USE OF ARTIFICIAL DIET SYSTEM TO ASSESS THE POTENTIAL BIO-INSECTICIDE EFFECT OF A FUNGAL LECTIN FROM *XEROCOMUS CHRYSENTERON* (XCL) ON *MYZUS PERSICAE*

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SUMMARY

Lectins are carbohydrate binding proteins which are widely distributed in nature: they have been isolated from microorganisms, fungi, plants and animals. Many of these proteins were tested for their potential biocide effect on lot of pests. Indeed, lectins can cause dramatic changes in the cellular morphology and metabolism, particularly on the digestive system of insect having ingested them, by lectin binding to membrane glycosyl groups of the digestive tract cells. A fungal lectin, namely *Xerocomus Chrysenteron* lectin (XCL) was previously purified and was shown to be toxic to several pests including aphids. At the cell level, an increase in the endocytosis, the induction of morphological changes such as the actin cytoskeleton shape was determined. In this work, the recombinant XCL was produced and was tested for its potential aphicide effect on *Myzus persicae*, a polyphagous aphid found on more than 400 host plant species and transmitting more than 100 viral diseases. We developed bioassays using different artificial diets incorporating a broad range of XCL concentrations (from 10 $\mu g.ml^{-1}$ to $1 mg.ml^{-1}$) to assess the potential negative effects of XCL on the development and reproduction of the *M. persicae* aphid. Significant mortality rates, changes of developmental durations and nymph production were observed depending on the XCL concentration in the artificial diet. Concanavalin A lectin was also used in a new range of experiments to compare the effects of the two lectins on the aphid biological parameters. According to the observed dose responses toward the XCL and Con-A lectins included in the artificial diet and the action mode of this kind of proteins, the perspectives of lectin use in pest control will be discussed.

Key words: *Myzus persicae*, artificial diet, fungal lectin, XCL.

INTRODUCTION

Lectins are carbohydrate binding proteins which are widely distributed in nature: they have been isolated from microorganisms, fungi, plants and animals. They are carbohydrate-binding proteins that bind glycans of glycoproteins, glycolipids or polysaccharides (Goldstein *et al.*, 1978; Sharon *et al.*, 1989) and possess at least one or more binding sites per subunit, which can reversibly bind to specific sugar segments through hydrogen bonds and Van Der Waals interactions (Peumans and Van Damme, 1995; Lis and Sharon, 1986 ,1998; Gatehouse *et al.*, 1995).

Recent years extensive studies have been carried out to identify proteins with insecticidal properties towards insect pests of major economic importance for expression in transgenic plants (Piek, 1990; Ferrari *et al.*, 1991; Aronson, 1994). Many plant lectins such as GNA (*Galanthus nivalis*; snowdrop), PSA (*Pisum sativum*; pea), WGA (*Triticum vulgare*; wheatgerm), ConA

(*Canavalia ensiformis*, jack bean), AIA (*Artocarpus integrifolia*, jack fruit), OSA (*Oriza sativa*, rice) and UDA (*Urtica dioica*, stinging nettle) have negative effect on different insect pests (Powel, 2001; Ripoll et al, 2003; Sauvion *et al.*, 1996); Hilder *et al.*, 1995). Nevertheless the mechanism of action is poorly known (Peumans and Van Damme, 1995; Sauvion, 2004).
Although a binding between lectins and glycoproteins of the epithelial cells of the midgut appears to be necessary, it is not sufficient to explain the disruption of cellular functions interfering with insects growth and survival (Harper *et al.*, 1995; Powel *et al.*, 1998).
Lectin-based strategies for the production of insect resistant transgenic crops are currently receiving much attention, particularly for control of hemipteran pests for which they comprise among the best available toxins and display the widest array of molecular targets (Hilder *et al.*, 1995; Gatehouse *et al.*, 1996; Rao *et al.*, 1998; Gatehouse *et al.*, 1999; Stoger *et al.*, 1999; Foissac *et al.*, 2000; Maqbool *et al.*, 2001; Loc *et al.*, 2002; Banerjee *et al.*, 2004).
Hemiptera orders are sensitive to mannose-glucose lectins including those from Canavalia ensiformis (ConA) and the family of Amaryllidaceae (van Damme *et al.*, 1988). Many studies have demonstrated deleterious effects of GNA expression in planta (potato, rice, wheat) towards different sap-sucking insects (Gatehouse *et al.*, 1996; Rao *et al.*, 1998; Stoger *et al.*, 1999; Foissac *et al.*, 2000). Also GNA had no significant effects on beneficial insects such as ladybirds, either in artificial diet or in planta at the tritrophic level (Down *et al.*, 2000).
In this work we used a previously purified lectin from a mushroom: *Xerocomus chrysenteron* (Trigueros *et al.*, 2003). This lectin (XCL) belongs to the group of AOL (*Arthrobothrys oligospora* lectin) and ABL (*Agaricus bisporus* Lectin), which are specific for N-acetyl-galactosamine and galactose (Francis *et al.*, 2003, Rosén *et al.*, 1992).
Despite current interest in the insecticidal properties of lectins, their modes of action are not clearly understood. If strategies based on the use of transgenic crops expressing specific lectins are to be adopted, more information on their precise modes of activity will be required to ensure durability in the field (Powell *et al.*, 1993).
The present paper aim to clarify the biological effects of lectins on *M. Persicae* development, fecundity and mortality.

MATERIAL AND METHODS

Aphid rearing

Myzus persicae were reared on young broad beans, *Vicia faba* at 20±2°C, L16:D8 before being used for feeding experiments on artificial diet. *Vicia faba* were grown in perlite: vermiculite mixture (50/50 V:V) in plastic pots 20×30×5 cm diameter, in the greenhouse.

Artificial diet

Different artificial diets was used in this experience, among them 2 types of artificial diets were appropriate for *M. persicae*, the first was 15% sucrose

(for short time) and the second was a complete diet which was presented by Febvay *et al.* (1988).
Different concentrations of XCL and ConA were used (from 10 $\mu g.ml^{-1}$ to 1 $mg.ml^{-1}$).
Diet sachets (two layers of parafilm enclosing 150 µl of diet) were changed every 2 days. Our experiment was carried out with 5 repetitions and each repetition with 20 aphids.

Material

Concanavalia ensiformis (ConA) was purchased from Sigma (C2010 T: IV). *Xerocomus chrysenteron*(XCL) was purified by Trigueros et al 2003

RESULTS

Comparison of different concentrations of sucrose on *M. persicae* survival

In this study we developed bioassays using different range of sucrose concentrations (from 5 to 25%) to assess the appropriate concentration of sucrose on the development and reproduction of the *M. persicae* aphid. Significant differences were observed depending on the sucrose concentration in the artificial diet

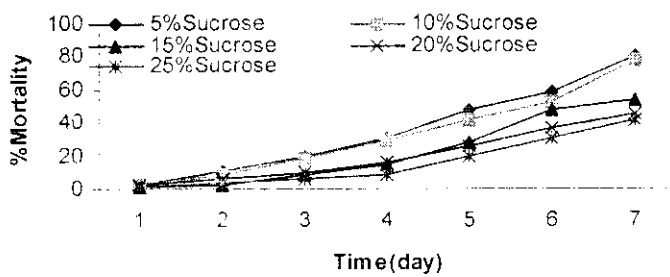


Figure 1. Effect of different concentrations (5, 10, 15, 20 and 25% v/w) of the sucrose on *Myzus persicae* survival

Ever 5 and 10% sucrose concentration did not allow to keep a sufficient survivals of the adult aphid. The high sucrose dosages which were used were all suitable in further toxicology test at short time. No significant difference was observed between the mortality rate observed with the 15% to 25% Sucrose concentration ($F=12.48$, $p<0.001$). Therefore, the 15% concentration was selected as appropriate concentration as a artificial diet for *M. persicae* after 4 days (Figure 1).

Effect of two artificial diets on the growth and development of *M. peresicae* in short term (after 4 days)

A more complete diet was tested to follow the biological parameters for a longer period than a few days. The test of 15% ucrose and complete diet (Febvay *et al.*, 1988) were carried out for comparison between this two artificial diet on the growth and development of *M. persicae*.

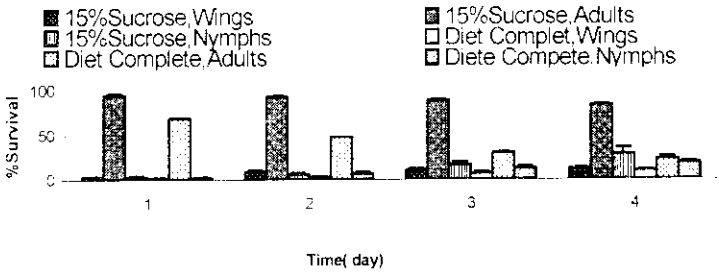


Figure 2. Comparison of two artificial diets a, 15% Sucrose with complete diet on *M. persicae* development after 4 days

No significant difference between 15% sucrose and complete diet was observed on survival of *M. persicae* with wing and nymph. Significant difference between the complete diet and 15% sucrose on survival of *M. persicae* adults was observed (Fgure 2).

Comparison of 2 artificial diets on long term, growth and development of *M. persicae*

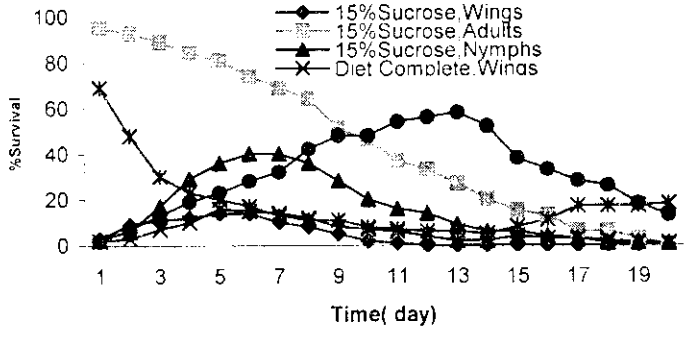


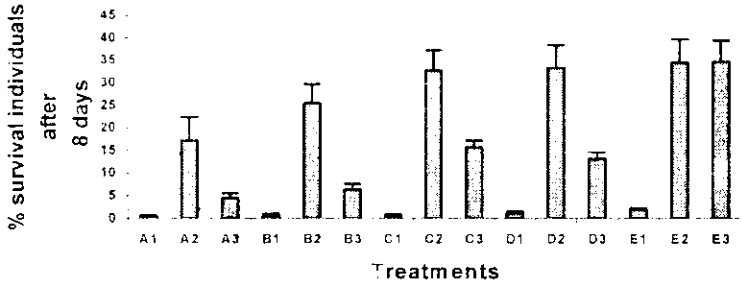
Figure 3. Effects of two artificial diets, 15% sucrose with complete diet on *M. persicae* development after 20 days

No significant difference was observed (Figure 3) between 15% Sucrose and complete diet on survival of *M. persicae* with wing. Nevertheless, significant difference between 15% Sucrose and complete diet on survival of adults and nymphs of *M. persicae* were observed after 20 days ($F=43,04$ $P<0.001$).

Effects of different concentrations of *Xerocomus chrysenteron* lectin (XCL) on mortality and development of *M. persicae*

Bioassays utilising artificial diet incorporating XCL were performed to assess the potential effects of this lectin on the development of the aphid, *Myzus persicae*.

We developed bioassays using different artificial diets incorporating a broad range of XCL concentrations (from 10 µg.ml^{-1} to 1000µg ml^{-1}) to assess the potential negative effects of XCL on the development and reproduction of the *M. persicae* aphid. Significant mortality rates, changes of developmental durations and nymph production were observed depending on the XCL concentration in the artificial diet (Figure 4).



A1 0.1%XCL,Wings	B1 0.05%XCL,Wngs	C1 0.025%XCL,Wings	D1 0.01%XCL Wing	E1 Control,Wings
A2 0.1%XCL,Adults	B2 0.05%XCL, Adults	C2 0.025%XCL Adults	D2 0.01%XCL,Adults	E2 Control,Adults
A3 0.1%XCL,Nymphs	B3 0.05%XCL Nymph	C3 0.025%XCL,Nymph	D3 0.01%XCL,Nymph	E3 control,Nymph

Figure 4. Different concentrations of *Crysentron lectin* (XCL) on mortality and development of *M. persicae* after 8 days

No significant difference between concentration of 0,1%, to 0,01% and control on development of *M. persicae* with wings was observed (Figure 3). Nevertheless, there is significant difference between concentration of 0,1% and 0,05%, with other concentration on adults survival. So there is a difference effect between control and other concentration on nymphs survival ($F=29.53$, $P<0.001$).

3.5. Effect of XCL and ConA on *M. persicae* mortality

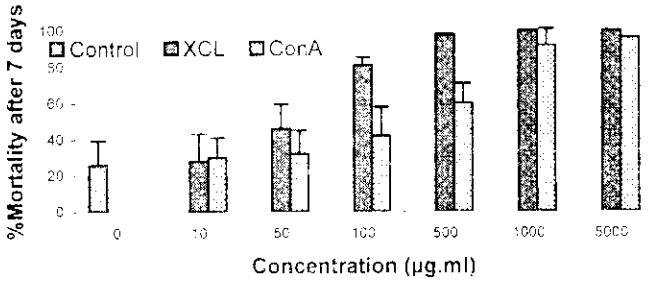


Figure 5. Comparison effect of the different concentrations (0,5% to 0, 0,01%) of XCL and ConA on *M. persicae* mortality

Different concentrations (from 10 to 5000 µg.ml^{-1}) of ConA and XCL on *M. persicae* mortality showed that in higher concentrations (100 to 5000 µg.ml^{-1}) of XCL on *M. persicae* mortality were similar (F=38.22, P<0.001 and Figure 5). However, there is high significant difference between concentrations of 100µg.ml^{-1} and other concentrations (10 and 50 µg.ml^{-1}) of XCL on *M. persicae* mortality. Higher concentrations (1000 to 5000 µg.ml-1) of ConA on *M. persicae* mortality were similar. Nevertheless, in other concentrations (10 to 500µg.ml^{-1}) of ConA on *M. persicae* mortality were similar (F=38.22, P<0.001 and Figure 5). Therefore, in low concentration, XCL is more effective than the ConA on *M. persicae* mortality.

DISCUSSION

The results from the present study provide a greater understanding of the use of the sucrose as an artificial diet. One aim of the present study was to better understand the appropriate concentration of sucrose as an artificial diet or incorporated with other artificial diets. Sucrose content of 20% was used as the control diet and the basal diet to which the test proteins were added (Febvay *et al.*, 1988). Previous studies were carried out with 20% sucrose by Triguéros *et al.*, 2003 and Rahbe *et al.*, 1995. Our results also showed that 15% sucrose was the most appropriate concentration as an artificial diet than other concentrations for biological tests on *M. persicae* (Figure 1). No significant difference was observed between the mortality rate observed with the 15% to 25% sucrose concentration (F=12.40, p<0.001). Our results showed that (Figure 2 and 3) 15% sucrose was the simple artificial diet to perform efficient toxicological and short term development tests on *M. persicae* aphid (F=128.41, P<0.001). However, more complex and complete diets are needed to be used for longer periods of test on *M. persicae* aphids (F=43.04, P<0.001). Results obtained from binding of the lectin concanavalin A (lectin from *Canavalia ensiformis*) clearly showed the ConA bound to the apical membranes (striated border) of stomach walls (Couvion *et al.*, 2004). Similar results were obtained from the effect of Concanavalin A on *A. pisum* showing considerable negative effects on the

growth and survival of this aphid (Rahbé *et al.*, 1995). The results of our experiment on *M. persicae* showed that different concentrations of ConA have different negative effects on mortality of *M. persicae* (Figure 4).

Fungi are an important group of microorganisms, some of which have beneficial effects while others are pathogenic. They include ribosome inactivating proteins, ribonucleases, ubiquitin-like proteins and peptides, lectins, cellulases, xylanases, laccases, invertases and trehalose phosphory lases (Barbieri *et al.*, 1993; Ng *et al.*, 2004).The lectin from *Xerocomus chrysenteron* (XCL) was isolated following a screening for new insecticidal molecules (Mier et al; 1996). Francis et al, 2003, previously tested XCL on cell proliferation of the adherent cell lines. Our results showed that effects of different concentrations of lectin from mushroom, *Xereocomus chrysentron* (XCL) on *M. persicae* mortality were different ($F=29.53$, $P<0.001$ & Figure 4).

Results obtained from different concentrations (from 10 $\mu g.ml^{-1}$ to 5000 $\mu g.ml^{-1}$) of ConA and XCL ($F=38.22$, $p<0.001$) on *M. persicae* mortality showed that in higher concentrations (1000 to 5000 $\mu g.ml^{-1}$) the effect of XCL & ConA on *M. persicae* mortality were similar. Nevertheless, in low concentration, XCL is more effective than the ConA on *M. persicae* mortality (Figure 5).

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