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Biotin Inhibitors, Avidin and Streptavidin: Their Influence on Lethality, Growth and Survival of *Callasobruchus maculatus* (Chrysomelidae: Coleoptera)

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ABSTRACT

Background: *C. maculatus*, the most important and cosmopolitan pest, severely attacking several stored pulses leading to weight loss, market value, as well as the germination potential of the infested grains. The present study was conducted to know the effect of biotin inhibitors, avidin and streptavidin on lethality, growth and survival of *Callasobruchus maculatus.*

Methods: The experiment was conducted in the laboratory of Department of Entomology, Agricultural College, Bapatla during 2017-18. Bio assays were conducted against *C. maculatus* with biotin binding proteins' (BBP's) *viz.,* avidin and streptavidin in comparison with spinosad by pellet feeding method. Similarly the effect of BBP's on growth development of *C. maculatus* was also studied.

Result: Based on bioassay studies, the LC_{50} for avidin and streptavidin were 14.733 and 11.192 ppm while LC_{90} was 76.031 and 86.134 ppm, respectively. The growth and development of *C. maculatus* was studied at approximately the respective LC_{25}, LC_{50}, LC_{75}, LC_{80} and LC_{90} values of avidin and streptavidin. Avidin and streptavidin incorporated pellets when fed to it, avidin at concentration of ≥ 75 ppm was found effective by recording lesser number of eggs (≤ 9 eggs), delayed day of first adult emergence (≥ 53 days), maximum development period (> 68 days), minimum adult emergence (≤ 13%), lower growth index (≤ 0.2) and minimum weight loss (≤ 16%) and streptavidin at concentrations of ≥ 75 ppm recorded comparatively minimum eggs (≤ 7 eggs), prolonged day of first adult emergence (≥ 66 days), maximum development period (≥ 82 days), minimum adult emergence (≤ 13%), lower growth index of ≤ 0.16 and minimum pellet weight loss of ≤ 12% respectively.

Key words: Avidin and streptavidin, Biotin binding proteins' (BBP's), Biotin inhibitors, *Callasobruchus maculatus*.

INTRODUCTION

Green revolution has increased food grain production in India to about 230 million tons of food grains. But Post-harvest losses in India amounts to 12 to 16 million metric tons of food grains each year, an amount that the World Bank estimates could feed one-third of India's poor. The monetary value of these losses amounts to more than Rs. 50,000 crores per year (Singh, 2010). Among these food grains, pulses are referred as "the poor man's meat", as they serve as a low-cost protein to meet the needs of large section of the people are the second most important group of crops worldwide. Pulses are excellent sources of proteins (20-40%), carbohydrates (50-60%) and are fairly good sources of thiamine, niacin, calcium and iron. One of the major constraints in the production of pulses are the insect pests and pulse beetle, *C. maculatus* is regarded as the most important and cosmopolitan pest, severely attacking several stored pulses leading to weight loss, market value, as well as the germination potential of the infested grains (Booker, 1967).

Biotin is an essential nutrients for vertebrates and insects which is also known as vitamin H or coenzyme R, is a water-soluble B-vitamin (vitamin B7). It is a co-factor of major carboxylases involved in lipogenesis, gluco neogenesis, fatty acid and amino acid catabolism, required for normal cellular metabolism and growth (Alban *et al.*, 2000), but its synthesis occurs only in plants, bacteria and certain fungi.

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Biotin is composed of a ureido (tetrahydroimidizalone) ring fused with a tetrahydrothiophene ring. A valeric acid substituent is attached to one of the carbon atoms of the tetrahydrothiophene ring. 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 nonglycosylated protein present in culture supernatant of *Streptomyces avidinii* (Stapley) (Bayer *et al.*, 1990) and these biotin binding proteins bind tightly to vitamins. They act as anti-nutritional proteins by inhibiting insect growth and causing biotin deficiency. The use of various synthetic insecticides, which

are considered as the source of serious health and ecological problems, such as development of pest strains resistant to pesticides, toxicity to mammals and detection of residues in human food can no longer reliable. Hence this experiment was conducted to know the effect of biotin inhibitors, avidin and streptavidin on lethality, growth and survival of *C. maculatus* which may be thought of as an alternative tool.

MATERIALS AND METHODS

Rearing of the test insect

The population *Callosobruchus maculatus* F. were maintained in the Laboratory of Department of Entomology, Agricultural College, Bapatla. Black gram grains were kept in an oven for one hour at 50°C for disinfestation. Black gram grains (250 g) were taken separately in jars (each of volume 500 ml) and 10 pairs of adults of *C. macculatus* were released into each jar and after allowing mating and oviposition for one week, these adults were removed. These containers were covered with muslin cloth and kept at room temperature (32±1°C) and 75±2% relative humidity throughout the period of study. The adults which have emerged from this culture were utilized for conducting experiments as well as for subculturing. Sub culturing of these insects was done at 20 days intervals to meet the continuous supply of insects for experiments.

Chemicals

Biotin binding proteins-avidin and streptavidin SRL chemicals and Himedia, Vijayawada and tested against the test insect by pellet feeding method.

Bioassay

The test insects were subjected to suitable bioassay method to work out the LC_{25}, LC_{50} and LC_{90} values for *C. maculatus* against avidin and streptavidin. Different concentrations of avidin and streptavidin were prepared *viz.,* 5, 15, 30, 75 and 100 ppm of avidin and 5, 20, 30, 75 and 100 ppm of streptavidin against *C. maculatus*; These concentrations were fixed based on a preliminary study using 10, 50, 100, 150, 200 and 300 ppm where zero adult emergence of *C. maculatus* was recorded from 150 ppm of avidin/streptavidin onwards, Hence, based on the mortality of larvae in the broad range concentrations, a narrow range was fixed between 0 to 100 ppm for *C. maculatus*. Different concentrations of avidin and streptavidin as mentioned above were prepared by serial dilution technique from the stock solution using double sterile distilled water.

Pellet feeding method

In this method, milled bengalgram flour was taken and sterilized by keeping in hot air oven at 60°C for one hour. One gram of flour was mixed with one ml of required concentration of avidin or streptavidin to form a paste. This prepared paste was then dried completely for 24 hours and the dried paste was ground into powder by using pestle and mortar. This ground powder was hydrated to 10% moisture using double sterile water and this flour blend was made into four small pellets. These pellets were coated by dipping in 8% aqueous gelatin solution and air dried overnight (Plate 1) (Shade *et al.*, 1986 and Murdock and Shade (2008). Untreated control was maintained by preparing flour pellets without mixing avidin/streptavidin. Four pellets were taken in glass vial in five replications and two mating pairs of freshly emerged adults of *C. maculatus* were released for oviposition. The adults were removed after three days ensuring oviposition. The treated pellets were dissected after three weeks to record the number of dead and live grubs.

Statistical analysis

The mortality data of the test insects were subjected to Abbott's correction (Abbott, 1925) and then to probit analysis (Finney, 1971) using SPSS (Statistical Package for Social Sciences) to calculate (Median Lethal Concentration) LC_{25}, LC_{50} and LC_{90}, heterogeneity (c^2), intercept (a), slope of the regression line (b), regression equation and fiducial limits.

Effect of BBP's on grain damage, growth and development of *C. maculatus*

Biotin inhibitors *viz*., avidin and streptavidin were tested at different concentrations approximately at their respective LC_{25}, LC_{50}, LC_{75}, LC_{80} and LC_{90} values on growth and development of *C. maculatus*. Avidin 5, 15, 30, 75 and 100 ppm; streptavidin 5, 20, 30, 75 and 100 ppm, spinosad 70 and 250 ppm were tested along with untreated control in three replications to study their effect on growth and development of *C. maculatus*. The LC_{25} and LC_{50} values of



Plate 1: Bengal gram flour made into pellets for testing BBPs against *C. maculatus*.

spinosad were determined by conducting bioassay with spinosad as these values were not available from literature. The above treatments were tested by bengal gram pellet feeding method as outlined obove. Two mating pairs of freshly emerged adults of *C. maculatus* were released into each replication having four pellets prepared from one gram bengal gram flour. The adults were removed after one week. The treatments were kept at ambient temperature (32±1°C) and relative humidity (75±2%) for further development of the pest. The number of eggs laid by *C. maculatus* in each test treatment sample was counted at ten days after release by observing under the illuminated magnifying lens. Later these treatments were kept for observation under laboratory conditions till the emergence of adults and 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 was ceased, after which the total number of adults emerged was pooled and the per cent adult emergence, development period, growth index, per cent weight loss was calculated by the following formulae:

Per cent adult emergence =

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

$$\text{Growth index} = \frac{\text{\% Adult emergence}}{\text{Development period}}$$

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

Where:
W1 = Initial weight of sample.
W2 = Final weight of sample.

Stistical analysis

The parameters *viz.*, number of eggs laid, 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 transformations and then subjected to analysis of variance (ANOVA) in completely randomized block design (CRBD) with three replications for the test of significance and LSD (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

Lethal concentrations of avidin against *C. maculatus*

Avidin incorporated pellets, when fed to *C. maculatus* with varying concentrations of 5, 15, 30, 75 and 100 ppm, the mortality observed in the grubs is 20, 47.6, 72.58, 89.65 and 92.68 per cent respectively. At concentrations of greater than 77 ppm of avidin, more than 90 per cent mortality is observed. The LC_{25}, LC_{50} and LC_{90} values of avidin against *C. maculatus* was 6.211, 14.733 and 76.031 ppm respectively (Table 1). The slope (b) of log concentration probit (lcp) line of avidin against *C. maculatus* was determined as 1.79 (Fig 1). The chi-square test indicated that the *C. maculatus* population used in this study was homogeneous.

Lethal concentrations of streptavidin against *C. maculatus*

It is seen from Table 2 that the mortality of *C. maculatus* larvae seen is 33.33, 58.06, 78.12, 86.95 and 92.1 per cent when fed with streptavidin incorporated pellets with concentrations of 5, 20, 30, 75 and 100 ppm respectively. The probit analysis of the mortality data revealed that the

Table 1: Lethal concentrations of avidin against *C. maculatus*.

Concentration (ppm)	Mean mortality %	LC_{25}(ppm) (95% FL)	LC_{50}(ppm) (95% FL)	LC_{90} (ppm) (95% FL)	Heterogeneity (χ^2)	Slope b (± S.E.)	Regression equation Y = a + bx
5	20.00	6.211	14.733	76.031		1.798±	
15	47.61	(1.811-	(7.396-	(53.635-	0.164	0.374	Y= -2.101+1.798x
30	72.58	10.803)	21.011)	148.550)			
75	89.65						
100	92.68						

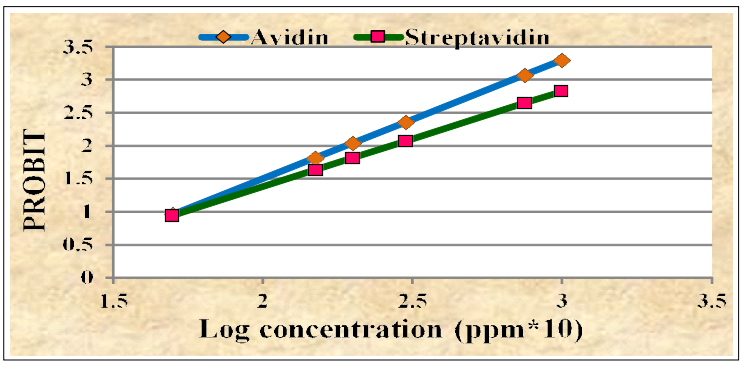

Fig 1: Log concentration probit line of avidin and streptavidin for *C. maculatus*.

respective LC_{25}, LC_{50} and LC_{90} values of streptavidin against *C. maculatus* were 3.824, 11.192 and 86.134 ppm. The population of the *C. maculatus* was found homogeneous as evident from the chi-square test. The slope of the lcp line of streptavidin against *C. maculatus* was determined as 1.44 (Fig 1).

It is clearly evident from bio-assay studies that avidin is more effective than streptavidin in causing mortality of *C. maculatus* with its lcp line more flat than that of streptavidin (Fig 1). Avidin could cause more than 90 per cent of *C. maculatus* at concentrations less than 77 ppm while streptavidin at 100 ppm. Similarly Murdock and Shade (2008) indicated that avidin at concentration of 15 ppm caused 50 per cent mortality in *C. maculatus* larvae which was in accordance and supported the present study. He also stated that at concentrations of 20 ppm and above, nearly complete mortality is seen with only few larvae reaching the grub stage before dying and in another experiment he reported that more than 83 per cent mortality at avidin concentration of 25 ppm and above. The results from Murdock and Shade (2008) revealed that streptavidin at a concentration of 10 ppm in the diet caused about 75 per cent mortality in *C. maculatus* and stated that streptavidin had toxic effects similar to that of the avidin. These results were in conformity with the findings of Murdock and Shade (2008) on *C. maculatus* where two and three adults emerged in avidin 25 ppm and streptavidin 10 ppm, respectively against 11 adults in untreated control.

Bioassay experiments proved that there is a slow increase in mortality of the three test insects and increased up to third week. But at higher concentration, larval mortality was observed immediately within one week. Similar results were obtained with the red flour beetle *T. confusum* and flat grain beetle *C. pusillus* (Kramer, 2000). It is also observed that the dead larvae turned to black, as the larvae appeared to stop feeding during ecdysis, died while still attached to a partially shed larval skin. Similar results were also observed in the case of *H. armigera* and *S. litura* larvae, fed on transgenic tobacco leaves expressing BBPs (Burgess *et al.*, 2002).

Effect of avidin and streptavidin on oviposition by *C. maculatus*

The number of eggs laid by *C. maculatus* on treated pellets differed significantly. Maximum number of, 26 eggs were laid on four pellets made with one gram of untreated bengal gram flour (Plate 2). The least number of eggs (6) were recorded on pellets treated with spinosad 250 ppm and streptavidin 100 ppm which were on par with 7.67 eggs recorded at streptavidin 75 ppm (Table 3). The next better treatments were 100 and 75 ppm of avidin and 30 ppm streptavidin with 9.33 and 9.67 and 10 eggs respectively which were similar without any significant difference between them. Pellets treated with 20 ppm streptavidin, 15 and 30 ppm avidin and 70 ppm spinosad recorded 11.33, 11.67, 10.67 and 11 eggs, respectively which were similar with each other. Lower concentrations of streptavidin 5 ppm and avidin 5 ppm recorded fairly higher number of eggs (15.33 and 19.67) but lesser than the untreated control and differed significantly with other treatments (Fig 2).

Effect of avidin and streptavidin on day of first adult emergence of *C. maculatus*

Significant difference was observed regarding the first adult emergence of *C. maculatus* on treated pellets compared to untreated one. First adult emergence of *C. maculatus* was observed on 36th day which was earliest/minimum in untreated control. There was no adult emergence, on pellets treated with spinosad at 70 and 250 ppm. There was only one adult emergence on 76th day in streptavidin

Table 2: Lethal concentrations of streptavidin against *C. maculatus*.

Concentration (ppm)	Mean mortality %	LC_{25}(ppm) (95% FL)	LC_{50}(ppm) (95% FL)	LC_{90}(ppm) (95% FL)	Heterogeneity (χ^2)	Slope b(± S.E.)	Regression equation Y = a + bx
5	33.33	3.824	11.192	86.134	1.019	1.446±	Y= -1.517+1.446x
20	58.06	(0.810-	(4.770-	(54.200-		0.309	
30	78.12	7.587)	17.429)	216.365)			
75	86.95						
100	92.10						

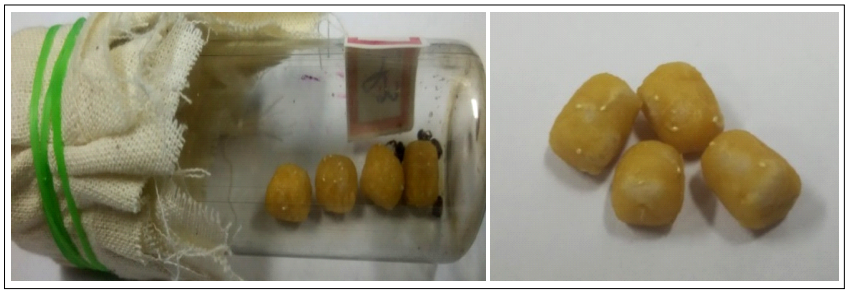

Plate 2: Oviposition on untreated pellets by *C. maculatus*.

100 ppm. Streptavidin 75 ppm and avidin 100 ppm have recoded first adult emergence on 66th and 81st day which were on par with streptavidin 100 ppm and differed significantly from other treatments. Streptavidin @ 30, 20 and 5 ppm recorded first adult emergence on 41st, 41st and 42nd day and showed no significant difference with lower concentration of avidin @ 5 and 15 ppm by recording first adult on 43rd and 37th day respectively and were found to be on par with 30 ppm avidin which recorded 43rd day of first adult emergence. Avidin at 75 ppm showed first adult emergence on 53rd day which differed significantly from other treatments (Fig 2) (Table 3).

Effect of avidin and streptavidin on development period of *C. maculatus*

The results revealed a significant difference among all the treatments regarding the development period of *C. maculatus* (Table 3). Spinosad treated pellets showed no development of *C. maculatus* when treated with concentrations of 70 and 250 ppm. Streptavidin 30 ppm showed minimum development period of 42.33 days, because on an average only three adults emerged out of 10 eggs laid within this development period in this treatment. The short development period with better growth was observed in untreated pellets with 55.33 days which

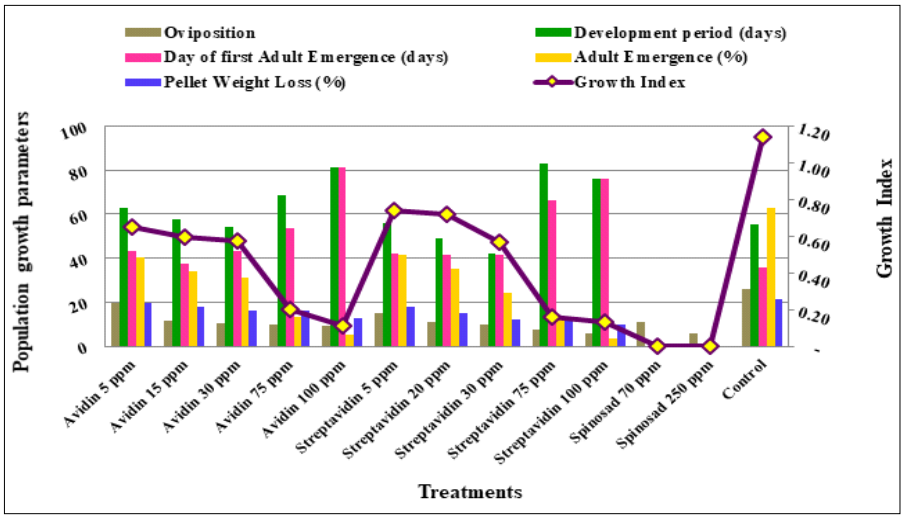

Fig 2: Effect of avidin and streptavidin on the growth and development of *C. maculatus*.

Table 3: Effect of avidin and streptavidin on the growth and development of *C. maculatus*.

Treatments (ppm)	No. of eggs laid/four pellets**	Day of firs adult emergence (in days)***	Development period (in days)***	Adult emergence (%)*	Growth index **	Weight loss (%)**
Avidin 5 ppm	19.67 (4.43)^{b}	43 (1.63)^{de}	63 (1.80)^{cd}	40.58 (39.57)^{b}	0.65 (1.07)^{bc}	19.54 (4.47)^{ab}
Avidin 15 ppm	11.67 (3.41)^{d}	37.33 (1.58)^{de}	57.33 (1.77)^{de}	33.93 (35.60)^{c}	0.59 (1.04)^{c}	17.95 (4.29)^{abc}
Avidin 30 ppm	10.67 (3.26)^{d}	43.33 (1.64)^{d}	54.33 (1.74)^{ef}	31.11 (33.89)^{c}	0.58 (1.04)^{c}	16.43 (4.11)^{bc}
Avidin 75 ppm	9.67 (3.11)^{de}	53.33 (1.73)^{c}	68.33 (1.84)^{bc}	13.47 (21.39)^{e}	0.20 (0.84)^{d}	16.14 (4.07)^{bcd}
Avidin 100 ppm	9.33 (3.05)^{de}	81.33 (1.92)^{a}	81.33 (1.92)^{a}	5.56 (13.63)^{f}	0.11 (0.78)^{e}	12.53 (3.59)^{def}
Streptavidin 5 ppm	15.33 (3.91)^{c}	42 (1.63)^{de}	56 (1.76)^{de}	41.35 (40.01)^{b}	0.74 (1.11)^{b}	17.82 (4.27)^{abc}
Streptavidin 20 ppm	11.33 (3.36)^{d}	41.33 (1.62)^{de}	49 (1.70)^{f}	34.94 (36.21)^{c}	0.72 (1.10)^{b}	15.24 (3.96)^{cde}
Streptavidin 30 ppm	10 (3.14)^{de}	41.67 (1.63)^{de}	42.33 (1.63)^{g}	24.17 (29.29)^{d}	0.57 (1.03)^{c}	12.29 (3.56)^{ef}
Streptavidin 75 ppm	7.67 (2.77)^{ef}	66.33 (1.83)^{b}	82.67 (1.92)^{a}	13.10 (21.21)^{e}	0.16 (0.81)^{de}	12.25 (3.56)^{ef}
Sreptavidin 100 ppm	6.00 (2.43)^{f}	76 (1.89)^{ab}	76 (1.89)^{ab}	3.33 (10.51)^{f}	0.13 (0.79)^{de}	9.84 (3.20)^{f}
Spinosad 70 ppm	11.00 (3.31)^{d}	0 (0.00)^{f}	0 (0.00)^{h}	0 (1.17)^{g}	0 (0.71)^{f}	0 (0.71)^{g}
Spinosad 250 ppm	6.00 (2.44)^{f}	0 (0.00)^{f}	0 (0.00)^{h}	0 (1.17)^{g}	0 (0.71)^{f}	0 (0.71)^{g}
Control	26.00 (5.10)^{a}	36 (1.57)^{e}	55.33 (1.75)^{def}	62.83 (52.43)^{a}	1.13 (1.27)^{a}	21.55 (4.70)^{a}
SEm (±)	0.13	0.03	0.02	1.13	0.02	0.17
CD (P=0.05)	0.39	0.07	0.06	3.30	0.05	0.48
CV%	6.85	3.12	2.22	7.60	3.44	8.23

*Values in parenthesis are angular transformed values.

**Values in parenthesis are square root transformed values column values with similar alphabet do not vary significantly at P=0.05.

***Values in parenthesis are logarithmic transformed values.

was on par with 15 and 30 ppm avidin and 5 and 20 ppm streptavidin with development period of 57.33, 54.33, 56 and 49 days respectively (Plate 3; Fig 2).

The longest development period with delayed growth was observed in avidin 100 ppm and streptavidin 75 ppm with 81.33 and 82.67 days respectively, showed no significant difference between them and were on par with streptavidin 100 ppm with 76 days respectively, followed by avidin 75 ppm with 68.33 days.

Among avidin treated pellets, the development period at 15 ppm was 57.33 days, which is on par with avidin 5 and 30 ppm with 63 and 54.33 days respectively and differed significantly from the maximum development period of 81.33 and 68.33 days with avidin 100 and 75 ppm respectively.

Among streptavidin treated pellets, development period was delayed maximum up to 82.67 days with 75 ppm which was on par with 100 ppm with a development period of 76 days respectively and differed significantly with shorter development period of 56, 49 and 42.33 days at lower concentrations of 5, 20 and 30 ppm, respectively.

When avidin treated pellets were compared with streptavidin treated pellets, no significant difference was seen regarding the development period at avidin 15 ppm with streptavidin 5 ppm and at avidin 100 ppm with streptavidin 75 ppm. Where, at 100 ppm of streptavidin no further development was seen after first adult emergence at 76th day.

Effect of avidin and streptavidin on adult emergence of *C. maculatus*

Adult emergence of *C. maculatus* differed significantly among all the treated pellets. The highest adult emergence of 62.83 per cent was noticed in untreated pellets and differed significantly from all other treatments. Zero adult emergence was observed with spinosad 70 and 250 ppm. The least number of adults emerged were 3.33 per cent in 100 ppm streptavidin, followed by 5.56 per cent in 100 ppm avidin which has no significant difference with each other and differed significantly from other treatments (Fig 2). These were followed by 75 ppm of avidin and streptavidin with adult emergence of 13.47 and 13.10 per cent respectively with no significant difference between them.

Among avidin treated pellets, highest number of adult emergence was found at lower concentrations of avidin 5 ppm with 40.58 per cent which differed significantly with 15 and 30 ppm avidin with similar adult emergence of 33.93 and 31.11 per cent respectively. It is followed by higher concentrations of 75and 100 ppm with 13.47 and 5.56 per cent adults respectively which differed significantly with one other.

Among streptavidin treated pellets, highest number of adults was observed at 5 ppm with 41.35 per cent followed by 20, 30, 75and 100 ppm with 34.94, 24.17, 13.1 and 3.33 per cent adults respectively which differed significantly with each other.

When adult emergence on avidin treated pellets were compared with streptavidin treated pellets, no significant

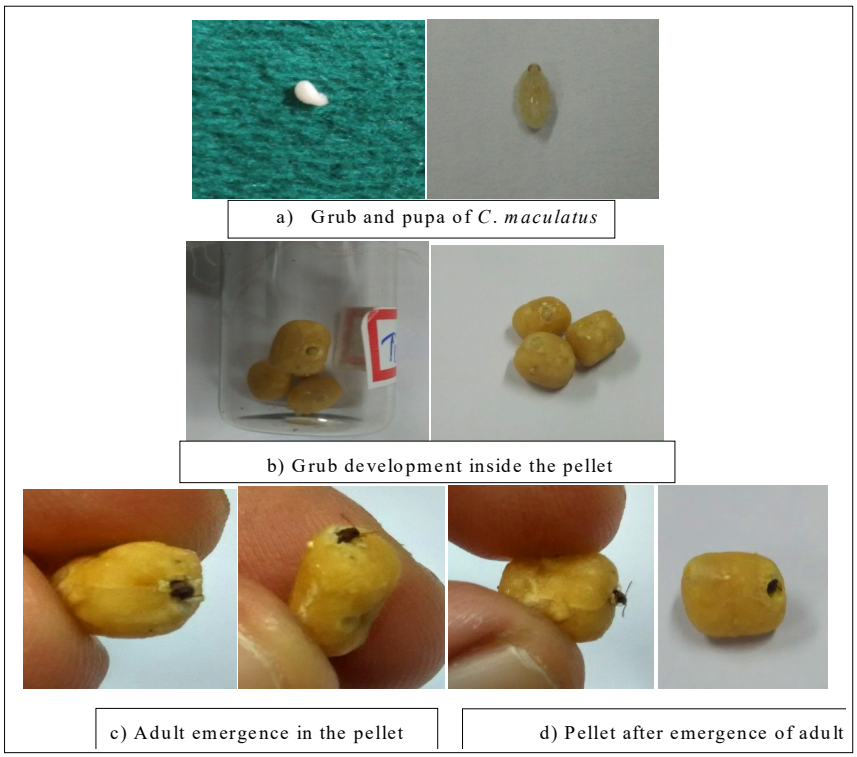

Plate 3: Growth and development of *C. maculatus* in untreated pellet.

difference was found at 5, 75 and 100 ppm of avidin when compared to 5, 75 and 100 ppm of streptavidin and also between 15 and 30 ppm of avidin compared with 20 ppm of streptavidin, which further differed significantly with 30 ppm of streptavidin and the untreated control.

The results conclude that the per cent adult emergence at lower concentration (30 to 5 ppm) of avidin and streptavidin were from 31 to 40 per cent and 24 to 41 per cent respectively and the per cent adult emergence at higher concentrations (100 and 75 ppm) of avidin and streptavidin were 5 to 13 per cent and 3 to 13 per cent respectively against the untreated control with 62 per cent (Table 3).

Effect of avidin and streptavidin on growth index of *C. maculatus*

The growth index of *C. maculatus* differed significantly among the treatments. The highest growth index of 1.13 was observed in the untreated pellets. It was followed by 5 and 20 ppm of streptavidin with growth index of 0.74 and 0.72 respectively, which were found to be similar and at par with 5 ppm avidin with 0.65 growth index and differed significantly with other treatments.

Zero growth index was recorded in spinosad treatments as there was no adult emergence. The lowest growth index of 0.11 is noticed at higher concentrations of 100 ppm avidin which is on par with growth index of 0.13 and 0.16 at streptavidin 100 and 75 ppm, respectively (Table 3).

Among avidin treated pellets, highest growth index of 0.65 was observed in lower concentrations at 5 ppm, which was on par with similar growth index of 0.59 and 0.58 at 15 and 30 ppm respectively and differed significantly from 75 ppm and 100 ppm avidin with growth index of 0.2 and 0.11 respectively.

Among streptavidin treated pellets, no significant difference in growth index was observed between lower concentrations of 5 and 20 ppm with higher growth index of 0.74 and 0.72 respectively and also between higher concentrations of 75 and 100 ppm with lower growth index of 0.16 and 0.13 respectively and differed significantly with 0.57 growth index at 30 ppm streptavidin.

When avidin and streptavidin treated pellets were compared with each other regarding the growth index, the lower concentrations of streptavidin at 5 and 20 were found on par with avidin at 5 and differed significantly with higher concentrations of avidin at 75 and 100 ppm respectively. Even the higher concentrations *viz.*, Streptavidin 75 and 100 ppm were on par with avidin 75 and 100 ppm and differed significantly with lower concentrations *viz.*, avidin 5 and 15 ppm respectively. There was no significant difference between 15 and 30 ppm avidin and streptavidin 30 ppm respectively.

The growth index recorded in 5 to 100 ppm avidin and streptavidin is in the range of 0.65 to 0.11 and 0.74 to 0.13 respectively against the control (1.13).

Effect of avidin and streptavidin on weight loss of Bengal gram flour pellet due to *C. maculatus*

Untreated Bengal gram pellets recorded the highest weight loss of 21.55 per cent which was on par with 5 and 15 ppm of avidin (19.54 and 17.95%) and 5 ppm of streptavidin (17.82%).

Weight loss was minimum (9.84%) in streptavidin 100 ppm and on par with 30 ppm and 75 ppm streptavidin with 12.29 and 12.25 per cent. No weight loss was recorded in pellets treated with spinosad 70 and 250 ppm, with no grub development (Fig 2).

Among avidin treated pellets, the highest weight loss was noticed at lower concentration of avidin 5 ppm with 19.54 per cent, followed by further higher concentrations of avidin 15, 30, 75 and 100 ppm with 17.95, 16.43, 16.11 and 12.53 per cent weight loss respectively, where weight loss at 30 ppm was at par with 5, 15 and 75 ppm respectively and differed significantly from 100 ppm of avidin with 12.53 per cent weight loss.

Among streptavidin treated pellets, streptavidin 20 ppm with 15.24 per cent was found on par with higher weight loss of 17.82 per cent at 5 ppm and with 12.29 and 12.25 per cent at 30 and 75 ppm of streptavidin, respectively. No significant difference regarding weight loss was found between 30 and 75 ppm streptavidin which was at par with 9.84 per cent at 100 ppm streptavidin.

When per cent weight loss in avidin and streptavidin treated pellets were compared with each other, streptavidin 5 ppm and avidin 15 ppm showed no significant difference. Minimum weight loss recorded at 100 ppm avidin, on par with 20, 30, 75 and 100 ppm of streptavidin.

From the present investigation, it is clearly evident that biotin inhibitors *viz.*, avidin at 75 and 100 ppm, streptavidin at 30, 75 and 100 ppm against *C. maculatus*; 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. There was no adult emergence at >100 ppm of BBP's and delayed adult emergence beyond 60 days at 75 ppm of Avidin and streptavidin. These results obtained were supported by the findings of Murdock and Shade (2008), where the first adult emergence of *C. maculatus* was delayed up to 35th day at concentration of 15 ppm and above when compared to 27th day of first adult emergence recorded in the control treatment.

There was zero adult emergence at 70, 250 ppm of spinosad and about 3.33 and 5.56 at 100 ppm of streptavidin and avidin with growth index 0.13 and 0.11. The findings of Morgan *et al.* (1993) were also in support to the results of the present study, where *R. dominica* when fed on 10 ppm avidin treated pellets caused 60 per cent reduction in emergence and 100 ppm avidin resulted in 100 per cent larval mortality with zero emergence. The above results of delayed development period obtained at higher concentrations of avidin and streptavidin were in conformity of the Bruins *et al.* (1991), who demonstrated that avidin increased the development time of *Drosophila melanogaster* and also by the findings of Murdock and Shade (2008) who reported that *C. maculatus* larval survival at 15 ppm dose of avidin with slight delay in their development. The results

obtained in this experiment were supported by the findings of Christeller *et al.* (2006) where the mean growth rates of the light brown apple moth, *Ephiphyas postvittana* larvae placed on diets at 14, 21 and 28 days later with avidin 5.52 mg were 0.61, 1.2 and 1.29 against the untreated control with 2.22, 4.17 and 4.74 growth rates respectively.

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), a physiological process absent in mammals. Insects normally survive the moult, during which time they do not feed, by storing lipid in the fat body during feeding and catabolizing these lipids during the non-feeding period to provide energy for the moulting process. Because both lipid synthesis and catabolism critically require biotin carboxylases, it is possible that due to avidin and streptavidin there was failure to deposit lipid and the inability to use it for energy production which accounted for insect death during moulting and provide an explanation for the heightened sensitivity that insects show to biotin depletion compared with mammals.

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.

Besides the above mentioned role, Biotin functions as an enzyme cofactor that acts as a one-carbon transfer agent via carboxybiotin (Attwood and Wallace, 2002; Jitrapakdee and Wallace, 2003). Biotin is covalently bound to various enzymes via a reaction catalyzed by holobiotin carboxylase (Zempleni and Mock, 1999). The enzymes that are known to use biotin as a cofactor are acetyl-CoA carboxylase, 3-methylcrotonyl CoA carboxylase, propionyl-CoA carboxylase and pyruvate carboxylase. These are enzymes involved in lipid and amino acid biosynthesis and degradation.

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Conflict of interest

All authors declare that they have no conflict of interest.

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