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EFFECT OF SECONDARY METABOLITES OF ACTINOBACTERIA STRAIN ON *LEUCINODES ORBONALIS* (GUEN.)

Helen Diana I, Syed Jahangir H*

Post Graduate and Research Department of Botany, Jamal Mohamed College (Autonomous), Tiruchirappalli, Tamil Nadu, India.

ABSTRACT: The present study was aimed to analysis the effect of isolated actinobacterial JMCHA8 strain secondary metabolites on 3rd and 4th instar larvae of *Leucinodes orbonalis* (Lepidoptera).The lethal concentration of the actinobacterial strain was identified by relative toxicity studies and the LC50 was found as 75ppm. Three sublethal doses of JMCHA8 strain (30, 35 and 40ppm) were mixed with brinjal fruit and used as feed for the larvae (n=16). Morphological and Behavioural changes of the larvae in the exposed group were observed as reduced feed intake, suddenly forms pupae, discolouration of epidermis and the hatched young ones showed various deformities resulted in affected adults. Total Glucose, Total cholesterol, Total protein (Albumin & Globulin) levels in the three treated groups were significantly (P<0.01) reduced than control group larvae. GPT:GOT ratio proved that GPT increased in treated groups than GOT, these two transaminase enzyme increases gluconeogenesis. Increased ALP and LDH indicated increased lysosomal activities to lyse the entered compound into their system. This study concludes that exposure of secondary metabolites of JMCHA8 strain disturbs the life cycle of *Leucinodes orbonalis* larvae to skip their 5th instar and altered biochemical composition in their tissues which resulted in deformed adults hatched from the pupae showed less viable.

KEYWORDS:*Leucinodes orbonalis*, actinobacteria, LC50, biochemical analysis, secondary metabolites.

Corresponding Author: Dr. Syed Jahangir H* Ph.D.

Post Graduate and Research Department of Botany, Jamal Mohamed College (Autonomous), Tiruchirappalli, Tamil Nadu, India. Email Address: Syedbot2000@gmail.com

1.INTRODUCTION

Actinobacteria belongs to the domain bacteria is one of the largest taxonomic lineages, are saprophytic, soil-dwelling organisms generally found on the surface and at depths of more than 2m

below ground [1-3], promotes plant growth either directly by uptake of soil nutrients into plant or indirectly by preventing the growth of one or more deleterious microbes [4], and which has bioactive compounds and routinely used as biological control or biocontrol against insects [5].In tropical and subtropical regions, the brinjal is considered as the important crop due to its ayurvedic medicinal and diabetic properties [6] and several varieties of brinjal are widely cultivated for 8 states in India [7], which is attacked by various kinds of insect pests such as defoliators, stem borers, stem girdlers, fruit borers and cell sap suckers [8]. From the past six decades various researchers studied about the chemical based insect pest management on brinjal plants [9-14] while for the past two decades, researchers focussed about the ecological pest control strategy to produce the microbial insect pest management [8]. *Leucinodesorbonalis* (Lepidoptera) phytophagous insect pest cause serious damage to the brinjal plants and reduces the annual productivity upto 60% [15, 16]. Integrated pest management (IPM) is an effective way to develop biological formulations like secondary metabolites to control the insects below the economic threshold levels and also never disturbs their species diversification [17, 18]. According to IPM program, chemical pesticides is not the only way to control pest, the preventive measures should emphasis on biological, cultural and physical ways [18].Instead of chemical pesticides as biocontrol agents, the bioagents like secondary metabolites of microbes can decrease the pesticidal pollution in agricultural soil and also through successive applications of sublethal doses produces remarkable values against insect pest control [19]. Many investigators studied the effect of different strains on lepidoterous insect showed remarkable effect ranging from antagonistic to additive effect [20, 21, 19]. Parte *et al.* [22] reported that actinobacteria are the prominent source of agro-active products, being employed as biocontrol. Total carbohydrates, protein and cholesterol are the essential biochemical components for the growth, development like moulting and reproduction like gamete formation. Transaminase enzymes like GOT and GPT are produced in the organisms during stress to induce gluconeogenesis and forms non essential amino acids resulted in increased production of nitrogen waste [23]. Katumuma *et al.* [24] described that the transaminase enzyme acts as a catalytic factors for protein and carbohydrate metabolisms and helps to produce energy [25]. The biochemical effect of actinobacterial secondary metabolites on insects was very scarce. As a novel attempt, this study focussed to find out the bioinsecticidal potent of selected JMCHA8 actinobacterial strain secondary metabolites on *Leucinodes orbonalis* by analyzing the biochemical parameters.

2. MATERIALS AND METHODS

2.1. Sample collection and processing

Brinjal field exposed to different type of insecticides such as Alanto, Exodus, Acetamiprid and Regent for more than 10 years is selected for this study. During the daytime, from four different locations five kilogram (each site) of insecticide exposed rhizospheric soil collected from brinjal field in Paithur village, Athur Taluk, Salem district, Tamil Nadu (Lat. Long). Aseptically samples

are transported to the Department of Botany, Jamal Mohamed College (Autonomous), Tiruchirappalli district for further preparation. Soil samples are air dried in the laboratory for four days and sieved in a mesh and stored in the refrigerator until further use. Based on the primary and secondary screening, the isolated actinobacteria designated as JMCHA8.

2.2. Lethal dose concentration and Experimental Dose

To identify the lethal concentration of secondary metabolites of the isolated actinobacterial strain, the Lepidopteran: *Leucinodes orbonalis* larvae were collected from the brinjal fruits purchased from Athur agricultural market. Various concentrations of secondary metabolites of the isolated strain exposed to *L. orbonalis* larvae (n=16) through brinjal fruits and observed for next 96hours. Each day the food source was replaced by new one impregnated with the secondary metabolites. By using probit analysis, the LD50 of the metabolites of the actinobacterial strain were identified and experimental dose were finalized. Control group also maintained without strain. During the experimental period, the morphological and behavioural activities of the larvae were observed manually.

2.3. Biochemical analysis

The larvae were pooled and homogenized with Physiological saline in glass mortar pestle placed in cold environment. Homogenized samples were centrifuged at 1200rpm for 15min in cooling centrifuge and supernatant collected in a separated tube. Total Glucose [26], Total cholesterol [27], Total protein [28], Transaminase enzyme [29], alkaline phosphatase [30], lactate dehydrogenase [31] enzymes were analyzed for control and three treated groups. Data were statistically analyzed by SPSS software (17.0 version) and subjected to one-way analysis of variance (ANOVA) and Post hoc Tukey test for homogensity between the experimental groups.

3. RESULTS AND DISCUSSION

3.1. Toxicity studies

Various concentrations of secondary metabolites such as 50, 70, 90, 110, 130 and 150ppm of JMCHA8 strain were used to identify the lethal concentration on *Leucinodes orbanolis* 3rd and 4th instar larvae. Each group consist of 16 larvae, mortality of the larvae considered as the endpoint within 96hrs. Figure 1 showed the lethal concentration of JMCHA8 strain as inbetween 70-90ppm. Finally LC50 of the selected JMCHA8 strain identified as 75ppm.

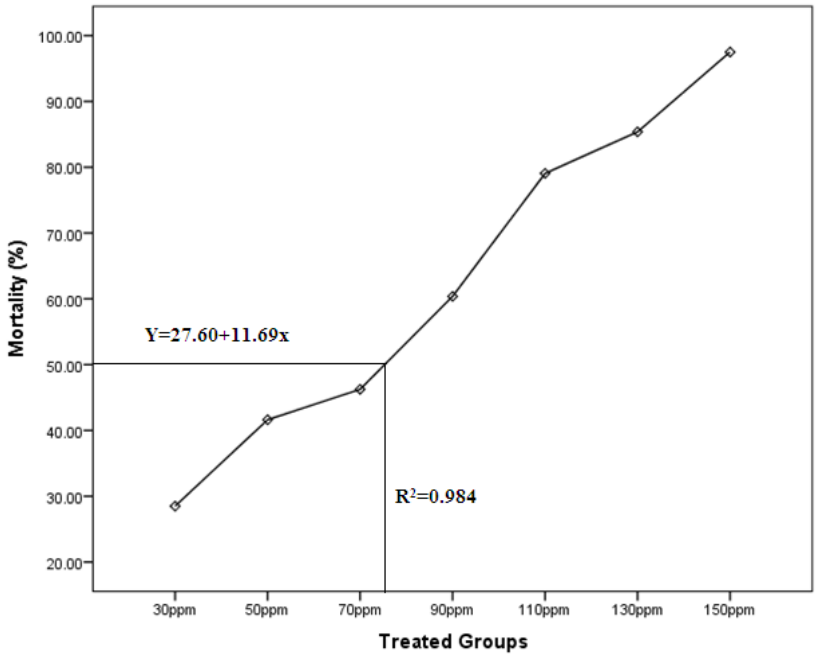

Figure 1: Relative toxicity (LC50) of secondary metabolites of actinobacterial JMCHA8 strain to 3rd and 4th instar larvae of *Leucinodesorbonalis*

Saruhan *et al.* [18] studied the biopesticidal effect for 3 to 7 days on *H. cunae* larvae showed increased mortality rate as dose and time increased. The isolated actinobacteria were investigated for larvicidal activity against *Aedes aegypti* and *Anopheles senphensi* mosquitoes, *S. griseus*, *S. albus*, *S. albofavus* and *S. Rochei* were identified as potential biopesticide producers [5]**.**

3.2. Morphological and Behavioural studies

Based on the LC50 values, three different concentrations such as 30ppm, 35ppm and 40ppm were selected for this study. One control group was maintained separately without impregnated of various concentrations of secondary metabolites. The different concentrations of extract of JMCHA8 strain mixed with brinjal fruit taken in a container (30×15cm) and collected larvae were introduced into it. In control group, larvae entered into the brinjal fruit and starts eat. Treated group larvae quickly move away from the food source mixed with extract of JMCHA8 strain and avoid the feed intake. Few larvae forms black coloured band on their body surface and the remaining larvae immediately forms bright silver coloured pupa around them after intake of treated fruit sample. After 8 days, the young ones emerges out showed various deformities such as antennae damage, deformed wings, dwarf males, less active females and mostly immature young ones were hatched out. Generally larvae showed reduced body size during the experimental period.Lepidoptera morphological and behavioural changes due to the parasitical infections were studied by Edward and Weaver [32]. The cuticular proteins tyrosine and proline forms polyphenol and quinones which are responsible for the dark band formation in the instar cuticles which enhances the pupation by the free amino acids from haemolymph [33].

3.3. Biochemical studies

Total Glucose levels were reduced (Figure 2) in extract of actinobacterial JMCHA8 strain treated groups nearly as 50% as 4.90±0.002, 6.01±0.002 and 7.61±0.003mg/dL in 30, 35 and 40ppm respectively than control glucose level as 18.37±0.008mg/dL (Table 1) and the ANOVA results showed significant as P>0.01. In treated groups, reduced glucose levels were due to energy demand developed in the larvae system. Increased glucose levels as increased concentrations due to the conversion of other biocompounds into glucose. Similarly reduced glucose levels evidenced in larva by Assar *et al.* [34], Ranjit and Dash [35], Abdel-Aal [36], and El-Sheikh [37].

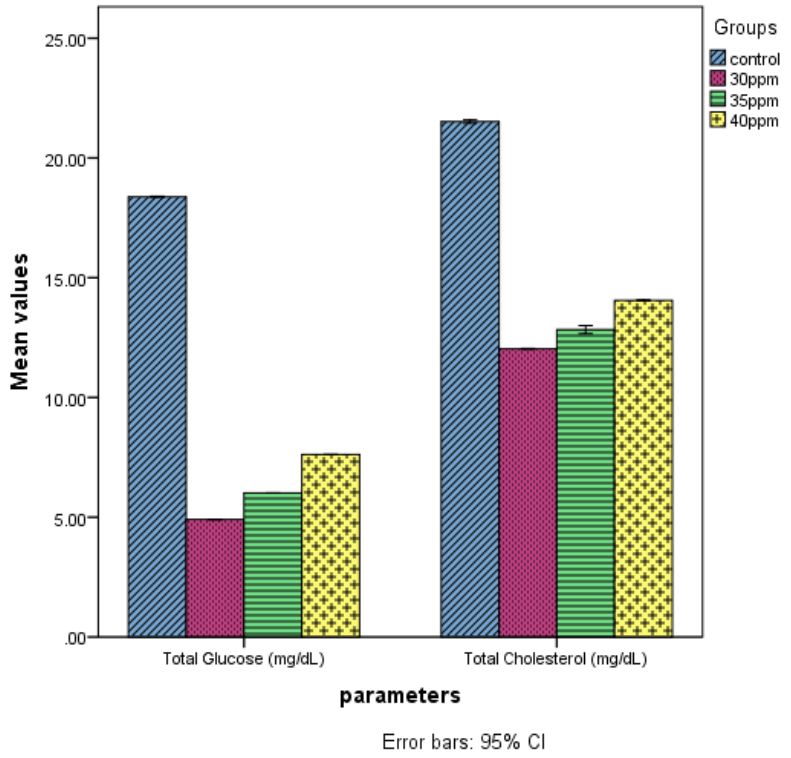

Figure 2: Effect of secondary metabolites of Actinobacterial isolate JMCHA8 on Total Glucose and Total cholesterol levels (mean±SE) of *Leucinodes orbonalis*(n=16)

Table 1: Effect of JMCHA8 Actinobacterial secondary metabolites on the Biochemical parameters (Mean±SE) of the 3rd and 4th instar larvae of *Lecuinodes orabonalis* (n=16)

Parameters	Control	JMCHA8		
		30ppm	35ppm	40ppm
Biochemical analysis				
Total Glucose (mg/dL)	18.37±0.008^{d}	4.90±0.002^{a}	6.01±0.002^{b}	7.61±0.003^{c}
Total Cholesterol (mg/dL)	21.51±0.031^{d}	12.03±0.007^{a}	12.83±0.076^{b}	14.05±0.010^{c}
Total Protein (gm/dL)	1.59±0.009^{d}	0.71±0.003^{b}	0.51±0.002^{a}	0.90±0.004^{c}
Albumin (gm/dL)	0.66±0.005^{c}	0.32±.003^{b}	0.22±0.004^{a}	0.31±0.003^{b}

Globulin (gm/dL)	0.94±0.003[d]	0.42±0.003[b]	0.33±0.002[a]	0.59±0.003[c]
Enzyme analysis				
GOT (U/L)	8.11±0.005[c]	8.84±0.009[d]	4.64±0.012[a]	6.18±0.002[b]
GPT (U/L)	101.81±0.324[b]	81.45±0.441[a]	301.14±0.244[c]	334.25±0.329[d]
ALP (U/L)	56.70±0.157[b]	93.13±0.513[a]	74.30±0.017[c]	45.76±0.037[a]
LDH (U/L)	96.62±2.00[a]	114.98±2.25[b]	140.81±0.247[c]	134.14±8.05[c]

a,b,c,d – least significant differences between the groups; P<0.01, significant

Similarly, the Total Cholesterol levels (Figure 2) also found as decreased levels as 12.03±0.007, 12.83±0.076 and 14.05±0.010mg/dL for the treated groups than control (21.51±0.031mg/dL). Larvae stores high content of fat in their dermal region, while exposed to the extract of actinobacterial strain, the stored fat were utilized by the tissues, resulted with increased cholesterol levels as increased concentrations of the extract of actinobacterial JMCHA8 strain. In control group, the protein level found as 1.59±0.009gm/dL whereas in treated larvae the protein levels found as 0.71±0.003, 0.51±0.002 and 0.90±0.004gm/dL respectively (Figure 3). Total protein levels were severely reduced in treated groups due to the high Protein assimilation to ruled out stress developed in the tissues.

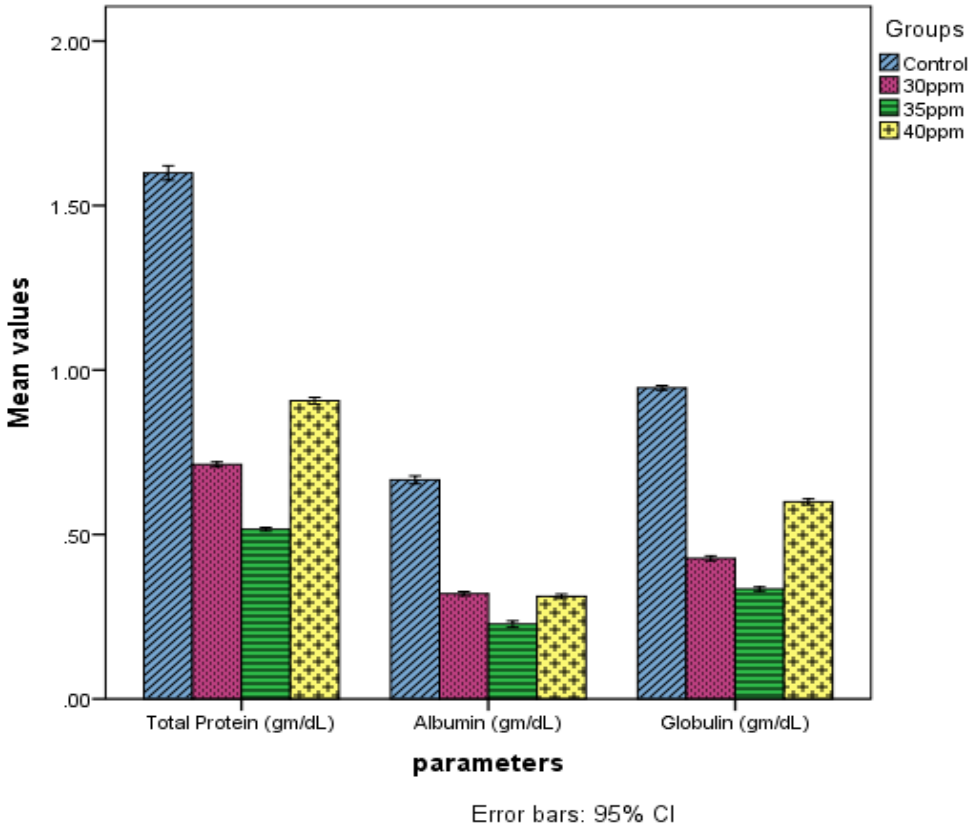

Figure 3. Effect of secondary metabolites of Actinobacterial isolate JMCHA8 on Total protein, Albumin and Globulin levels (mean±SE) of *Leucinodes orbonalis*(n=16)

Decreased albumin and globulin levels (Figure 3) ends with decreased total protein levels in treated larval tissues which was resulted in increased free amino acids in the haemolymph. Treated groups showed significant ($P<0.01$) level of total protein than control total protein (1.59±0.009gm/dL) level. Various studies proved that the presence of free aminoacids enhances detoxification [38] in the tissues which resulted as increased lactate dehydrogenase enzyme from the affected tissues.Reduced GOT levels observed in treated groups than control larvae. Kaur [39] suggested that decreased GOT levels were due to the significant reduction of free amino acids. The increased GPT levels (Figure 4) due to the increased catabolytic activities in the tissue than control. One-way ANOVA results also showed significant results between the experimental groups. Assar *et al.* [34] reported significant decrease in glucose, protein level simultaneously increased GOT and GPT in 0.1ppm Cyromazine treated dipteran insect. Increased activity of alkaline phosphate increases the Ecdysone process by increasing number of lysosomes which was evidenced by Radford and Misch [40], Van Pelt-Verkuil [41] and Assar *et al.* [34].

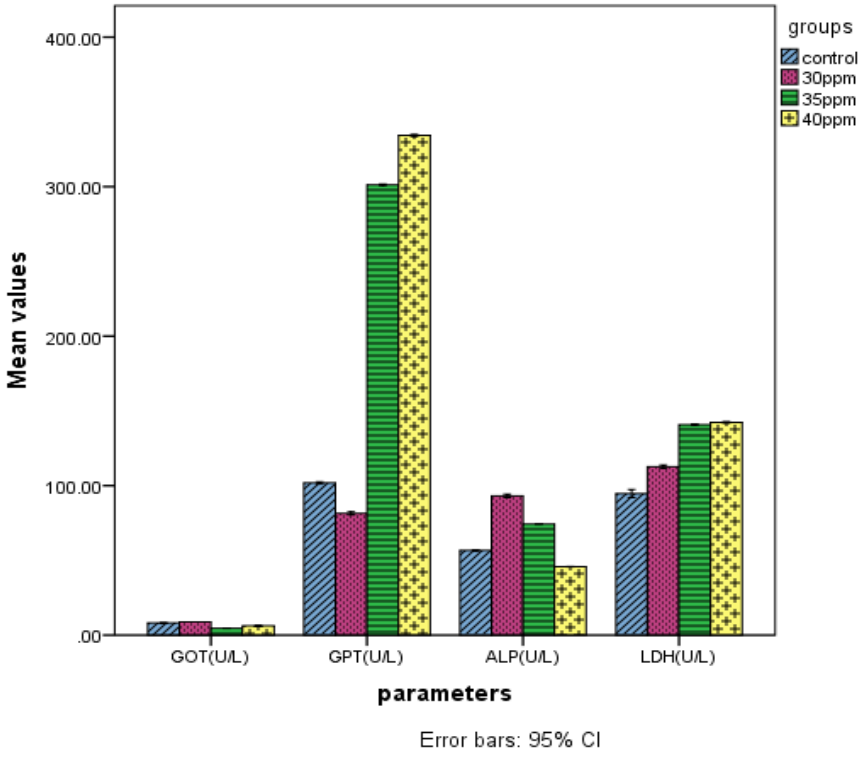

Figure 4.Effect of secondary metabolites of Actinobacterial isolate JMCHA8 on GOT, GPT, ALP and LDH levels (mean±SE) of *Leucinodes orbonalis*(n=16)

Increased activity of GOT parallels with increased protein levels in treated groups indicated that the 3rd and 4th instar larvae immediately enters into the pupae formation but due to their absence of 5th instar formation finally the larvae ends with deformities. This was evidenced by Gilbert [42], Pant and Morris [43] and Sidhu [44].

4. CONCLUSION

In insects, mobilization of major biocompounds from reserve was due to the acute stress developed by the JMCHA8 actinobacterial secondary metabolite exposure. The relative toxicity (LC50) was found as 75ppm. Sublethal dose exposed groups showed reduced feed intake, sudden pupae formation, discolouration and dark band formation and the hatched young ones showed various deformities resulted in affected adults. ANOVA results showed significantly altered biochemical levels in the treated larvae. Exposure of secondary metabolites of JMCHA8 strain disturbs the life cycle of *Leucinodes orbonalis* larvae by skipping their larval stage and altered biochemical composition in their tissues which resulted in deformed adults hatched from the pupae showed less viable.

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CONFLICT OF INTEREST

None

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