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Influence of biotin binding proteins on growth and development of *Tribolium castaneum* Herbst and *Corcyra cephalonica* Stainton

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Abstract
Biotin binding proteins like avidin and streptavidin bind to dietary biotin, making it unavailable to the insects, which then die from a deficiency of this vitamin. Avidin is found in chicken egg white and streptavidin is present in culture supernatant of *Streptomyces avidinii* (Stapley). When these biotin binding proteins were fed by mixing with flour against *T. castaneum* and *C. cephalonica*, dose dependent changes in growth parameters was observed. Avidin @ ≥ 10 ppm and streptavidin @ ≥ 7.5 ppm were found to be effective against *T. castaneum* with zero development period, adult emergence and growth index and in case of *C. cephalonica*, avidin ≥ 25 ppm and streptavidin ≥ 10 ppm recorded adult emergence, growth index and development period as zero.

Keywords: biotin, avidin, streptavidin, *Tribolium castaneum* and *Corcyra cephalonica*

1. Introduction

Biotin binding proteins like avidin and streptavidin bind to dietary biotin, making it unavailable to the insects, which then die from a deficiency of this vitamin. Avidin is a water soluble tetrameric glycoprotein found in chicken egg white and streptavidin is a non-glycosylated protein present in culture supernatant of *Streptomyces avidinii* (Stapley) (Bayer *et al*., 1990) [1] and these biotin binding proteins bind tightly to vitamins. They act as anti-nutritional proteins by inhibiting insect growth and causing biotin deficiency. The equilibrium dissociation constant of both avidin and streptavidin for each interaction is ≈ 10^{-15} M (Green, 1990) [4], the smallest value for any known biological bimolecular interaction. This value indicates that biotin binding is essentially complete at equimolar concentrations and irreversible, which is one of the property responsible for their insecticidal character.

The effects of avidin on insects were first studied by feeding antimetabolites to insects and other organisms to determine the nutritional requirements for their growth and development. Levinson and Bergmann (1959) [6] observed the vitamin deficiency in housefly produced by treating with egg albumin containing avidin and observed 37 % survival with emergence of few deformed adults. Morgan *et al*. (1993) [10] reported the insecticidal and growth inhibiting properties of avidin against five species of Coleoptera and two species of Lepidoptera. Insects currently shown susceptible to avidin belongs to five major insect orders (Lepidoptera, Diptera, Hymenoptera, Coleoptera and Orthoptera) and numerous families within those orders. Kramer *et al*. (2000) [5] reported that, the flour and meal made from transgenic avidin maize showed resistance to various insects, and observed > 90 % mortality of the lesser grain borer *Rhyzopertha dominica* (Fab.), sawtoothed grain beetle *Oryzaephilus surinamensis* (L.), red flour beetle *Tribolium castaneum* Herbst, confused flour beetle *T. confusum* Jacquelin du Val, flat grain beetle *Cryptolestes pusillus* Schonherr, Indianmeal moth *Plodia interpunctella* (Hubner), and Mediterranean flour moth *Anagasta kuehniella* Zeller and observed the suppressed larval development of warehouse beetle *Trogoderma variabile* Ballion.

In the present study, effect of avidin and streptavidin on the growth & development of *T. castaneum* and *Corcyra cephalonica* Stainton was evaluated.

2. Material and Methods

2.1 Chemicals

Biotin binding proteins- avidin and streptavidin were procured from the SRL chemicals and Himedia, Vijayawada, Andhra Pradesh, India and tested against the test insects by mixing with flour against *T. castaneum* and *C. cephalonica*. Stock solution of 1000 ppm avidin and streptavidin was prepared by using sterile distilled water.

2.2 Rice flour mixing and infestation

Milled rice flour was taken and sterilized, then 10g of flour in three replications were mixed with one ml of 10 times the required concentration of avidin and streptavidin. Avidin at 1.5, 2.5, 5, 7.5 and 10 ppm were tested against *T. castaneum* and at 0.5, 1.5, 5, 10, 25 and 50 ppm against *C. cephalonica*. Streptavidin was tested at 1.5, 2.5, 5 and 7.5 ppm against *T. castaneum* and 0.5, 1.5, 3, 5, 10 and 25 ppm against *C. cephalonica* along with spinosad 270 and 700 ppm. Treated flour was dried and ground to fine powder using pestle and mortar. Ten grams of treated flour is taken in a glass vial in three replications into which 25 neonate larvae of *T. castaneum* or 25 eggs of *C. cephalonica* were released.

Untreated control was maintained without avidin or streptavidin. The treated flour was kept for incubation at room temperature (32 ± 1ºC) and relative humidity (75 ± 2%) for further development of the pest. The larval weights were recorded at 17th day for *C. cephalonica* and 20th day for *T. castaneum*.

2.2.1 Adult Emergence
The treatments were checked daily for adult emergence data. The day of first adult emergence was noted. From then onwards, the number of adults that emerged were counted and separated every day till the emergence of adults ceased, after which the total number of adults emerged was pooled and the per cent adult emergence was calculated by using the following formula.

$$\text{Per cent adult emergence} = \frac{\text{Total no.of adults emerged}}{\text{Number of eggs/neonate larvae in the sample}} \times 100$$

2.2.2 Development period
The development period was recorded from the day of egg laying upto cessation of adult emergence.

2.2.3 Growth index
The growth index was calculated with these two parameters as follows,

$$\text{Growth index} = \frac{\text{\% Adult emergence}}{\text{Developmental period in days}}$$

2.2.4 Weight Loss
Weight loss was calculated by deducting the final weight of sample from initial weight and then converted to the percentage.

$$\text{Weight loss} = \frac{W1 - W2}{W1} \times 100$$

Where,
W1 = initial weight of sample
W2 = final weight of sample

2.3 Data analysis
The parameters *viz*., growth index, larval weight and weight loss were subjected to square root transformations, day of first adult emergence and development period were subjected to logarithm transformations, while per cent adult emergence was transformed into arc sine transformation and then subjected to analysis of variance (ANOVA) in completely randomized block design (CRBD) with three replications for the test of significance and calculation of critical differences (Snedecor and Cochran, 1967) [12].

3. Results
The results of growth & development of *T. castaneum* and *C. cephalonica* are furnished hereunder.

3.1 *T. Castaneum*
3.1.1 Day of first adult emergence
T. castaneum when released into the treated flour showed a significant difference regarding the first adult emergence. The earliest first adult emergence was observed at lower concentration (1.5 ppm) of avidin and streptavidin which were on par with the untreated control by recording the first adult emergence on 30th, 30th and 32nd day respectively and these differed significantly with other treatments (Table 1).

Zero adult emergence was observed in spinosad at concentrations of 270 and 700 ppm along with 10 ppm avidin and 7.5 ppm streptavidin respectively (Fig 1). Delayed and similar first adult emergence was noticed in the slightly higher concentrations of avidin (7.5 ppm) and streptavidin (5 ppm) with their respective adult emergence on 64th and 63rd day and differed significantly from other treatments. Streptavidin @ 2.5 ppm was found to be similar with avidin 5 ppm regarding the first adult emergence on 46th and 49th day and these differed significantly with 2.5 ppm avidin recording the first adult emergence slightly earlier on 38th day respectively.

3.1.2 Development Period
A significant difference in the development period of *T. castaneum* was observed among all the treatments. Spinosad treated flour along with 10 ppm avidin and 7.5 ppm streptavidin showed zero development period as no adults of *T. castaneum* emerged in these treatments. The larvae in the above avidin (10 ppm) and streptavidin (7.5 ppm) treatments turned black in colour and died without development (Table 1).

The longest development period was observed in 1.5 and 2.5 ppm avidin with 67.67 and 65.67 days respectively, which were found similar with each other and differed significantly with 56.33 days development period at 5 ppm avidin (Fig 1).

Streptavidin @ 1.5, 2.5 and 5 ppm, avidin 7.5 ppm and untreated control were on par and showed a similar development period of 60, 62.67, 63, 64 and 59.67 days respectively which differed significantly from the zero development period found in higher concentrations of spinosad, avidin (10 ppm) and streptavidin (7.5 ppm).

Avidin 5 ppm has lesser development period (56.33 days) than the untreated control (59.67 days) because the total adults emerged were less in avidin treated flour (0.93 per cent) when compared to the untreated control (55.43 per cent).

At 7.5 ppm avidin, the development period is same as that of the first adult emergence because after the first adult emergence at 64th day, there was no further adult emergence in this treatment.

Even among streptavidin treated flour, a similar development period is observed in 1.5, 2.5 and 5 ppm concentrations because in all these treatments a significant decrease in the adult emergence of 50.4, 23.5 and 0.7 per cent respectively is observed at these concentrations.

These results obtained were in accordance with the findings of Kramer *et al*. (2000) [5], wherein he reported that when several species of beetles were fed with avidin maize grains expressing 100 ppm and above concentrations, these insects failed to develop, with no pupation and showed significantly higher mortality.

The darker colour of the dead larvae seen in the experiment may be due to the reason that the larvae were unable to successfully complete the process of moulting from one instar to another. Larvae appeared to stop feeding during the ecdysis process and then turn black and died while still attached to the partially shed larval skin (Burgess *et al*., 2002) [2].

3.1.3 Adult Emergence
Adult emergence of *T. castaneum* was found to be significant among all the treatments observed. The highest number of adult emergence was observed in the untreated control with 55.43 per cent followed by the lower concentration (1.5 ppm) of streptavidin and avidin recording 50.44 and 47.53 per cent adult emergence respectively which differed significantly with one other and also among the other treatments.

Zero adult emergence was noticed in the higher concentrations of spinosad (270 and 700 ppm) along with avidin (10 ppm) and streptavidin (7.5 ppm) respectively (Table 1).

The adult emergence at 5 ppm streptavidin was 0.7 per cent which is on par with 5 and 7 ppm avidin with 0.93 and 0.5 per cent adult emergence respectively and differed significantly from other treatments. The adult emergence observed at 2.5 ppm of both avidin and streptavidin was 33.81 and 23.5 per cent respectively, which differed significantly with all the treatments and also among each other (Fig 1).

These results of decrease in adult emergence with increase in concentrations of avidin and streptavidin obtained in the present study were in conformity with the findings of Yoza *et al*. (2005) [13], where *S. cerealella*, reared on avidin rice of 1800 ppm resulted in zero adult emergence when compared to 23 adults in the untreated control.

3.1.4 Growth Index
The results revealed a significant difference in growth index of *T. castaneum* among the treatments.The growth index of *T. castaneum* was found to be the highest in the untreated control with 0.92 followed by both streptavidin and avidin at 1.5 ppm with 0.84 and 0.7 growth index respectively (Table 1).

Zero growth index was observed in spinosad treated flour at 270 and 700 ppm and also at higher concentrations of avidin (10 ppm) and streptavidin (7.5 ppm), as there was no adult emergence. These treatments were found to be on par with next higher concentrations of avidin (5 and 7.5 ppm) and streptavidin (5 ppm) with growth index of 0.02, 0.01and 0.01 respectively (Fig 1).

The growth index recorded at 2.5 ppm of both avidin and streptavidin was 0.52 and 0.38 is found to be significantly different among all the treatments.

The results obtained regarding the growth index of *T. castaneum* were supported by Morgan *et al*. (1993) [10] where it is concluded that when avidin and streptavidin were added to wheat germ and fed to *T. castaneum*, larval growth rates were substantially slower than that of control and at concentrations of 100 and 1000 ppm of avidin caused severe growth inhibition and about 70 and 90 per cent mortality respectively.

3.1.5 Larval Weight
A significant difference was observed regarding the larval weights (n=10) among all the treatments recorded at 20 days interval. The highest larval weight of 24.43 mg was recorded in the lower concentration (1.5 ppm) of streptavidin which was similar with 23.37 mg larval weight recorded in the untreated control and these differed significantly with other treatments.

The lowest larval weight of 2.63 mg was seen in 700 ppm of spinosad, followed by 270 ppm spinosad and 7.5 ppm streptavidin with 8.27 and 12.07 mg larval weight respectively (Table 1). Avidin @ 1.5, 2.5, 5, 7.5 and 10 ppm recorded 23.23, 20.57, 20.83, 22.43 and 20.5 mg larval weight which were found non-significant with each other. Streptavidin @ 2.5 ppm recorded 18.3 mg larval weight which was on par with 17.53 mg larval weight at 5 ppm streptavidin and also with all the treatments of avidin. Among streptavidin treated pellets, gradual decrease in larval weight is observed in case of *T. castaneum* from concentrations ranging from 1.5 to 7.5 ppm respectively (Fig 1).

The results of lower larval weight seen at relatively higher concentrations of avidin and streptavidin were in conformity by Morgan *et al*. (1993) [10]. He reported that the larval weight of *T. castaneum* at 1000 ppm of avidin and streptavidin (0.7 and 0.57 mg) were significantly lower when compared to the untreated control (2.55 mg).

3.1.6 Weight Loss
Among all the treatments, the highest weight loss of 27.36 per cent was recorded in the lowest concentration (1.5 ppm) of avidin, which was on par with the untreated control (27.17 per cent) and 2.5 ppm avidin (26.71 per cent) respectively (Table 1). Zero weight loss was seen in the spinosad treated flour as there was zero adult emergence and minimum larval weights, followed by streptavidin 7.5 ppm with the lowest weight loss of 2.63 per cent which differed significantly among all the treatments. Though there was zero adult emergence at streptavidin 7.5 ppm, some larvae could survive and recorded minimum larval weight of 12.07 mg which could cause 2.63 per cent weight loss (Fig 1).

Streptavidin @ 1.5 ppm recorded a weight loss of 15.78 per cent which is similar with 19.07 and 16.8 per cent weight loss recorded at 7.5 and 10 ppm avidin respectively and was on par with avidin 5 ppm with 21.17 per cent weight loss. Streptavidin @ 2.5 and 5 ppm recorded 11 and 6.17 per cent weight loss which differed significantly among all the treatments.

A significant decrease in weight loss was observed among the streptavidin treated flour when the concentrations increased from 1.5 to 7.5 ppm respectively. Streptavidin is found effective when compared to avidin, where the weight loss recorded at 1.5 ppm streptavidin showed no significance with the weight loss recorded at slightly higher concentrations of avidin at 7.5 and 10 ppm respectively.

3.2 *C. Cephalonica*
3.2.1 Day of first adult emergence
The results revealed that the first adult emergence of *C. cephalonica* varied significantly among all the treatments. The first adult emergence was recorded on 59th day in the untreated control which was found to be similar with streptavidin 0.5 ppm which recorded first adult emergence on 63rd day and differed significantly with other treatments (Table 2).

Zero adult emergence was noticed in spinosad 270 and 700 ppm, avidin 25 and 50 ppm and streptavidin 10 and 25 ppm (Fig 2).

Among avidin treated flour, shortest day of first adult emergence of *C. cephalonica* was recorded on 65th day at 0.5 ppm avidin which differed significantly with other treatments, whereas avidin @ 1.5 and 5 ppm recorded first adult emergence on 95th and 91st day which were similar with each other and were on par with 10 ppm avidin recording first adult emergence on 84th day, respectively.

Among streptavidin treated flour, shortest day of first adult emergence was seen at 0.5 ppm which was on par with 66th day of first adult emergence recorded at 1.5 ppm streptavidin and these were followed by 76th day and 93rd day of first adult emergence recorded at 5 and 3 ppm streptavidin respectively.

The first adult emergence of *C. cephalonica* recorded at 1.5 ppm streptavidin was found similar to 0.5 ppm avidin and the first adult emergence recorded at 3 ppm streptavidin was found similar to 1.5 and 3 ppm avidin respectively.

3.2.2 Development Period

The development period of *C. cephalonica* was found to be significant among all the treatments.The shortest development period of 76.33 days was recorded at streptavidin 5 ppm which was equal to the first adult emergence recorded in this treatment, because only one deformed adult emerged in this treatment. The development period recorded above was similar with the development period of 80.67 days at lower concentration of streptavidin 1.5 ppm and on par with streptavidin 0.5 ppm recording 83 days respectively (Table 2).

Zero development period was recorded at higher concentrations of avidin (25 and 50 ppm) and streptavidin (10 and 25 ppm) along with spinosad (270 and 700 ppm), as there were no adults emerged in these treatments.

No significant difference was noticed among 0.5, 1.5, 5 and 10 ppm avidin with delayed development period of 106, 107.33, 109.33 and 112 days respectively which were on par with 101.67 days development period recorded at streptavidin 3 ppm.

The development period observed in the untreated control (92.67 days) was found to be on par with lower concentrations of streptavidin at 0.5 and 3 ppm by recording 83 and 101.67 days development period respectively (Fig 2).

The observations of delayed and zero development period obtained in the present study were in accordance with in findings of Morgan *et al*. (1993) [10] where the Indianmeal moth (*P. interpunctella*) when fed with 100 and 1000 ppm avidin incorporated wheat germ, prevented the development and resulted in complete inhibition of the larval development.

3.2.3 Adult Emergence

Regarding the adult emergence of *C. cephalonica* on the treated flour, a significant difference was observed among all the treatments. Untreated control recorded significantly highest number of adult emergence *i.e*., 25.33 per cent, followed by avidin 0.5 ppm with 18.66 per cent adult emergence respectively.

Zero adult emergence was noticed in higher concentrations of spinosad 270 and 700ppm, avidin 25 & 50 ppm and streptavidin 10 & 25 ppm respectively (Table 2).

Lowest adult emergence of 4 per cent was noticed in 5 ppm streptavidin which was on par with 5.33 per cent adult emergence recorded at 10 ppm avidin and differed significantly from other treatments.

Among avidin treated flour, 0.5 ppm recorded highest number (18.66 per cent) of *C. cephalonica* adults followed by 14.66, 8 and 5.33 per cent adults recorded at 1.5, 5 and 10 ppm avidin respectively.

Among streptavidin treated flour, maximum adult emergence of *C. cephalonica* (12 per cent) was noticed at 0.5 ppm followed by fairly lower adult emergence of 8, 6.66 and 4 per cent at 1.5, 3 and 5 ppm respectively (Fig 2).When adult emergence was compared between avidin with streptavidin treated flour, the adult emergence recorded at 10 ppm avidin was on par with lower concentrations of 3 and 5 ppm streptavidin respectively.

The above results are indicating, a gradual decrease in the adult emergence with increase in concentrations of avidin and streptavidin, were in conformity with the findings of Morgan *et al*. (1993) [10] in case of the Rice weevil (*S. oryzae*) when reared on pellets made from whole wheat flour containing 100 ppm avidin, showed no adult emergence compared to 57 adults recorded in the untreated control.

3.2.4 Growth Index

The growth index of *C. cephalonica* varied significantly among all the treatments. The highest growth index of 0.27 with better growth was observed in the untreated control followed by 0.18 growth index at lower concentration of 0.5 ppm avidin respectively (Table 2). Zero growth index was observed at higher concentrations of 270 and 700 ppm spinosad, avidin 25 & 30 ppm, and streptavidin 10 and 25 ppm, respectively.

Among avidin treated flour, the highest growth index was recorded at 0.5 ppm concentration, followed by fairly lower growth index of 0.14, 0.07 and 0.05 noted at 1.5, 5 and 10 ppm avidin, respectively.

Among streptavidin treated flour, highest growth index of 0.14 was observed at 0.5 ppm streptavidin followed by growth index of 0.1, 0.06 and 0.04 recorded at 1.5, 3 and 5 ppm respectively. The growth index of *C. cephalonica* recorded in streptavidin treated flour were comparatively lower and found to be better when compared to avidin treated flour, where growth index at 3 ppm streptavidin was similar with growth index at 10 ppm avidin and the growth index at 0.5 ppm streptavidin was on par with 0.5 and 1.5 ppm avidin respectively (Fig 2).

Zero and negligible growth index obtained in the present study were in accordance with the findings of Markwick *et al*. (2001) [9] on *E. postvittana* larvae, where the larvae fed on diet containing 100 μg ml^{-1} avidin, observed after 28 days caused ≥ 90 per cent mortality with lesser growth when compared to control recording maximum growth with zero mortality per cent respectively.

3.2.5 Larval weight

The larval weights (n=6) recorded after 17 days differed significantly among all the treatments. Streptavidin @ 5 and 10 ppm recorded the highest larval weight of 27.17 and 26.37 mg which was non-significant with each other. It is observed that most of these larvae even though recorded the highest larval weight, showed mortality before pupation and turned black in colour.

The larval weight recorded in spinosad treatments (270 and

700 ppm) was zero since there was no larval development noticed in these treatments and at 270 ppm spinosad, a slight webbing was noticed initially with no further growth and development (Table 2).

Among avidin treated flour, no significance in larval weight noticed between 1.5 and 5 ppm and also between 0.5 and 10 ppm concentrations recording larval weight of 12.93, 13.07, 13.7 and 17.27 mg respectively. These treatments differed significantly with lowest larval weight of 5.5 mg recorded at highest concentration of 50 ppm avidin which was at par with 9.1 mg at 25 ppm avidin respectively (Fig 2).

Streptavidin @ 0.5, 1.5, 3 and 25 ppm were on par and recorded almost similar larval weight of 17, 18.4, 17.27 and 16.63 mg respectively and these larval weights were also found to be on par with the untreated control recording 17.63 mg larval weight. Among all the treatments observed, a decrease in the larval weight was noticed only after 25 ppm of both avidin and streptavidin respectively.

These results of larval weight loss recorded at higher concentrations were in accordance with the findings of Morgan *et al*. (1993) [10] wherein he demonstrated that the weight of the *Ostrinia nubilalis* larvae when fed on avidin and streptavidin incorporated diet showed significant reduction in larval weights of 2.5 and 4.3 mg at 100 ppm concentration compared to their respective control larval weights of 7.1 and 9.6 mg respectively.

3.2.6 Weight Loss

A significant difference in weight loss of rice flour was observed among all the treatments. Untreated control recorded the highest weight loss of 69.33 per cent due to *C. cephalonica* which differed significantly with other treatments.

There was no significant weight loss at 700 ppm spinosad, followed by 270 ppm spinosad with 3 per cent weight loss with slight larval webbing but no larval development. Fairly lower weight loss of 17.51 and 19.2 per cent was recorded at 50 ppm avidin and 25 ppm streptavidin which were at par with each other (Table 2).

Avidin @ 0.5, 1.5, 5, 10 and 25 ppm recorded 51.9, 62.5, 52.8, 64.1 and 42.05 per cent weight loss respectively, whereas streptavidin @ 0.5, 1.5, 3, 5 and 10 ppm recorded 31.2, 29.36, 27.34, 45.64 and 33.53 per cent respectively (Fig 2). It can further be concluded that the weight loss recorded by streptavidin treated flour were lower when compared to avidin treated flour, where weight loss recorded at 5 ppm streptavidin was on par with 25 ppm avidin.

4. Discussion

From the present investigation, it is clearly evident that biotin inhibitors *viz*., avidin 7.5 and 10 ppm, streptavidin 5 and 7.5 ppm against *T. castaneum*; and avidin 25 and 50 ppm, streptavidin 10 and 25 ppm against *C. cephalonica* have inhibited the growth and development by recording delayed first adult emergence, longer development periods, zero or least adult emergence, reduced larval weights and weight loss. During development, the larvae have showed reduced feeding, inactivity, failure to moult and pupation. It is also evident that insects affected by BBPs generally die during the moulting period (Markwick *et al*., 2001, Burgess *et al*., 2002) [9, 2].

This mortality depends on the concentration of biotin binding proteins, avidin and streptavidin which bind to biotin in the insect diet. They may not be able to remobilize the fat during ecdysis process. Similarly the larvae may die as they may not be able to cope with the high energy expenditure in the absence of active feeding. It is clearly known that biotin dependent carboxylases are required for the deposition of fat reserves during active feeding and for subsequent utilization of these reserves. Yoza *et al*. (2005) [13], stated that, avidin extracted from the transgenic rice kernel lost most biotin-binding activity after five min of heating at 95ºC. Active avidin was only 3% of non-heated avidin rice. A similar result was obtained with the authentic avidin. similar avidin produced in rice kernels lost most activity after heating, avidin rice might be usable as a food.

The larval weights of *T. castaneum* recorded after 20 days showed no significant difference among the avidin treated flour and decreased gradually among the streptavidin treated flour. In case of *C. cephalonica*, the larval weights recorded after 17 days increased gradually from 0.5 to 10 ppm avidin and 0.5 to 10 ppm streptavidin and a decrease in larval weights was noticed from 25 ppm of avidin and streptavidin respectively. At higher concentrations of BBPs, the increase in larval weights observed may be due to more consumption of food to overcome the deficiency of biotin. Beyond these concentrations of BBPs, there was decrease in larval weight which might be due to increased effect of BBPs as well as low fat metabolism. The effects of avidin and/ or streptavidin have so far been investigated on non-target organisms, and found that the earthworms, honeybees, nematodes parasitoid and mice were not affected when fed with transgenic avidin or in the form of semi artificial diet containing avidin fed to them (O’Callaghan *et al*., 2008; Malone *et al*., 2002a, 2002b; Flinn *et al*., 2006; Kramer *et al*., 2000) [11, 5, 7, 8, 3].

Table 1: Effect of avidin and streptavidin on the growth and development of *T. castaneum*

S. No.	Treatments (ppm)	Day of first Adult Emergence (in days)***	Development Period (in days)***	Adult Emergence (%)*	Growth Index**	Larval Weight (in mg)**	Weight Loss (%)**
1.	Avidin 1.5 ppm	30.33 (1.50)d	67.67(1.84)a	47.53(43.56)c	0.70(1.10)c	23.23(4.80) ab	27.36(5.28)a
2.	Avidin 2.5 ppm	38(1.59)c	65.67(1.82)a	33.81(35.53)d	0.52(1.01)d	20.57(4.53) abc	26.71(5.22) ab
3.	Avidin 5.0 ppm	49.33(1.70)b	56.33(1.76)b	0.93(5.53)f	0.02(0.72)f	20.83(4.56) abc	21.17(4.65) bc
4.	Avidin 7.5 ppm	64(1.81)a	64(1.81)ab	0.50(4.04)g	0.01(0.71)f	22.43(4.73) abc	19.07(4.42) c
5.	Avidin 10 ppm	0(0)e	0(0)c	0(0)h	0.00(0.71)f	20.50(4.52) abc	16.8(4.15) c
6.	Streptavidin 1.5 ppm	30(1.50)d	60(1.79)ab	50.44(45.23)b	0.84(1.16)b	24.43(4.94) a	15.78(4.03) c
7.	Streptavidin 2.5 ppm	46.33(1.67)b	62.67(1.80)ab	23.50(28.97)e	0.38(0.94)e	18.30(4.26)bc	11(3.38)d

8.	Streptavidin 5.0 ppm	63.67(1.81)^{a}	63(1.81)^{ab}	0.70(4.79)^{fg}	0.01(0.71)^{f}	17.53(4.19)^{c}	6.17(2.44)^{e}
9.	Streptavidin 7.5 ppm	0(0)^{e}	0(0)^{c}	0(0)^{h}	0.00(0.71)^{f}	12.07(3.45)^{d}	2.63(1.73)^{f}
10.	Spinosad 270 ppm	0(0)^{e}	0(0)^{c}	0(0)^{h}	0.00(0.71)^{f}	8.27(2.86)^{e}	0(0.71)^{g}
11.	Spinosad 700 ppm	0(0)^{e}	0(0)^{c}	0(0)^{h}	0.00(0.71)^{f}	2.63(1.62)^{f}	0(0.71)^{g}
12.	Control	32.33(1.52)^{d}	59.67(1.78)^{ab}	55.43(48.10)^{a}	0.92(1.20)^{a}	23.37(4.83)^{a}	27.17(5.26)^{ab}
	SEm (±)	0.02	0.02	0.51	0.01	0.19	0.21
	CD (P=0.05)	0.067	0.06	1.47	0.03	0.54	0.61
	CV %	3.65	3.02	4.87	1.80	7.92	10.49

* Values in parenthesis are angular transformed values

** Values in parenthesis are square root transformed values

*** Values in parenthesis are logarithmic transformed values

In each column mean values with similar alphabet do not vary significantly at P=0.05 using Fisher's protected LSD. Analysis was done with ANOVA in CRBD.

Table 2: Effect of avidin and streptavidin on the growth and development of *C. cephalonica*

S. No.	Treatments (ppm)	Day of first adult emergence (in days)***	Development period (in days)***	Adult emergence (%)*	Growth index**	Larval weight (mg) **	Weight loss (%) **
1.	Avidin 0.5 ppm	65.67(1.82)^{cd}	106(2.03)^{a}	18.67(25.56)^{b}	0.18(0.82)^{b}	13.7(3.75)^{b}	51.90(7.22)^{bc}
2.	Avidin 1.5 ppm	95.33(1.98)^{a}	107.33(2.03)^{a}	14.67(22.50)^{c}	0.13(0.79)^{c}	12.93(3.66)^{bc}	62.50(7.94)^{ab}
3.	Avidin 5 ppm	91.67(1.97)^{a}	109.33(2.04)^{a}	8.00(16.42)^{e}	0.07(0.76)^{de}	13.07(3.67)^{bc}	52.88(7.29)^{bc}
4.	Avidin 10 ppm	84.33(1.92)^{ab}	112(2.05)^{a}	5.33(13.16)^{fg}	0.05(0.74)^{ef}	17.27(4.17)^{b}	64.10(8.03)^{ab}
5.	Avidin 25 ppm	0(0)^{e}	0(0)^{e}	0(0)^{h}	0(0.71)^{g}	9.1(3.06)^{cd}	42.05(6.45)^{cde}
6.	Avidin 50 ppm	0(0)^{e}	0(0)^{e}	0(0)^{h}	0(0.71)^{g}	5.5(2.44)^{d}	17.51(4.14)^{h}
7.	Streptavidin 0.5 ppm	63.33(1.81)^{d}	83(1.92)^{cd}	12.00(20.14)^{d}	0.14(0.80)^{bc}	17(4.18)^{b}	31.2(5.61)^{ef}
8.	Streptavidin 1.5 ppm	66.33(1.83)^{cd}	80.67(1.91)^{d}	8.00(16.42)^{e}	0.10(0.77)^{d}	18.4(4.32)^{b}	29.36(5.37)^{fg}
9.	Streptavidin 3 ppm	93.33(1.97)^{a}	101.67(2.01)^{ab}	6.66(14.89)^{ef}	0.06(0.75)^{ef}	17.27(4.21)^{b}	27.34(5.27)^{fg}
10.	Streptavidin 5 ppm	76.33(1.88)^{bc}	76.33(1.88)^{d}	4.00(11.53)^{g}	0.04(0.73)^{f}	27.17(5.26)^{a}	45.64(6.77)^{cd}
11.	Streptavidin 10 ppm	0(0)^{e}	0(0)^{e}	0(0)^{h}	0(0.71)^{g}	26.37(5.17)^{a}	33.53(5.83)^{def}
12.	Streptavidin 25 ppm	0(0)^{e}	0(0)^{e}	0(0)^{h}	0(0.71)^{g}	16.63(4.08)^{b}	19.20(4.41)^{gh}
13.	Spinosad 270 ppm	0(0)^{e}	0(0)^{e}	0(0)^{h}	0(0.71)^{g}	0(0.71)^{e}	3(1.81)^{i}
14.	Spinosad 700 ppm	0(0)^{e}	0(0)^{e}	0(0)^{h}	0(0.71)^{g}	0(0.71)^{e}	0(0.71)^{j}
15.	Control	59.67(1.78)^{d}	92.67(1.97)^{bc}	25.33(30.19)^{a}	0.27(0.88)^{a}	17.63(4.25)^{b}	69.33(8.35)^{a}
	SEm (±)	0.02	0.016	0.710	0.01	0.23	0.34
	CD (P=0.05)	0.06	0.049	2.051	0.02	0.682	0.998
	CV %	3.24	2.47	10.79	1.69	11.43	10.53

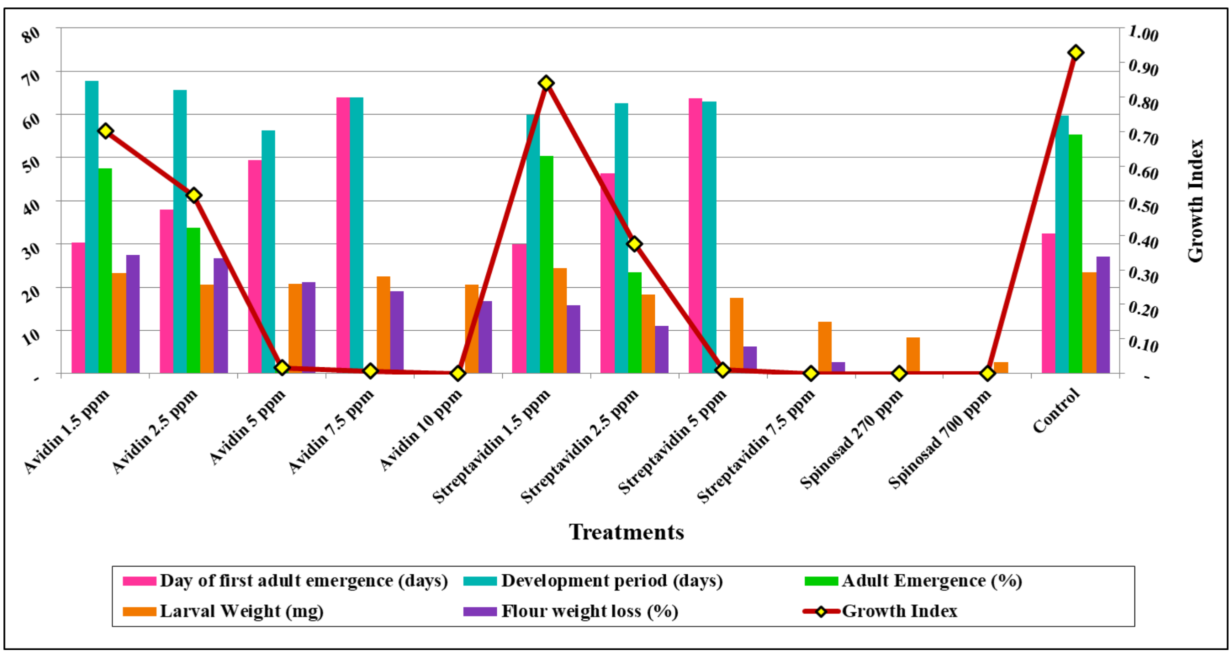

Fig 1: Effect of avidin and streptavidin on the growth and development of *T. castaneum*

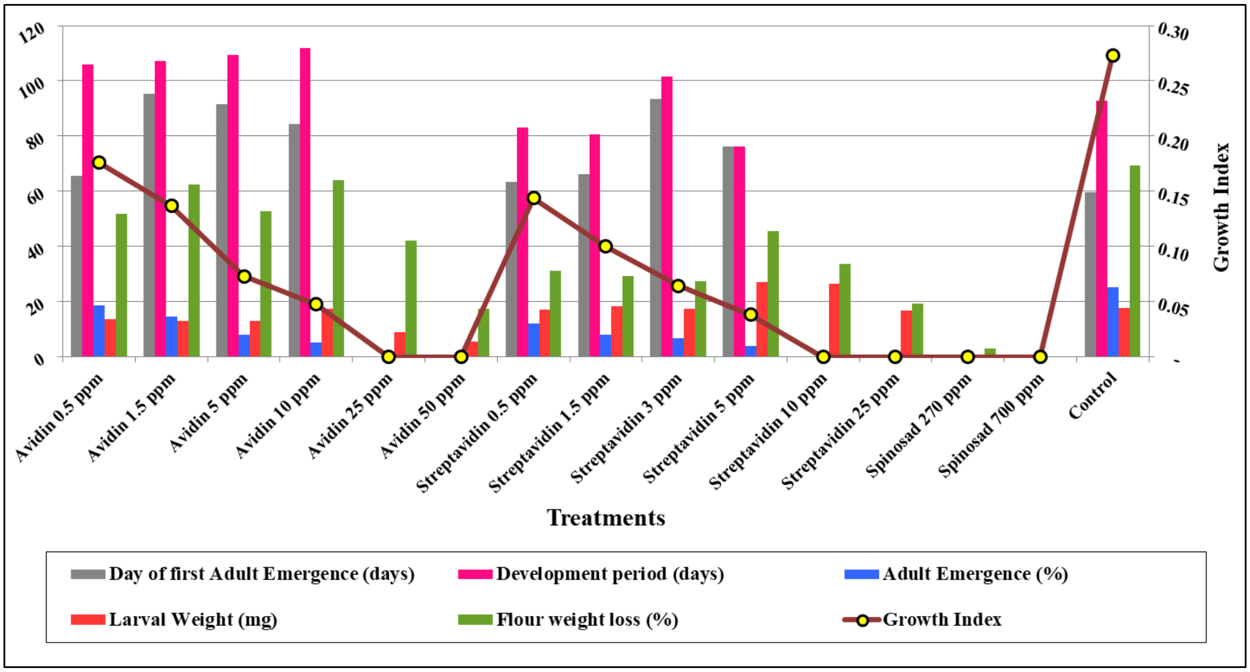

Fig 2: Effect of avidin and streptavidin on the growth and development of *C. cephalonica*

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