SUMMARY

At present, the use of chemical pesticides is still necessary to control and increase the agricultural yields, although in the last years the abusive use of these products has caused a series of environmental alterations, which have influenced the deterioration of soil, the loss of biodiversity, and the increase in the number of diseases that attack the crops, due to the development of species resistant to the pesticides.

The consumers, too, are more and more concerned about the direct, indirect and accumulative effects that pesticides have on the quality of food, health and the environment. Thus, there is a solid agreement within the scientific community about the cultivation methods based on the use of chemical synthetic products, which become unacceptable, and, consequently, new systems more ecological for the protection of the crops are being researched. The present approach to the control of diseases is based on the compatible, rational and sustainable integration of all the available control systems (cultivation control, chemical control and biological control). These control systems seek to reduce the dependence on the chemical pesticides considerably, and they are based on the use of biological control methods and the mechanisms of protection of plants. The research resources in this field are increasing in a spectacular way, and it is reflected in the number of available biocontrol agents in the market or those being studied.

Due to the need to find new methods that substitute the chemical products for the blight control and cultivation diseases, the use of fungi as biocontrol agents, compared to other control systems, either by themselves or by their metabolic compounds, would be an interesting alternative. On the other hand, the biological control takes place in a natural way without man's control, since blights have their natural antagonists. Thus, if the interaction mechanisms between a pathogen and its antagonistic organisms are known, the biological control could keep the balance among the populations. Some organisms hinder the pathogenic activity of other organisms using a wide variety of action mechanisms, such as the antibiosis, the competition for the nutrients and the space, and the parasitism. Sometimes, it is not easy to determine, accurately, the interaction mechanisms between antagonistic fungi and pathogens, although, in general, there is not a unique action type. Moreover, if the antagonist

presents several of them, the risk that the pathogen develops resistance systems becomes reduced. Thus, when the antagonist presents different mechanisms, there are more possibilities for its use as a biocontrol agent.

Out of fungic species with more potential for the control of plant diseases, *Penicillium* and *Trichoderma* genera are outstanding. *P. oxalicum* species is active compared to *Fusarium oxysporum* f.sp. *lycopersici*, which causes the fusariosis of the tomato, and as regards *Trichoderma*, it has already reported the antagonistic behaviour of *T. harzianum* compared to other pathogenic fungi of plants, being the micoparasitism its main action mechanism.

The aim of this doctoral dissertation was to obtain an efficient organism for the development of a environment-friendly agriculture, able to control pathogen agents that cause diseases in the cultivations. A study *in vitro* was carried out in order to achieve this goal, about the behaviour of *Penicillium oxalicum* LBVB 1001 and *Trichoderma harzianum* LBVB 1010 strains, evaluating their capacity to compete and to dominate under determined environmental conditions of water activity (a_w) and temperature (T), when they meet some different phytopathogenic strains: *Rhizoctonia solani* CECT 2819, *Verticillium dahliae* CECT 2694, *Fusarium oxysporum* f.sp. *lycopersici* CECT 2715 and CECT 2866, *Fusarium oxysporum* f.sp *gladioli* CECT 2867 and CECT 2868.

In the first place, the behaviour of single growing fungic strains as regards the environmental factors was studied. Next, for dual growth experiments, the influence of these environmental factors was evaluated when *P. oxalicum* interacted with the four different strains of *F. oxysporum*, as *T. harzianum* interacted with *R. solani*, *F. oxysporum* f.sp. *lycopersici* CECT 2715 and *V. dahliae*. Also, according to the type of interaction found for species studied in the interspecific interactions, the Index of Dominance (I_D) values for each one of the tested strains in this study were calculated. Finally, the results obtained allowed us to determine the antagonistic capacity of the *P. oxalicum* and *T. harzianum* strains as opposed to the studied phytopathogenic agents.

The results obtained from the single growth experiment for all the studied strains, at different a_w (0.85, 0.90, 0.95, 0.98 and 0.995) and T (15 and 25ºC) conditions, revealed that an increase in the growth rate takes place when water availability of the medium increases, being established the maximum value of growth rate for the highest a_w value (0.995). However, for *P. oxalicum* strain, the growth rate increases as a_w

increases, too, up to 0.98, where optimum growth is obtained, and a decrease for higher a_w values (0.995) is observed. In all cases, a decrease in the growth rate was observed when temperature was lowered from 25 to 15ºC. It can be noticed that *V. dahliae* strain behaviour showed lowest growth rate values, whereas *T. harzianum* reached the highest ones.

For *P. oxalicum* strain, when interacting with different *F. oxysporum* strains, higher growth rates were obtained when, at 25ºC, a_w was equal to or lower than 0.98. At 15ºC experiments, *P. oxalicum* yielded higher growth rate than *F. oxysporum* 2715 and 2867 when a_w was lower or equal to 0.95 and 0.90, respectively. However, at 15ºC, no significant differences in growth rate were observed when *P. oxalicum* interacted with *F. oxysporum* 2866 and 2868.

The *T. harzianum* strain presented in the dual growth experiments compared to *R. solani* and *V. dahliae*, for both 15 and 25ºC, higher growth rate values for those a_w in which both strains showed growth (0.98 and 0.995), suggesting the total predominance of *T. harzianum* on the testing strains. For the interaction between *T. harzianum* and *F. oxysporum* 2715, *T. harzianum* also showed higher growth rates in the testing experimental conditions, except when a_w was 0.95 at 15ºC, where no significant differences were observed for both growth rate values.

The Type of Interaction found between *P. oxalicum* and the different strains of *F. oxysporum*, as well as the Index of Dominance obtained for each one of them, showed that, when the a_w value was not higher than 0.95 at 25ºC, the *P. oxalicum* strain would be able to inhibit the growth of those ones, by dominance at a distance (value of 5) or on contact (value of 4), showing further growing over the inhibited colonies. Moreover, both parameters demonstrated the total predominance of *T. harzianum* on *R. solani*, *V. dahliae* and *F. oxysporum* 2715, inhibiting the growth of those by dominance on contact (value of 4), when, for both temperatures, a_w was in the 0.95-0.995 range, except as opposed to *F. oxysporum* strain, when they interacted at 15ºC for a_w of 0.95, in which both strains established a mutual antagonism on contact, without predominance of any species, and being the I_D (value of 2) the same one for both of them.

The results obtained allow us to conclude that the *T. harzianum* strain presents an extraordinary antagonistic capacity compared to *R. solani*, *V. dahliae* and *F.*

oxysporum 2715 under all the testing conditions, and, consequently, *T. harzianum* could be considered as a very good biocontrol agent.

The results obtained using *P. oxalicum* strains were not as spectacular as the ones found for *T. harzianum*, though they were also interesting. This strain brings forward very useful data, because it showed higher competitiveness when a_w conditions are not optimum for its growth, suggesting that the competitiveness between species is not related to growth. This circumstance is the one that will allow *P. oxalicum* strains to control the studied *F. oxysporum* strains, and its antagonistic capacity against them and its role as biocontrol agent has been clearly demonstrated.