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Research Article

The Natural Bioactive Products against Scald in Barley

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ABSTRAT

The biological fight with the antagonists became one of the most favorable alternative to the chemical fight against the fungal diseases. The rhynchosporiose is a foliar fungous disease met in all the Barley producing regions generated by R. secalis. The application of this method has involved using five herbs (Sygylium aromaticum, Alium sativum, Daphne gnidium, Artemisia herba alba and Eucalyptus.sp) in seven concentrations and two isolates originating in Khemisset and Gharb.

The aqueus extracts of Sygylium aromaticum and Alium Sativum induced in vitro the total inhibition of two isolates of the pathogen from 20g/l and 100g/l respectively. The isolats inhibited by the two aquous extracts transferred to the culture medium alone did not come back to life. The effect of Sygylium aromaticum and Alium sativum on R. secalis is there fore fungicide. However, a decoction of Daphné gnidium induced a very significant reduction in mycelial growth of both strains. The percent inhibition of diametric growth reached 71.58% and 68.44% for isolate RS1 at the same concentration 140g/l.

Keywords: Scald, Rhynchosporium secalis, biological control, fungicide.

INTRODUCTION

Barley is the most important crop in most regions of the world (Dizkirici 2006) with world production of more than 144 million tonnes (FAOSTAT 2014). It is the basis of animal feed and a raw material for the production of malt. In Morocco, barley farming accounts for 26.04% of cereal production, which represents 25,000 million quintals (Qx) in the 2016/2017 season(O.N.I.C.L 2017).

Diseases are the main factor affecting the quantity and quality of barley yield, in fact, the damage goes from 10 up to 40% (McDonald et al., 1999). One of the most common and harmful diseases of barley is scald, which causative agent is Rhynchosporium secalis. The prevalence of this disease in Morocco was 37% during the surveys of 2009/2010 and it is becoming more and more present in our fields, with high severity in 50% of the fields visited in 2014. (Bentata et al., 2014).

Foliar fungicides were applied successfully against the rhynchosporiose however the number of R. secalis resistant increased thus limiting the effectiveness of this fighting method (look et al. 1995, Zhan et al. 2008). Moreover these methods induce in the majority of the cases undesirable effects on the Mean and the environment. Indeed, a private interest was granted to the phytotherapy which calls on natural substancesfrom vegetable origin.

Many studies brought the different biological activities out of the aromatic and medicinal plants, in particular their antifungal (Taibi et al. 2014; Bentata et al., 2015; Essouaadi et al., 2015), antibacterial (Bourkhiss et al., 2007; Magina et al., 2009), antioxidant (Bouzouita et al., 2008) and insecticide powers (Erler et al ., 2006; Tang et al., 2007; Cheng et al., 2009).

Sygylium aromaticum, Allium sativum, Daphne gnidium Artemisia herba Alba and Eucalyptus sp, five plants more used in the conventional medicine; their antifungal power has been demonstrated by many studies (Javidinia et al., 2003; Colack, 2004; Kolai et al., 2012). However the evaluation of their antifungal power towards R.secalis has not been studied before. The goal of this study is to test their antifungal activity in vitro towards to R. secalis.

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MATERIALS AND METHODS

Vegetal material

The five medicinal plants were harvesting at random in the Moroccan Maamora forest during two months March and April, 2014. The plants were drying in the shade during 15 days.

Fungal material

Pathogen isolation was achieved from barley leaves with scald symptoms collected from the Khemisset (Isolate RS1) and Gharb (Isolate RS2) regions respectively in March 2014. The leaves are cut into small pieces of 10 mm. The latter are disinfected and then dried on sterile filter paper to be subcultured in the medium (LBA). The Petri dishes were incubated in the dark at a temperature of 18 ° C for 10 days.

After the incubation period, confirmation of the identity of the fungus is made using a determination key (Barnett & Barry, 2005). The choice of these two isolates is based on the one hand on the evaluation of their severity in the field and on the other hand, on the geographical distribution.

Preparation the plant extract

The tests are realized on the LBA medium. Before the sterilization, different quantities of each five plants were introduced in the growth mediums. The concentrations used range from 20 to 140g/l.

The plants parties used are S.aromaticum, A.herba alba, D. gnidium, Eucalyptus.sp leaves and A.sativum bulb.

Biological test

The barley isolates are transplanted on the growth mediums in the presence of the different four plants concentrations. The both isolates are also transplanted on the growth medium as a witness. Three repetitions are retained for each concentration. The Petri dishes are incubated in the darkness and at 25°C.

Evaluation the antifungal activity of four plant extracts

The effect of five plant extracts on growth of the both isolates is determinated by the calculation of percentage of diametric growth inhibition according to Leroux and Gredet (1978) formula. The results are analyzed by Excel 2013.

$$P.I.C.D = (Ø_t - Ø_e / Ø_t) \times 100$$

P.I.C.D = percentage of diametric growth inhibition.

Øt = average diameter of witness thallus.

Øe= average diameter of thallus exposed to plant extracts.

Nature of antifungal activity

Isolates completely inhibited by S. aromaticum and A.sativum extracts are transferred to LBA alone to verify whether it is a fungistatic or fungicidal effect. The extracts are fungistatic when the fungus comes back to life after a stop phase under the action of the plants extracts. It is fungicidal when it causes irreversible and total inhibition of the diametrical growth of the fungus.

RESULTS

1-Effet of five extracts plants on diametric growth of Rhynchosporium secalis

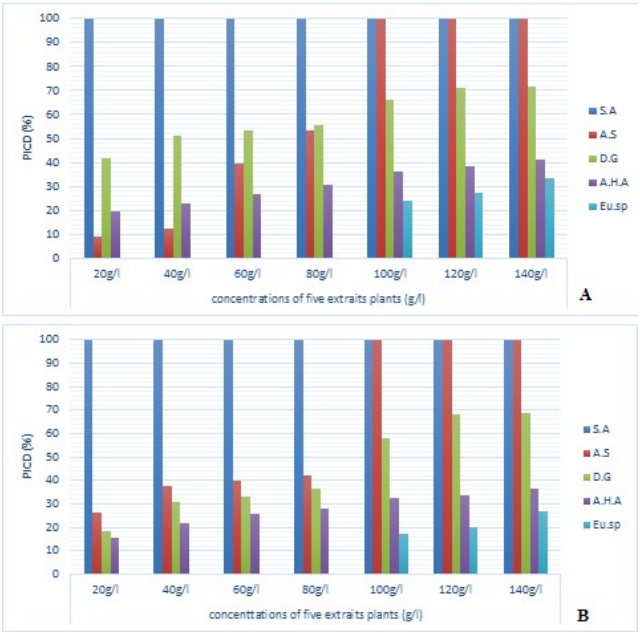
The achieved results with four different aqueous extracts concentrations revealed that the inhibitory activity rises as the concentration increases.

However, both aqueus extracts of S.aromaticum and A. sativum showed complete inhibition of both isolates (RS1 and RS2) of R.secalis from 20g/l and 100g/l respetively (Fig1. A,B).

The both isolates are inhibited under the action of D. gindium extract. Nevertheless, the most significant inhibitory activity against RS1 and RS2 is obtained respectively at the concentration 140g/l in order of 71.58% and 68.44% (Fig1. A,B) .

The A. herba alba and Eucalyptus sp extract show a limited antifungal power against to both isolates. Indeed the inhibitive activity of A. herba alba is more important, it reaches respectively 41.1% and 36.44% against RS1 and RS2 at 140 g/l. At the same time Eu.sp extract show respectively a very limited antifungal power against both isolates (RS1 and RS2) at 140g/l. So we conclude that A.herba alba extract show a significant antifungal power against both isolates than Eu.sp extract (Fig. 1 A, B).

Figure 1: The effects of five extract plants on both isolates of Rhynchosporium secalis RS1 (A) and RS2 (B).



Fungicidal fungistatic effect

Both isolates RS1 and RS2 completely inhibited by the two aqueous extracts of medicinal plants are transformed to LBA alone. The test shows that none of the two inhibited isolates has revived. Therefore aqueous extracts of S.aromaticum and A.sativum has a fungicidal effects against R. secalis (Tabl1).

Table 1: Effect of the transfert of two isolates inhibited by S.aromaticum and A.sativum on LBA alone.

R. secalis isolates	PICD under the action of S.aromaticum for 7 concentrations 20, 40, 60, 80, 100, 120, 140g/l	PICD under the action of Alium sativum For 3 concentrations 100, 120, 140g/l.	Result of the transfer of the isolates of R.secalis inhibited on LBA alone	Effect
RS1	100%	100%	-	Fongicidal
RS2	100%	100%	-	Fongicidal

(- : absence de croissance)

DISCUSSION AND CONCLUSION

The biological fight is the use of living organisms with an aim of limiting the pullulation and/or theharmfulness of various cultures enemies, such as “rodent, insects, nematodes, diseases and weeds”

Several attempts at biological fight calling on microbial antagonism against R. secalis gave good performances. Indeed, the biological control of the rhynchosporiose by bacteria reduced the germination of the spores of this disease-causing agent (Rotem et al., 1976). Work of Kulichova (1997), showed that the treatment of the seeds with Trichoderma inhibited the germination of the spores of R. secalis of 30% up to 90%.

The medicinal plant extract contains an antimicrobial activity which mainly depends on their chemical composition. They inhibit as well the bacteria as the fungi due to their wide spectrum action (Saravanan & Valluvaparidasan, 2001; Klingauf, 2005). In Morocco, the aromatic and medicinal plants present a varied and diverse ecological area which extends over all bio climates types and all geographic substrates. The most used plants in the Moroccan conventional medicine are S. aromaticum, A. sativum, D. gnidium, A. herba alba and Eu sp.

The aqueous extract of S.aromaticum showed a total inhibition of the two isolates of R.secalis at a concentration of 20g/l. this antifungal power with respect to this fungus is explained by the richess of the aqueous extract of the eugenol chemical compound (Devi, et al., 2010). These results agree with the work found by sofia and al (2007), that tested the antimicrobial activity of different plants such as A.sativum and S. aromaticum. They found that the extract of S.aromaticum had a complete bactericidal effect against all foodborne pathogens tested (Sofia et al ., 2007).

Our study revealed a total antifungal power of the decoction of A. sativum with respect to two isolates of R. secalis, RS1 and RS2. This is in agreement with the work of krichen et al., (2004) which showed the strong activity of the garlic extract against the spores of Fusarium oxysporum F. sp. Niveum, responsible for the fading of water melon. This same medicinal plant also expresses a strong antifungal power against Fusarium which attacks the olive-tree (Triki et al., 2012). This is explained by A. sativum wilth in suffers and its several phenolic compounds (Grainge & Ahmed, 1988). Another study showed the inhibiting power importance of the aqueous extract of a medicinal plant, Daphne gnidium, on the same pathogenic mushroom R.secalis (Essouaadi et al., 2015).

Our study revealed significant antifungal power towards both isolates of R.secalis. this is in agreement with the work of Javidnia and al (2003) which demonstrated the antimicrobial activity of the extract of D. mucronata against other pathogenic microorganisms for humans, among others, Escherichia coli and Staphylococcus aureus.

In addition, D.gnidium has antifungal activity against several other phytopathogenic fungi as an example : Pythium, Verticillium and Fusarium (Lauk et al., 1996; Sawadogo, 2011; El Makhfi, 2012).

Concerning the Artemisia herba alba extract revealed a limited antifungal activity against Rhynchosporium secalis. However Kolai and al., 2012 noticed that its extract contains a strong antifungal power against Fusarium oxycysporum f sp. Radicis lycopersici. Eucalyptus sp extract didn’t show any antifungal activity against Rhynchosporium secalis at the highest concentrations but Himri and al., 2011 showed that the essential oil of Eucalyptus camaldulensis presents an antifungal power on Alternaria alternata and on Penicillium expansum.

This work is original as much as that we did not find any report or paper testing the impact of five plants medicinals (S.aromaticum, A. sativum, D.gnidium ,A.herba alba and Eu.sp) on R.secalis the causative agent of scald of barley. Moreover S.aromaticum, A. sativum and D. gnidium can present a potential protect way against Scald but new research should focus on they should be confirmed their antifungal power in vivo.

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