

EFFECT OF A FUNGAL LECTIN FROM *XEROCOMUS CHRYSENTERON* (XCL) ON THE BIOLOGICAL PARAMETERS OF APHIDS

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SUMMARY

Aphids are important pests of crop plants in Europe. Increasing resistance of aphids to insecticides and their side effects on the environment and non target organism's including human's stimulated research on alternative methods of aphid control, including the use of entomotoxic proteins. Lectins are carbohydrate binding proteins that are widely distributed in nature; they have been isolated from microorganisms, fungi, plants and animals. Several of these proteins were tested for their potential biocide effect on plenty of pests. A fungal lectin, namely *Xerocomus chrysentron* lectin (XCL) was previously purified and was shown to be toxic for several pests including aphids. XCL was clearly the most toxic lectin against *M. persicae*. In this work, bioassays using artificial diets incorporating a broad range of XCL concentrations (from 10 µg.ml⁻¹ to 5000 µg.ml⁻¹) were developed to assess the negative effects of XCL on the biological parameters (development duration, weight and fecundity) of *M. persicae* a polyphagous aphid found on more than 400 host plant species and transmitting more than 100 viral diseases. A significant mortality of aphids was observed, corresponding to the LC₅₀ and LC₉₀ of 0, 46 and 6, 02 mg/ml respectively after 24hrs. Significant differences of *M. persicae* weight, development duration and fecundity (P<0.05) was observed between the tested XCL concentrations. *Conavalia ensiformis* lectin (ConA) was included as lectin reference on the bioassay experiments and was shown to be less toxic and induced lower negative changes in *M. persicae* biological parameters when compared with XCL.

Key words: *Myzus persicae*, *Xerocomus chrysentron*, fungal lectin

INTRODUCTION

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). A mannose-binding lectin found in peanut tissues, *Arachis hypogaea*, was compared with mannose-binding lectin of pea, *Pisum sativum*, for toxic effects on larvae of stem borer, *Chilo Partellus* (Swinhoe). After 10 days, the larvae mortality due to the artificial diet containing 0.5% peanut lectin was 46.2%. Also, the larvae mortality related to 1.0% peanut lectin in artificial diet was similar (48.1%), and the insects were significantly smaller. Larvae of both lectin treatments stopped feeding within three days (Rami, 1997). GNA had a significant effect on parthenogenesis fecundity as well as on insect development (Sauvion *et al.*, 1996). Fitches

et al. (1997) shows that GNA exerts a significantly detrimental effect upon larval development, growth, and consumption of tomato moth (*Lacanobia oleracea*), but it has less effects on survival of this insect. This suggestion is similar to the effect of GNA on potato aphids (*Aulacorthum solani*), where GNA was seen to be significantly affected on development and fecundity (Down *et al.*, 1996).

Powell *et al.* (1993) and Habibi *et al.* (1993) reported that the harmful effects of lectins on biological parameters of insects include larval weight, mortality, feeding inhibition, metabolism, honeydew excretion, pupation, delays in total developmental duration, adult emergence and fecundity on the first and second generation. 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 (Powell *et al.*, 1998). Lectin-based strategies for the production of insect resistant transgenic crops are currently receiving much attention, particularly for control of Hemiptera 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; Foissac *et al.*, 2000).

Hemiptera species 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; Foissac *et al.*, 2000). Also GNA had no significant effects on beneficial insects such as ladybirds, neither in artificial diet or in planta at the third trophic level (Down *et al.*, 2000)

The effects of concanavalin A (ConA) on insect crop pests from two different orders, Lepidoptera and Homoptera, were investigated. When fed larvae of tomato moth (*Lacanobia oleracea*) at a range of concentrations (0.02-2.0% of total protein) in artificial diet, ConA increased up to 90% mortality observed at the highest dose level, and delayed development. When fed peach-potato aphids (*Myzus persicae*) in artificial diet, ConA reduced aphid size by up to 30%, retarded development to maturity, and reduced fecundity by 35% (Gatehouse *et al.*, 1999). 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). XCL may cross the midgut epithelial barrier and pass into the insect circulatory system, like the *G. nivalis* lectin (Fitches *et al.*, 2001). 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 aimed at clarifying the effects of XCL on *M. Persicae* weight, development duration, fecundity and mortality.

MATERIAL AND METHODS

Concanavalia ensiformis lectin (ConA) was purchased from Sigma. *Xerocomus Chrysenteron* lectin (XCL) was purified by Trigueros *et al.*, (2003).

Artificial diet

Artificial diets incorporating different concentrations (from 10 to 5000 $\mu\text{g}\cdot\text{ml}^{-1}$) of XCL and ConA were prepared according to Febvay *et al.*, (1988) and used immediately or aliquots and kept frozen at -20°C until used. Diet sachets (two layers of parafilm enclosing diet) were changed every 2 days.

Statistical analysis

Experiments in this study were analyzed by Minitab Software (version 13.1). Before variance of analyses each experiment was tested by equation of variance and Dunnett and Tukey tests.

Aphid rearing

The *Myzus persicae* used in this study were reared on young broad beans, *Vicia faba* at $20\pm 2^{\circ}\text{C}$, L16:D8 before being used for feeding experiments on artificial diet (Figure 1). These aphids have been reared continuously for many years on *V. faba* plant under environmentally controlled conditions ($20\pm 2^{\circ}\text{C}$, L16:D8). *V. faba* were grown in perlite/vermiculite mixture (50/50, W/W) in plastic pots, $20\times 30\times 5$ cm. Neonate aphids (aged 0-24 h) were used for all the artificial diet experiments and the protocol for standardising experimental aphid production was described previously by Rahbé & Febvay (1998).

Hemagglutination assays

Rabbit erythrocytes were used for the determination of hemagglutination activity during the isolation procedures and for inhibition assays. Fifty microliters of serial twofold dilutions of the fungal extracts was mixed with an equal volume of a 4% erythrocyte suspension in wells of U-shaped microtiter plates. After gentle shaking, the plates were allowed to settle at room temperature for 2 h and agglutination was recorded visually. PBS was used as a negative control and ConA as a positive control.

Toxicological test

Twenty neonate aphid (with 5 replicates) were deposited at day 0 on artificial diets supplemented with lectin. At day 7, mortality was determined and LC50 of XCL was determined.

Fecundity test

This experiment was carried out on 20 replicates (individual aphids previously adapted on artificial diet). Different XCL concentrations (from 10 to 5000 $\mu\text{g}/\text{ml}$) were used and the number of new nymphs was daily counted and removed.

Development duration test

This experiment was performed on 20 replicates (individual aphids). Different XCL concentrations (from 10 to 5000 $\mu\text{g}/\text{ml}$) were used and duration between first nymph and adult was observed.

RESULTS

Effects of XCL on *M. persicae* biological parameters

High significant differences ($F=102.12$, $P<0.001$) were observed between XCL concentrations in diet on aphid mortality. The LC_{20} , LC_{50} and LC_{90} were 31, 468 and 6026 $\mu\text{g/ml}$ respectively. The linear regression equation related to *M. persicae* mortality toward XCL was $y = 8.9819x - 2.2577$.

Weight

High significant differences on *M. persicae* weights ($F=20.33$, $P<0.001$) were observed between XCL concentrations from 1 to 7 days (Figure 1).

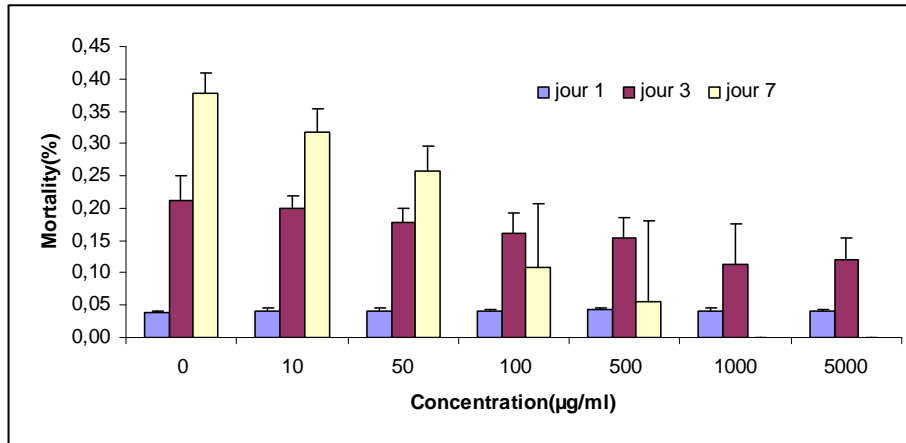


Figure 1. Effects of XCL concentrations in artificial diet on *M. persicae* weight

No significant difference was observed between XCL concentrations in diet on *M. persicae* weight after 1 day ($t=3.081$, $P>0.05$) and 3 days ($t=0.057$, $P>0.05$). Significant differences ($t= 4.20$, $t=5.02$, $t=3.01$ and $P<0.05$) were observed between 500, 1000 and 5000 $\mu\text{g/ml}$ of XCL with control on *M. persicae* weight. No significant difference ($t=-1.32$, $P>0.05$,) was observed among concentrations of 10 and 50 $\mu\text{g/ml}$ with Control after 3 days. High significant differences were observed among concentrations of 100 to 5000 $\mu\text{g/ml}$ of XCL on *M. persicae* weight after 7 days.

Mortality

High significant differences ($F=56.28$, $P<0.001$) were observed according to the XCL concentrations on *M. persicae* mortality (Figure 2).

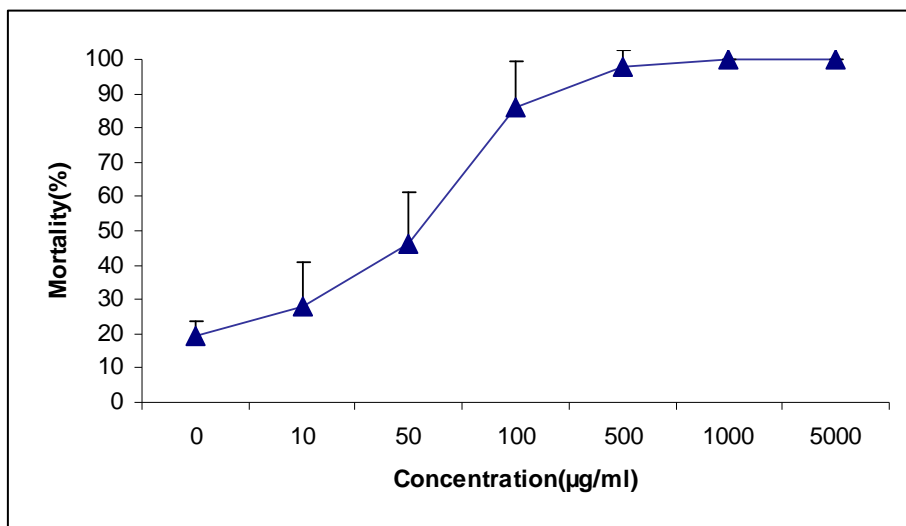


Figure 2. Mortality rates of XCL concentrations on *M. persicae*

There is no significant difference ($t=1.24$, $P>0.05$) between concentrations of 10 µg/ml of XCL with control on *M. persicae* mortality, but there were high significant differences ($t=4.54$, $t=10.33$, $t=11.57$, $t=11.98$, $t=11$, 98, $P<0.001$) among concentrations of 50 to 5000 µg/ml of XCL with control.

Development duration

High significant differences ($F=17.82$, $P<0.001$) were observed between XCL concentrations on *M. persicae* development durations (Figure 3). No significant difference ($t=0.36$, $t=1.09$, $t=-2$, 54, $P>0.05$) was observed between concentrations of 10, 50 and 100 µg/ml of XCL with Control on *M. persicae* development duration. High significant differences ($t=-5.45$, $p<0.001$) were observed among concentration of 500 to 5000 µg/ml with control on *M. persicae* development duration.

Fecundity

High significant differences ($F=34.72$, $P<0.001$) were observed among different concentrations of XCL on *M. persicae* fecundity. A significant difference ($t=-2.94$, $P<0.05$) was observed between concentration of 10 µg/ml of XCL with control. As well, high significant differences ($t=-5.14$, $t=-8.81$, $t=-10.27$, $P<0.001$) were observed among concentrations of 50 to 5000 µg/ml of XCL with control on *M. persicae* fecundity. The fecundity of *M. persicae* was highly susceptible to XCL even at lowest concentrations.

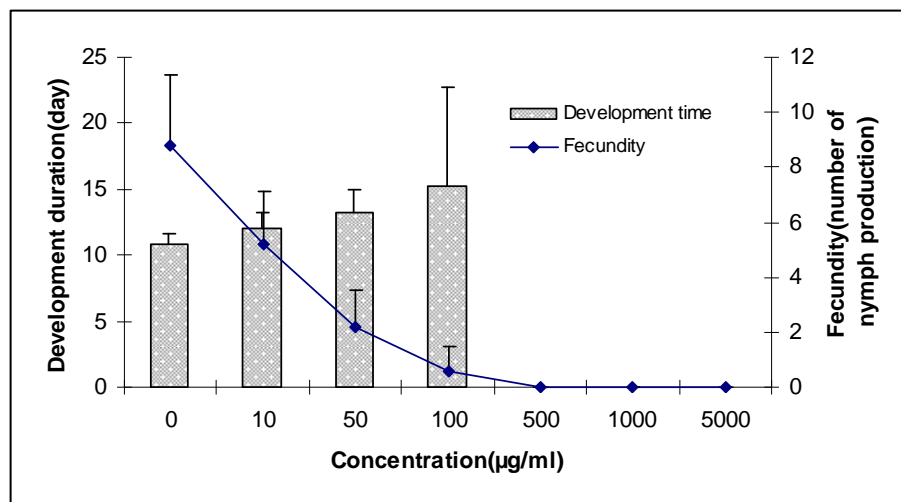


Figure 3. Effect of XCL concentrations on *M. persicae* development duration and fecundity after 7 days

COMPARISON OF XCL AND CONA EFFECTS ON *M. PERSICAE* BIOLOGICAL PARAMETERS

Weight

Significant differences ($F=22.08$, $P<0.001$) were observed among different XCL and ConA concentrations on *M. persicae* weight after 7 day (Table 1). High significant differences ($t=7.63$, $t=6.11$, $P<0.001$) were observed among concentration of 10 and 50 µg/ml of XCL and ConA on *M. persicae* weight after 7 days.

Mortality

A significant difference ($F=37.57$, $P<0.001$) was observed among concentrations of XCL and ConA on *M. persicae* mortality after 7 days. No significant difference ($t=0.36$, $t=2.91$, $t=0.73$, $t=0.36$, $P>0.05$) was observed between 10, 1000 and 5000 µg/ml of both XCL and ConA on *M. persicae* mortality after 7 days respectively. High significant difference ($t=5.47$, $P<0.001$) and significant difference ($t=4.17$, $p<0.05$) were observed between concentrations of 100 and 500 µl/ml of XCL and ConA. XCL was significantly more toxic for *M. persicae* than the ConA (Table 1).

Development duration

Significant difference ($t=3.58$, $P<0.05$) was observed between concentrations of 50 µg/ml of XCL and ConA on *M. persicae* development duration.

Fecundity

Significant differences ($t=-3.66$, $t=-1.66$, $P<0.05$) were observed between concentrations of 50 and 100 µg/ml of XCL and ConA respectively on *M. persicae* fecun-

dity. Likewise at low concentrations, XCL was more active than the ConA on *M. persicae* fecundity (Table1).

Table 1. Comparison of XCL and ConA effects on *M. persicae* biological parameters (\pm SD)

XCL				
Concentration (μ g/ml)	Weight (mean \pm SD)	Mortality (%)	Development time (day)	Fecundity (number of new nympe)
0	0.38 \pm 0.03	19 \pm 3.93	10.80 \pm 0.84	8.80 \pm 2.59
10	0.32 \pm 0.04	28 \pm 13.04	12.00 \pm 1.22	5.20 \pm 1.92*
50	0.26 \pm 0.04	46 \pm 15.17**	13.20 \pm 1.79	2.20 \pm 1.30**
100	0.11 \pm 0.09**	86 \pm 13.42**	15.30 \pm 7.42	0.60 \pm 0.89**
500	0.28 \pm 0.13**	98 \pm 4.47**	N	N
1000	N	100 \pm 0.00**	N	N
5000	N	100 \pm 0.00**	N	N
ConA				
Concentration (μ g/ml)	Weight (Mean)	Mortality (%)	Development time (day)	Fecundity (Number of new nymph)
0	0.38 \pm 0.03	19 \pm 3.93	10.80 \pm 0.84	8.80 \pm 2.59
10	0.34 \pm 0.01	30 \pm 12.25	11.20 \pm 1.10	6.00 \pm 2.12
50	0.31 \pm 0.02	32 \pm 10.95	12.40 \pm 1.14	4.20 \pm 1.79*
100	0.20 \pm 0.03**	42 \pm 13.04*	13.40 \pm 1.67	1.80 \pm 1.10**
500	0.20 \pm 0.03**	60 \pm 15.81**	15.00 \pm 8.22*	0.60 \pm 0.89**
1000	0.12 \pm 0.11**	92 \pm 10.95**	N	N
5000	0.11 \pm 0.08**	96 \pm 8.94**	N	N

N: no observation; *: P > 0.05; **: P > 0.001

Dunett test: was used to compare the significant difference between the applied doses in each lectin

Tukey test: was used to compare the significant difference between the applied same doses in each lectin

DISCUSSION

Previous studies demonstrated that artificial diet with combination of amino acid, mineral and vitamin was appropriate for biological parameters of *M. persicae* (Dadd and Mittler, 1996). We have previously demonstrated that XCL in moderate and high concentrations induced significant mortality on *M. persicae* when it was compared with control (Karimi *et al.*, 2006). The ingestion of ConA with different ranges of concentrations (0.02-2.0% of total protein) in artificial diet was used. When *M. persicae* fed in liquid artificial diet with ConA, aphid size was reduced by up to 30%, retarded development, mortality and reduced fecundity by 35% but had little effect on survival (Gatehouse *et al.*, 1999). Sauvion *et al.* (1996) reported in the use of highest concentration (1500 μ g/ml) of GNA on *M. persicae* the mortality rate was observed by 19% and 58% after 3 days and 8 days respectively. In this study, we found that artificial diets incorporating with different concentrations of XCL influence significantly on *M. persicae* mortality after 7 days when it was compared with control. In moderate and high concentrations (100 to 5000 μ g/ml) of XCL, significant effects on *M. persicae* mortality were observed when compared with the control. For low concentrations (10 to 50 μ g/ml) of ConA, no significant effect was observed on *M. persicae* mortality after 7 days. But in moderate and high concentrations (100 to 5000 μ g/ml), significant difference was observed. Sauvion *et al.*, (2004) reported that only ConA in high concentrations (800 to 1500 μ g/ml) had negative effects on *M. persicae* mortality. Therefore our result confirms this suggestion that is presented by Sauvion *et al.* (2004). As a result, ConA

only in higher concentrations have negative effects on *M. persicae* mortality. Result obtained from the effects of ConA on *A. pisum* showed that there were negative effects on the growth and survival of these aphids (Rahbé *et al.*, 1995; Sauvion *et al.* (2004). Rahbé *et al.* (1995); Sauvion *et al.* (1996) demonstrated that the weight of *M. persicae* nymph at the higher concentration (>1000 µg/ml) of ConA and GNA was reduced. In this study, result shows that *M. persicae* weights were significantly influenced by different concentrations of XCL in different times when compared with the control. Consequently, *M. persicae* weights were significantly reduced in comparison with control after 7 days.

Our result shows that no significant difference was observed among concentrations of 10 to 100 µg/ml of XCL on *M. persicae* development time. But for high concentrations (1000 to 5000 µl/ml), significant difference was observed. Down *et al.* (1996) reported that snowdrop lectin (GNA) inhibited development and decreased fecundity of the potato aphid (*Aulacorthum solani*) when administered in vitro and via transgenic plants both in laboratory and glasshouse trial. In this study, *M. persicae* development time increased gradually with increasing concentrations of XCL and decreased their fecundity. Sauvion *et al.* (1996) and Fitches *et al.* (1997) showed that the effects of snowdrop lectin (*Galanthus nivulius* agglutinin, GNA) on *A. solani* were tested by bioassays, where the protein was incorporated in an artificial diet at a single concentration of 0.1% (w/v). The presence of GNA in the diet throughout insect life decreased the fecundity of adult aphids, as measured by nymph production, by up to 65%, but normally caused only a marginal decrease (~10%) in aphid survival. The presence of GNA in the diet decreased the rate of length and weight of aphids by up to 40% (Down *et al.*, 1996). We found that with increasing concentration of lectin (XCL or ConA), the weight nymphs were decreased. Our result demonstrates that for low concentrations (50 to 500 µl/ml), XCL influenced more than the ConA towards *M. persicae*. However, the effects of XCL and ConA in high concentrations (1000 to 5000 µg/ml) were similar. XCL induced high mortality rates in several insect species from different orders (Wang *et al.*, 2002). Trigueros *et al.* (2003) demonstrated that XCL is highly toxic towards two insect models (*D. melanogaster*, *A. pisum*) when tested on drosophila, this lectin exhibited a higher insecticidal activity than the GNA lectin, which is one of the most toxic lectins to insects. Our result showed that the development time of *M. persicae* was increased by low concentrations of XCL and by moderate concentrations of ConA. Therefore, our results showed that in low and moderate concentration XCL was more influence than the ConA on biological parameters of *M. persicae*. Consequently XCL was one of the important lectin with property of insecticide, especially for their property of aphicid.

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