008; Liefting *et al*., 2008; Venkatesan *et*

al., 2010) and confirms that the main causal agent of PMP in Mexico, as in other countries, is the bacterium *Candidatus* Liberibacter solanacearum.

Diseased plants show abnormal development, some show dwarfism, in others the branches or stems protrude, the upper leaves curl, turn yellow or purple, there is a thickening of the stem nodes, the distance between stem nodes is shortened, the stem grows in a zigzag pattern, aerial tubers are formed and the plant may die early (Cuesta *et al*., 2021). When dealing with striped potato, tubers show light brown striations in the flesh that are formed due to an alteration in the concentration of sugars and become more evident when the tubers are fried, so they are not useful for the production of fried flakes, which is the main form of potato processing in Mexico (Rubio *et al*., 2013). Tubers used as seed generally do not sprout and if they do, they have very elongated or tapered sprouts, as a result yields decrease significantly and tuber size is reduced. Depending on the stage of development at which the plants are infected, tuber yields can decrease between 10 and 100% and, as a consequence, economic losses are very high, considering that production costs average $100,000/ha (Rubio *et al*., 2013).

The vector of CaLso is the psyllid *Bactericera cockerelli*, which is a sucking insect that feeds on the sap of the plants it attacks. When feeding, both adults and nymphs inject a toxin that causes yellowing and curling of leaves and also transmits the bacterium that the psyllid carries inside its body (Cuesta *et al*., 2021). Currently, the control of PMP is based almost exclusively on the intensive application of chemical insecticides, so it is necessary to establish an integrated disease control system that includes the use of tolerant varieties, biological insecticides and cultural practices (Rubio *et al*., 2013). For this reason, the objective of this work was to determine the efficiency of the microtunnel and the application of biological products to produce tubers with quality to be used as seed.

MATERIALS AND METHODS

Clone 8 - 29 M, considered tolerant to late blight, was used for planting. Five treatments were evaluated and the total area consisted of 12 furrows with a length of 12 meters per furrow. Treatment 1: Cultivation in microtunnel (anti-affidus mesh), application of entomopathogens and biofertilizer; Treatment 2: Cultivation in microtunnel, application of entomopathogens and no application of biofertilizer; Treatment 3: Open field cultivation without microtunnel), application of entomopathogens and biofertilizer ; Treatment 4: Open field cultivation, application of entomopathogens and no application of biofertilizer; Treatment 5: Open field cultivation, no application of entomopathogens and biofertilizer (Table 1).

For the detection of the bacterium *Candidatus* Liberibacter solanacearum considered to be the causal agent of PMP, the Polymerase Chain Reaction-Endpoint (PCR) technique was used. DNA extraction from tubers collected in the field was performed using the CTAB technique modified by Almeyda *et al*. (2001). PCR reactions used primers Lp16s--2F/Lp16s-2R (Hansen *et al*., 2008), designed on the sequence of the gene coding for the bacterial 16S ribosomal RNA and amplifying a fragment of approximately 872 bp. PCR reactions were prepared in a final volume of 25 µl containing: PCR Buffer (1X), primer (25 pMoles each), Taq-DNA Polymerase enzyme (1.5 U) and 50 ng of DNA. The run conditions in the thermal cycler were: One cycle at 94º C for 3 min, 35 cycles at 94º C for 1min, 60º C for 30sec and 72º C for 1min, a final cycle at 72º C for 10min. The amplified fragments were fractionated on 1.5 % agarose gels and run at 100 Volts, for 60 sec. The gels were stained with Gel-Red dye and visualized for analysis under ultraviolet light.

Treatment	Microtunnel	*Trichoderma harzianum*	*Beauveria bassiana*	*Metarhizium anisopliae*	*Rhizophagus intraradices*
1	X	X	X	X	X
2	X	X	X	X	-
3	-	X	X	X	X
4	-	X	X	X	-
5	-	-	-	-	-

Table. 1. Treatments evaluated in the production of potato (Clone 8 - 29 M), for use as seed.

RESULTS AND DISCUSSION

The results of the percentage and intensity of tuber browning were variable depending on the treatment evaluated. The lowest browning percentages and intensity were recorded in the treatments grown in microtunnel (Table 2). The highest percentage of tuber browning, as well as its intensity, was recorded in the treatments conducted in open field; thus, in treatment three, the intensity of browning was recorded even in the strong category, although the number of tubers at this level of intensity was low (Table 2). These results allow inferring that the vector population was much lower in the treatments conducted in microtunnel in relation to the treatments that did not have the anti-affid netting. Based on the above, we can infer that the inoculum concentration in the treatments conducted in the open air was high due to a higher population density of the vector *Bactericera cockerelli* since no control of the insect was carried out.

Treatment	% of tubers with browning	Intensity of browning			
		Zero	Slight	Median	Fort
1	33.3	20/30	10/30		
2	36.7	19/30	11/30		
3	86.6	4/30	11/30	14/30	1/30
4	60.0	12/30	9/30	9/30	
5	93.3	2/30	14/30	14/30	

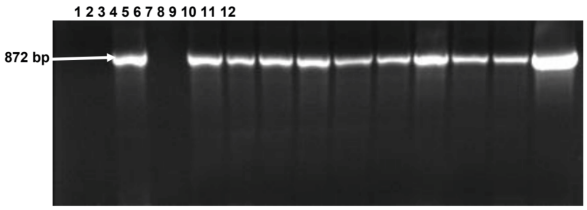
Table 2. Percentage and intensity of browning recorded in the treatments evaluated at the field level against purple top of potato.

The results shown in Table 2 show that the application of entomopathogens and the biofertilizer probably have a protective effect against soil pests and pathogens, in addition to promoting greater root development, but their effect in reducing the percentage and intensity of tuber browning caused by CaLso is not observed. This is due to the fact that the data obtained for these variables were similar among the treatments that did not have anti-affid netting, including the highest intensity of browning in treatment three, which places it in the strong category, surpassing treatment five, which had no application of entomopathogens or biofertilizer.

On the other hand, the percentage of tuber sprouting was affected according to the treatment evaluated. Tubers collected in the three treatments without protection of the anti-affid netting showed 5 to 10% sprouting, while tubers collected in the treatments protected with the anti-affid netting showed 100% sprouting. It is important to note that the 5 or 10% of sprouting recorded in the treatments without the mesh protection were thin and thinned sprouts, which probably do not generate a healthy and vigorous plant for good potato production and quality. Likewise, although the tubers from the treatments with protection showed 100% of apparently vigorous and healthy sprouting, the risk of being infected with the bacterium is highly probable, since a percentage of browning of these tubers was recorded (Table 2), which is an indicator that the bacterium is present. This was confirmed by PCR-endpoint tests,

where fragments of the gene coding for the 16S ribosomal gene of this pathogen were amplified in all the treatments evaluated (Figure 1). Therefore, it is recommended that tubers from lots with records of infection, even if mild, by CaLso should not be used as seed, even if 100% values are obtained in sprouting tests, since even at low concentrations the bacterium is present and constitutes the first source of inoculum to infect the crop.

The detection of CaLso in tuber samples collected in the microtunnel treatments may be due to two probabilities, firstly, the seed used was infected by the bacterium even at low concentrations and there was no dispersal of the pathogen throughout the treatment because the anti-affid netting prevented the presence of the vector and secondly, probably some specimens of the insect were able to penetrate the anti-affid netting infecting some plants but their density was low so that the dispersal of the bacterium was not significant to generate a high percentage and intensity of tuber browning.



Amplification of fragments of the gene coding for the 16S ribosomal RNA of *Candidatus* Liberibacter solanacearum from DNA extracted from different potato samples by PCR using primers Lp16s-2F/Lp16s-2R. Lane 1: Negative control, Lanes 2 and 6: DNA extracted from tubers collected in treatment 3, Lanes 3 and 7: DNA extracted from tubers collected in treatment 4, Lanes 4, 5, 8 and 9: DNA extracted from tubers collected in treatment 5, Lane 10: DNA extracted from tubers collected in treatment 1, Lane 11: DNA from tubers collected in treatment 2, Lane 12: Positive control (*Cataranthus roseus*).

CONCLUSIONS

The microtunnel was efficient in producing tubers with 100% sprouting, but its use as seed in commercial sowings is not recommended if the presence of the bacterium causing PMP is detected even in a small percentage of the tubers produced, since they constitute the primary source of inoculum in the infection of the entire crop, but its use is feasible in the production of potatoes for self-consumption. The entomopathogens and arbuscular mycorrhiza used in this study have no effect in reducing the percentage and intensity of tuber browning caused by the bacterium *Candidatus* Liberibacter solanacearum.

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