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Evaluation of methanol and acetone bark extracts from *Acacia nilotica* (Linn.) as a source of growth inhibitors against *Bactrocera cucurbitae* (Diptera: Coquillett)

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Abstract
Growth inhibitory potential of methanol and acetone bark extracts from *Acacia nilotica* was determined using artificial diet bioassay against first, second and third instar larvae of *Bactrocera cucurbitae* at concentrations ranging from 1-625ppm. Both extracts adversely affected the larval period and total developmental period of *B. cucurbitae*, a serious pest of cucurbit crops. Percentage pupation and emergence were significantly inhibited. Significant effects of the extracts were observed on the activity of GSTs, esterases and catalases in the second instar larvae of *B. cucurbitae*. The GST activity was induced with both the extracts, while an induction in the esterases activity was observed only with acetone extract. Elevated levels of catalase were noticed on prolonged treatment of the larvae with both extracts. These studies indicated the effectiveness of both extracts against the melon fruit fly and further investigations are needed to evaluate this activity against wide range of insect pests.

Keywords: *Bactrocera cucurbitae*, pest management, bark extracts, *Acacia nilotica*, cucurbitaceae crop pest

1. Introduction
The age old tradition of using plant extracts to repel insects is gaining considerable attention as non-judicious use of organic pesticides has deteriorated the environment and jeopardized public health. Plants are, in effect, natural laboratories in which a great number of chemicals are biosynthesized. Some of these chemicals discourage feeding by insects and other herbivores. Others provide protection or immunity from diseases caused by various pathogens. A number of compounds have been discovered from plants and are being examined for their use as biopesticides.

Acacia nilotica (Linn.) belongs to the family: Leguminosae, sub family: Mimosaceae and is widely distributed in tropical and subtropical countries. A number of medicinal properties have been assigned to various parts of this highly venerated plant. The plant has been found to exhibit antioxidant [1], antimalarial [2], anticancer [3], antiplasmodial, antimolluscicidal, antifungal, anti-microbial activity and also inhibitory activity against HCV and HIV-I [4]. Phytochemical investigations have revealed that stem bark of *A. nilotica* contains terpenoids, alkaloids, saponins, cardiac glycosides and tannins [5]. These secondary metabolites play an important role in plant-insect interactions and are responsible for plant resistance to insects [6]. These compounds can act as attractive, repellent or toxic agents as well as growth regulators, affecting physiological processes of insects [7, 8].

Insects have coexisted with plants over a long period of time and have evolved mechanisms to resist the toxic effects of plant allelochemicals. They possess detoxification enzymes which act as a barrier to the toxic allelochemicals and help the insect to adapt to plant [9]. However, some allelochemicals have an inhibitory effect on detoxification enzymes thereby increasing the toxicity of plant compounds [10, 11]. Therefore, present study was envisaged to investigate the effect of methanol and acetone extracts from bark of *A. nilotica* on the development of Melon fruit fly, *Bactrocera cucurbitae* (Coquillett). In addition studies were carried out to assess their influence on some detoxification and antioxidant enzymes in the insect. *B. cucurbitae* is distributed widely in temperate, tropical and sub-tropical regions of the world but India is considered as its native home [12]. It damages over 81 plant species specially of family

Cucurbitaceae which include fruits of bitter gourd (*Momordica charantia*), muskmelon (*Cucumis melo*), snap melon (*Cucumis melo* var. *momordica*) and snake gourd (*Trichosanthes anguina* and *T. cucumeria*) [13]. It is hoped that the study would help in evaluating its potential as a biopesticide against the insect pests.

2. Material and methods

The present study was conducted in the Insect Physiology Lab, Department of Zoology, Guru Nanak Dev University in the months of June-August. Crude methanol and acetone bark extracts of *A. nilotica* were procured from the Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar. The culture of *B. cucurbitae* was procured in larval stage from infested bitter gourds at local vegetable market and reared to adult stage. Newly emerged flies were identified according to the characters given in taxonomic keys [14, 15]. Adult flies were maintained in Rescholar's fruit fly wire mesh cage (L45×B45×H40cm) in insect culture room at 25±2 ºC, 70-80% R.H. and a photoperiod of 10h:14h L:D. Flies were provided 20% sugar solution, Protinex (Pfizer India), vitamin E as food and pieces of pumpkin fruit, *Cucurbita moschata* (Duschesne ex Poir.) for oviposition. The artificial medium used for rearing of larval stages of melon fruit fly was slightly modified version of the formula and methodology suggested by Srivastva [16].

2.1 *Larval treatment*

In order to procure the first, second and third instar larvae, pumpkin pieces were placed in wire mesh cages having more than 100 gravid females. After 24h charged pumpkin pieces were removed from the cages and were kept in the battery jars (D10×L15cm) containing moist sand. The larvae were dissected out with fine forceps from the pumpkin pieces after an interval of 48h, 64h and 88h, washed in distilled water and dried on the filter paper. These larvae were transferred to the experimental vials (D25×L100mm) containing artificial diet incorporated with the different concentrations (1, 5, 25, 125 and 625ppm) of methanol and acetone bark extracts as well as respective control. There were six replications with 15 larvae in each vial for each concentration (n=540) and all the experiments were repeated twice. These experimental vials were kept in B.O.D and observations were made daily for various developmental parameters such as time taken for pupae formation, number of pupae formed, time taken for emergence of flies and number of flies emerged.

2.2 *Biochemical analysis*

In order to gain some insight into the metabolic adaptations occurring at the biochemical level the activity of three enzymes viz. GST, catalase and esterase was estimated after feeding second instar larvae (64-72h old) on artificial diet incorporated with methanol (LC_{40}= 10ppm) and acetone (LC_{40}= 11ppm) bark extract of *A. nilotica* for 24h, 48h and 72h. GST was estimated and extracted by homogenizing the larvae (2% w/v) in 0.1M sodium phosphate buffer (pH 7.6) [17]. The ethanolic CDNB solution was used as the substrate solution and the absorbance was recorded at 340nm. The method of Bergmeyer [18] was used for extraction and estimation of catalase. The homogenates (5% w/v) were prepared in 0.05M potassium phosphate buffer (pH 0.7). Substrate used was 0.05% H_2O_2 and the decrease in absorbance was recorded at 240nm. Esterases are carboxylic ester hydrolases and estimated by following the methodology of Katzenellenbogen and Kafatos [19]. The homogenate (1% w/v) of larvae was prepared by using 0.1M phosphate buffer (pH 6.5). Assay mixture contained 1mM α- naphthyl acetate as the substrate solution. The optical density of the mixture was recorded at 540nm. Percentage of control for enzyme activities was calculated using formula given below:

$$\frac{T - C}{C} \times 100$$

Where T- enzyme activity in treated larvae and C- enzyme activity in control larvae.

2.3 *Statistical analysis*

Statistical analysis was performed using the computer program Minitab (version 14) for academic use. Results are expressed as mean±SE. Means were compared using one way analysis of variance (ANOVA). Linear regression analysis was done using Microsoft office excel 2007 (Microsoft Corp., USA). Means obtained for enzyme activities at all the three exposure intervals were compared for their significance by the t-test.

3. Results and Discussion

In the management of fruit flies, till recently major emphasis had been on the use of insecticides. But because of the various ill effects of these conventional insecticides, plant derivatives for the management of insect pests are gaining a lot of importance.

3.1 *Bioassay studies*

The current study demonstrated significant effects of methanol and acetone extract from bark of *A. nilotica* on the development and enzymes of *B. cucurbitae*. Varied effects of methanol and acetone extracts from bark of *A. nilotica* were observed on larval and total development period of *B. cucurbitae*. While the methanol extract shortened the larval period of all instars (by 3.65 days in first instar, 3.18 in second instar and 6.2 days in 3rd instar), the acetone extract prolonged the larval period of first and second instar by 3.89 (r^2=0.97) and 1.9 (r^2=0.71) days at highest concentration of 625ppm respectively (Table.1).

Table 1: Larval Period of *B. cucurbitae* when first instar, second instar and third instar larvae were treated with different extracts of *A. nilotica*

Concentrations (ppm)	Larval period after treatment of different age group larvae with methanol and acetone extracts					
	First Instar		Second Instar		Third Instar	
	Methanol extract	Acetone extract	Methanol extract	Acetone extract	Methanol extract	Acetone extract
Control	18.50±0.093	19.58±0.233	15.78±0.331	17.15±0.287	12.58±0.226	6.43±0.143
1ppm	18.33±0.112	20.33±0.357	14.62±0.083	16.60±0.219	8.30±0.136	5.70±0.197
5ppm	17.63±0.193	21.08±0.287	14.57±0.102	16.78±0.286	7.97±0.229	4.90±0.186
25ppm	17.23±0.175	21.87±0.364	13.93±0.256	17.40±0.093	7.25±0.043	4.90±0.198
125ppm	16.82±0.114	22.12±0.031	12.83±0.173	18.05±0.099	6.67±0.163	4.50±0.118
625ppm	14.85±0.180	23.47±0.182	12.60±0.063	19.05±0.191	6.38±0.087	3.83±0.214
f-value r^2	91.22* 0.87	23.76* 0.97	55.79* 0.95	21.94** 0.71	186.03* 0.74	22.92** 0.94

Figures are mean ± SE. *- Significant at 1% level, ** - Significant at 5% level, r^2- Coefficient of determination

Observations on total development period showed prolongation in all the larval age groups treated with methanol extract whereas it decreased with acetone extract (Table. 2). In a similar study, Kaur *et al*. [20, 21] had reported prolongation in the larval and total development period of *B. cucurbitae* after the treatment of 24-32h to 44-48h old larvae with *Acacia auriculiformis* methanol and acetone extracts. However, when 88-96h old larvae were given extract treatment, larval and total development period declined. Such response of larvae to both extracts could be an age group definite response to the diverse phytochemical constituents in the crude extracts.

Table 2: Total Development Period of *B. cucurbitae* when first instar, second instar and third instar larvae were treated with different extracts of *A. nilotica*

Concentrations (ppm)	Total Developmental Period after treatment of different age group larvae with methanol and acetone extracts					
	First Instar		Second Instar		Third Instar	
	Methanol extract	Acetone extract	Methanol extract	Acetone extract	Methanol extract	Acetone extract
Control	29.08±0.393	29.67±0.645	21.18±0.154	28.80±0.288	25.57±0.169	14.18±0.135
1ppm	30.18±0.407	28.95±0.249	20.03±0.251	28.87±0.255	26.88±0.212	13.72±0.263
5ppm	30.97±0.194	28.83±0.235	21.78±0.239	28.58±0.515	27.33±0.314	12.65±0.141
25ppm	32.05±0.367	28.95±0.395	22.47±0.105	27.33±0.677	28.05±0.290	11.68±0.111
125ppm	33.23±0.323	28.52±0.257	22.77±0.171	26.62±0.383	28.48±0.241	10.92±0.265
625ppm	34.53±0.167	28.10±0.343	24.80±0.253	26.55±0.318	29±0.300	10.13±0.233
f-value	33.68*	$1.69^{N.S}$	57.92*	5.83**	21.97*	55.20**
r^2	0.99	0.85	0.79	0.89	0.96	0.99

Figures are mean ± SE.*- Significant at 1% level, ** - Significant at 5% level, N.S – Non Significant, r^2- Coefficient of determination

Percentage pupation and emergence was significantly inhibited with both extracts (Table 3, Fig. 1, 2&3). Maximum inhibition in pupation was noticed with methanol extract (52.87%, r^2=0.95) and in emergence was observed with acetone extract (70.18%, r^2=0.96) in the first instar larvae. Chaubal *et al.* [22] and Taura *et al*. [23] too had reported a dose dependent lethal effect of aqueous extracts of *A. nilotica* on *Aedes aegypti* and *Culex quinquefasciatus* mosquito larvae. Methanol and ether extracts of *Rhazya stricta* resulted in lower pupation rate and complete inhibition of adult emergence in *Culex pipiens*, whereas both extracts of *Syzygium aromaticum* showed remarkable influence on adult emergence [24]. Aqueous extracts of neem seed kernel had an inhibitory effect on the adult emergence of *B. cucurbitae* and *B. dorsalis* [25]. Ethanol and petroleum ether extracts of *Valariana officianalis* significantly lowered pupal and adult emergence in peach fruit fly, *B. zonata* [26]. Percentage pupation, percentage emergence, oviposition and egg hatching decreased in a dose dependent manner in *B. cucurbitae* when treated with *A. auriculiformis* bark extracts [21]. Plants synthesize a broad array of compounds, a number of which are used as weapons of defense against pests [27]. The bark of *A. nilotica* has been reported to have high phenolic content [28]. Phenols are the most potent group of allelochemicals that unfavourably affect insect growth, development or feeding behavior [29, 30]. Their implication in resistance mechanisms of plants against insects has been demonstrated in different studies [31]. The adverse effects of the methanol and acetone extracts of *A. nilotica* on development of *B. cucurbitae* might be due to the toxic effects of these compounds in the extracts.

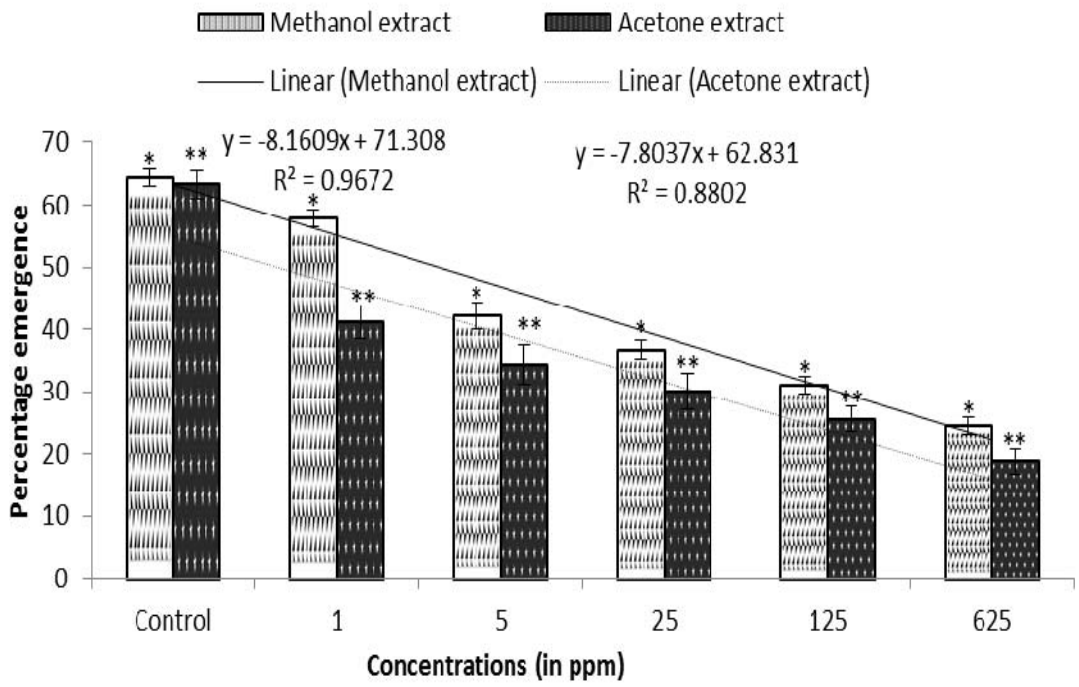

Fig 1: Effect of methanol and acetone extract of *A. nilotica* on percentage emergence of *B. cucurbitae* when first instar larvae were given extracts incorporated diet. Columns and bars represent the mean ± SE, *- Significtant at 5% level, **- Significant at 1% level.

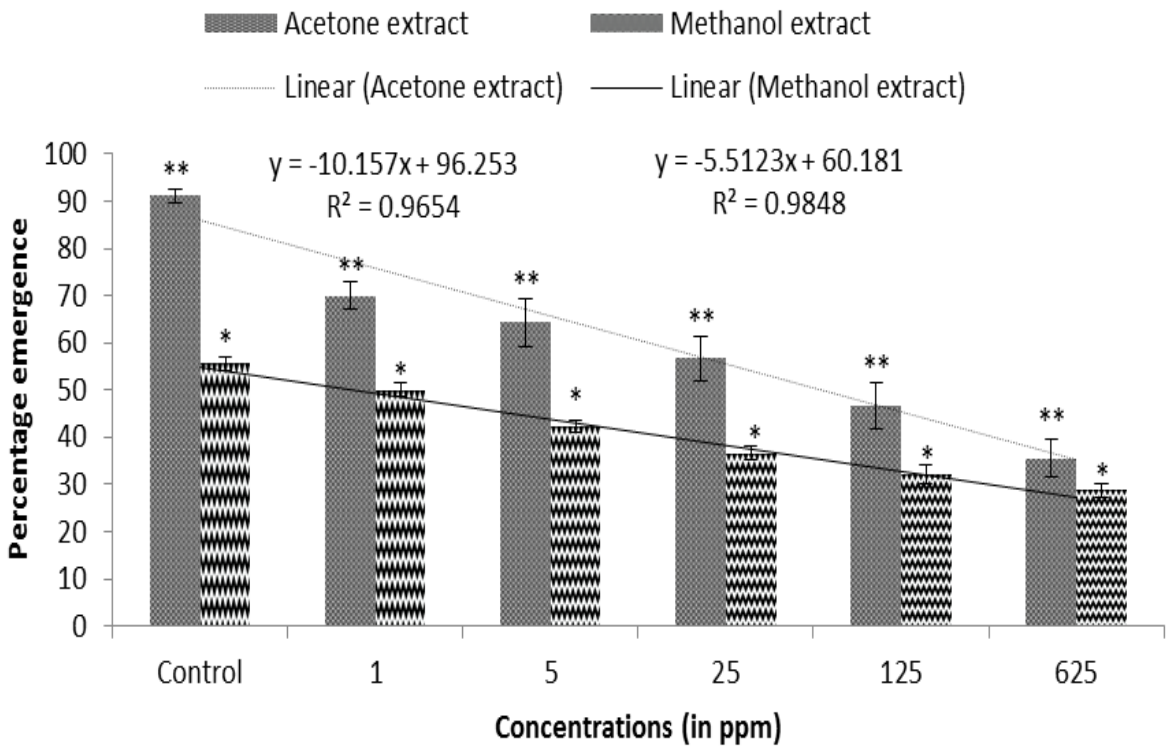

Fig 2: Effect of methanol and acetone extract of *A. nilotica* on percentage emergence of *B. cucurbitae* when second instar larvae were given extracts incorporated diet. Columns and bars represent the mean ± SE, *- Significtant at 5% level, **- Significant at 1% level.

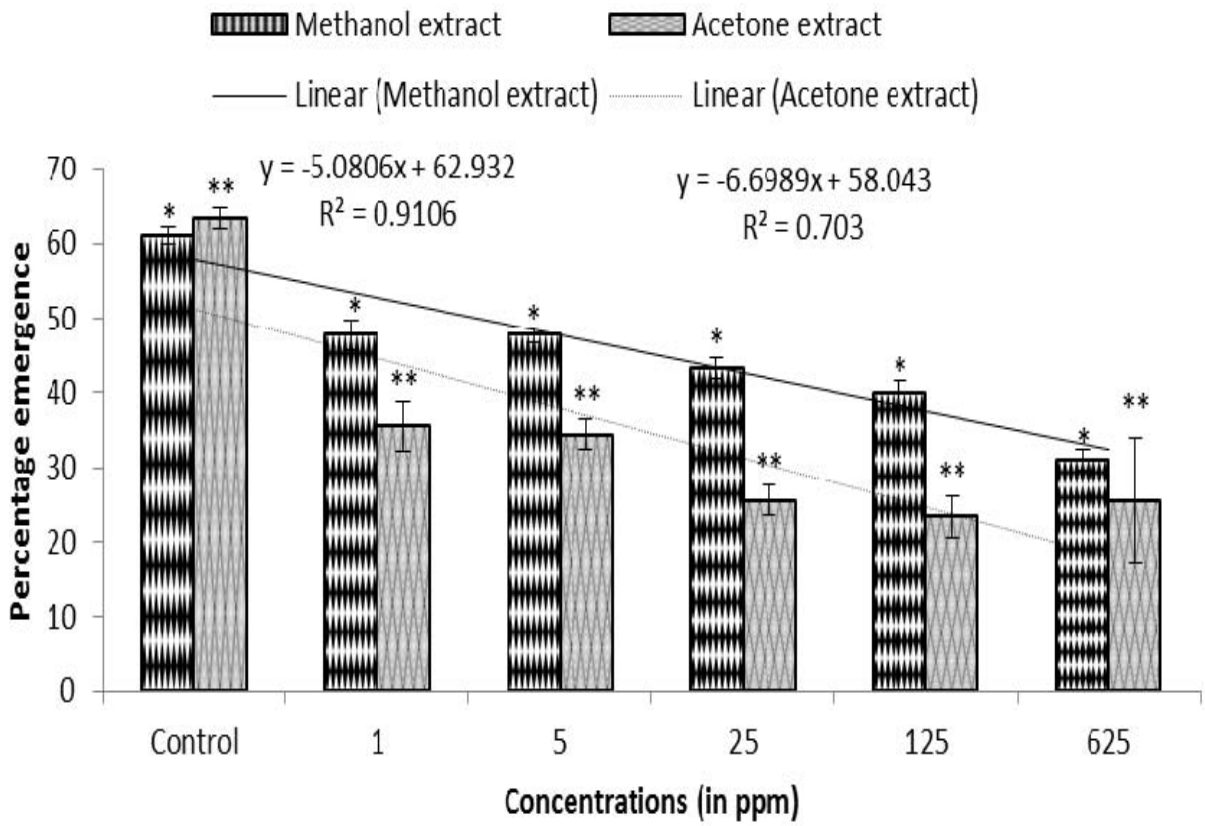

Fig 3: Effect of methanol and acetone extract of *A. nilotica* on percentage emergence of *B. cucurbitae* when third instar larvae were given extracts incorporated diet. Columns and bars represent the mean ± SE, *- Significtant at 5% level, **- Significant at 1% level.

Table 3: Percentage pupation of *B. cucurbitae* when first instar, second instar and third instar larvae were treated with different extracts of *A. nilotica*

Concentrations (ppm)	Percentage Pupation after treatment of different age group larvae with methanol and acetone extracts					
	First Instar		Second Instar		Third Instar	
	Methanol extract	Acetone extract	Methanol extract	Acetone extract	Methanol extract	Acetone extract
Control	77.77±1.412	71.07±1.413	75.53±1.412	68.83±1.413	75.53±1.412	95.53±1.412
1ppm	64.40±2.807	66.63±2.428	59.98±1.717	53.30±2.447	63.30±1.476	91.08±2.812
5ppm	53.30±1.730	63.30±1.476	58.85±2.670	53.30±1.730	62.17±2.804	85.52±6.317
25ppm	47.72±±1.117	54.42±2.059	56.65±2.274	53.30±2.446	58.85±2.670	89.52±2.857
125ppm	43.30±1.476	54.42±2.689	55.53±1.413	44.40±1.391	48.83±1.412	85.53±6.303
625ppm	36.65±1.498	47.75±2.670	44.42±2.213	42.20±1.391	42.20±1.391	73.30±4.216
f-value r^2	69.78* 0.95	16.95** 0.96	24.71* 0.83	25.09** 0.82	31.45* 0.94	2.87** 0.76

Figures are mean ± SE.*- Significant at 1% level, ** - Significant at 5% level, r^2- Coefficient of determination

3.2 *Enzymatic studies*

Though significant effects of the plant extracts were observed on the antioxidant and detoxifying enzymes of *B. cucurbitae*. The extracts varied in their effect on the activity of these enzymes which could be due to different phytochemical constituents present in the extracts. GST activity was induced with both methanol and acetone extracts of *A. nilotica* (Fig. 4, 5). However, while the methanol extract caused GST induction after 48h, the acetone extract after showing a 1.6 fold increase at 24h, declined when the treatment was prolonged for 48h and 72h. An induction in GST activity had also been reported in the third instar larvae of lepidopteran insect, *Plutella xylostella* after its treatment with root extract of *Derris elliptica* [32]. Sintim *et al.* [33] had also reported a general induction in GST activity in *Spodoptera litura* larvae when treated with leaf extracts of *Sesame indicum*. GSTs play a central role in the detoxification of both endogenous and xenobiotic compounds and are also involved in intracellular transport, biosynthesis of hormones and protection against oxidative stress [34]. They play an important role in insecticide resistance. The activity of esterases, which play an important role in contributing to xenobiotic resistance in insects, was suppressed with methanol extract at all treatment intervals but was induced significantly when the larvae were treated with acetone extract. Reports by Lindroth [35] indicated substantial induction of microsomal esterases in response to food plant species. On the other hand, Brever *et al.* [36] reported 31% inhibition in esterases activity in *Spodoptera frugiperda* when treated with neem extract. The present findings indicate that while esterases might be involved in the metabolism of phytochemicals in the acetone extract, the methanol extract could be interfering in the synthesis of the enzyme. The catalase activity which was less than control during the initial treatment interval was induced significantly after 48h of treatment with methanol extract (Fig. 4) and after 72h of feeding on acetone incorporated diet (Fig.5), which might be due to the high free radical generation property of crude bark extracts [37].

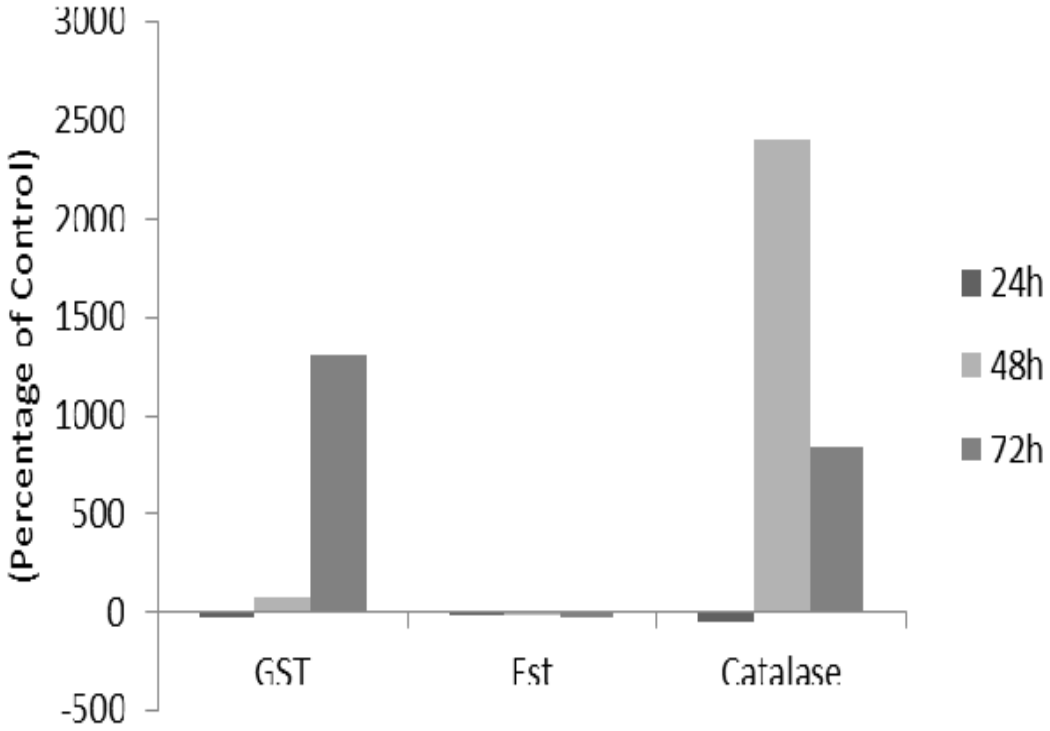

Fig 4: Effect of methanol extract of *A. nilotica* on activity of Glutathione S-transferase (GST), Esterases (Est), Catalase (CAT) in second instar larvae of *B. cucurbitae* at 24h, 48h and 72h of treatment

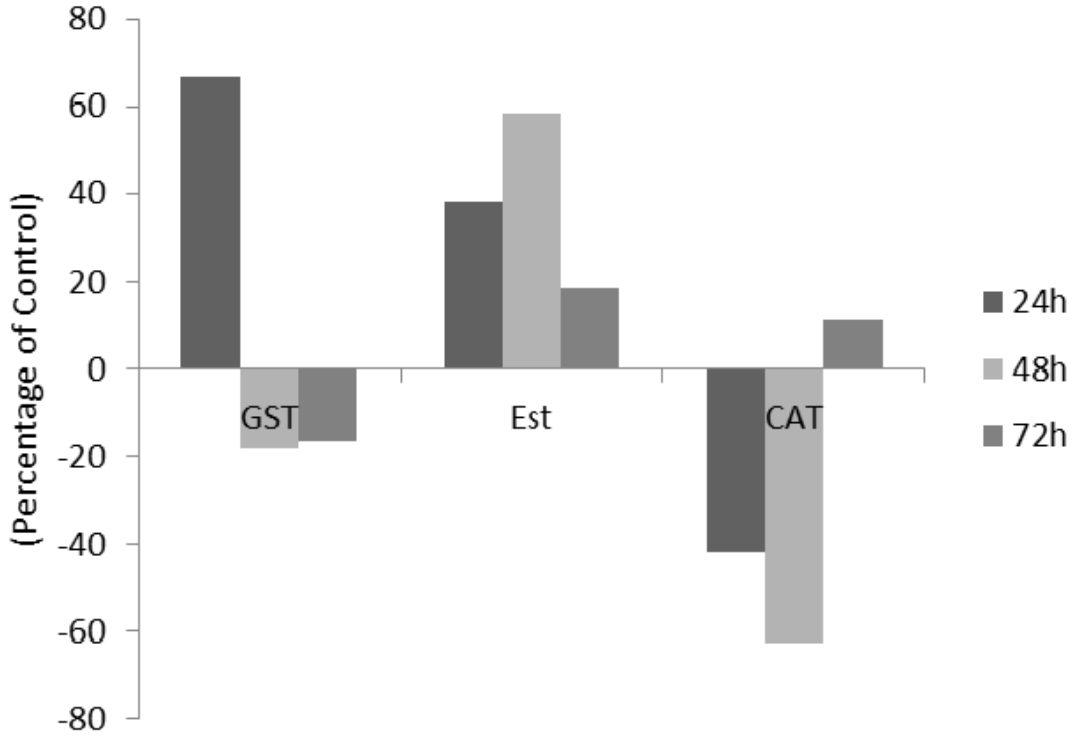

Fig 5: Effect of acetone extract of *A. nilotica* on activity of Glutathione S-transferase (GST), Esterases (Est), Catalase (CAT) in second instar larvae of *B. cucurbitae* at 24h, 48h and 72h of treatment

4. Conclusion

The present study clearly revealed the susceptibility of *B. cucurbitae* to both the extracts of *A. nilotica* as they showed inhibitory effect on growth and development of melon fruit fly. This could be attributed to the high phenolic content, comprising of tannin, gallic acid, protocatechuic acid, pyrocatechol, (+) -catechin, (-) epi- gallocatechin-7-gallate and (-) epigallocatechin-5, 7-digallate [38]. It is clear from the current study that the *A. nilotica* crude bark extracts have toxic compounds which need to be explored for the management of fruit flies.

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