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Effect of different combinations of antimicrobial agents on biological fitness and fecundity of tobacco caterpillar (*Spodoptera litura*) (Lepidoptera: Noctuidae) reared on meridic diet

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ABSTRACT

Four antifungal and four antibacterial agents in 48 combinations in the meridic diet for investigating their effects on developmental stages of tobacco caterpillar (*Spodoptera litura*) (Fab.). The antimicrobial combination of D43 recorded significantly higher survival, fecundity, larval (LGI) and total developmental index (TDI). Increasing streptomycin sulphate to 400 ppm in D26 has improved the growth and developmental parameters of insect. Boric acid substituting either formaldehyde (D38) or streptomycin sulphate (D35) in the diet had statistically enhanced the performance of test insect. It is evident that boric acid being an effective antifungal and antibacterial agent at lower concentration can be used to substitute harmful carcinogenic formaldehyde which is needed at high concentration. However propionic acid, chloramphenicol and oxytetracyclin showed significant negative effects on the biological performance of insect. This work brings out diets, D26, D35, D38, D42, D43 and D46 giving good results as far as biological parameters are concerned.

Key words: Antimicrobial agents, Biological attributes, Meridic diet, *Spodoptera litura*

The tobacco caterpillar, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) is an economically important polyphagous pest in India. It heavily infests crops like cotton, tobacco, tomato, citrus, groundnut, maize, chillies, cabbage, cauliflower and many other vegetables (Alexander *et al.* 2010). Cohen (2003) said that the success of entomology over the past century is founded on our ability to rear insects on artificial diets. Artificial diets ensure high quality and healthy insects that are essential for conducting meaningful laboratory and field studies (Lapionte *et al.* 2008). Nutritional studies and resistance management operations require insects with uniform feeding so as to have equal strength in the insects for getting correct results means no cannibalism or starvation. However the successful mass rearing is limited by the microbial contamination which directly or indirectly affects insect health, size, delay the development, incite mortality and decrease the quantities of amino acids and fatty acids (Sikorowski and Thompson 1984 and Cohen 2003). Often antimicrobials are added based on traditional insect diet recipes rather than taking into account the additive's effect on insect health, shelf life of diet and the effectiveness of the compound (Alverson and Cohen 2002). Ryuda *et al.* (2008) reported the cannibalism of tobacco cutworm larvae; hence there is a necessity to rear larvae separately on artificial diet.

The main objective of this study was to investigate the optimum levels of antimicrobial agents and their right combination that support better growth and development without any adverse effect on the biological attributes of insect. Boric acid was chosen as it is an effective antifungal and antibacterial agent which is cheap, easily available and needed in low concentration to substitute formaldehyde.

MATERIALS AND METHODS

The experiments were carried out between November 2009 and January 2011. The culture was maintained from a single egg patch artificial diet in glass vials under controlled conditions (temperature 27±1°C, relative humidity 65±5% and a scoot/photophase regime of 16:8 h).

Eight antimicrobial agents (Table 1) studied in detail were practically working concentrations, selected after initial screening. Methyl paraben is an antifungal agent inhibits the absorbtion of essential nutrients and amino acids and destroys the cell membranes of microbes (Luke and Jager 1997). Sorbic acid, (2, 4-hexadienoic acid) controls bacteria, molds and yeasts in moist foods by interfering with carbohydrate metabolism, binds to the thio functional group of enzymes and destabilize the cell membrane of microbes. Propionic acid inhibits mold and bacterial growth by lowering the pH

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of the diet and has got unspecified mode of action (Ando and Stodola 2001). Boric acid an antifungal and antibacterial cause damage to microbes by generation of oxygen free radicles and by reducing the relative ergosterol content of the yeast cells (De Seta *et al.* 2009). Streptomycin sulphate exhibits its effects through the production of hydroxyl free radicles and other reactive oxygen species which induce oxidative stress damage to microbes. Formaldehyde at low concentrations exhibits bacteriostatic properties while at high concentrations it is lethal to bacteria, yeasts and molds (Chapman 1987). Chloramphenicol, is a bacteriostatic antimicrobial effective against a wide variety of gram-positive and gram-negative bacteria through the inhibition of bacterial protein synthesis. Hence it has been widely used in the artificial diets as preservative (Roeder *et al.* 2010). Oxytetracycline is a broad spectrum antibiotic active against a wide variety of bacteria.

Total 47 diets were studied in eight experiments (Table 1) consists of six treatments (except experiment 8 with 5 treatments) and a control with three replications. Diet 48 in Fig 1 was the average of control of eight experiments.

The meridic diet used in the study consists of mainly four fractions. The diet preparation and insect rearing was followed as described by Gupta *et al.* (2005) with suitable modifications. The diet ingredients, chickpea and kidney bean were procured from single batch for all the experiments and were autoclaved to avoid microbial contamination. The glass vials were sterilized before use to remove external contamination. Double distilled water was used throughout the experiment.

Twenty five newly emerged first instar larvae were taken for each replicate and were inoculated into diet in sterilised glass vials individually. The larval weight was recorded on 8th and 14th day of the larval period which is standardized as optimal time to measure the diet accessibility and growth. The newly formed pupae (6-16 hr old) were weighed daily and transferred to glass jars covered with cloth and required humidity was maintained. The one pair of newly emerged uniform adults were transferred to ovipositional jars provided with 10% sucrose solution (fortified with 2% vitamin solution) as adult diet. The effects of the test diets containing different antimicrobial agents on growth and developmental parameters of insect, such as larval and pupal weight (mg), larval, pupal and developmental periods, per cent pupation, adult emergence, survival was recorded. After transferring to ovipositional chamber, fecundity and adult longevity (days) were noted. The developmental parameters were computed from formulae given by Srinivas and Bhattacharya (1999).

Completely randomized design (CRD) was used for data analysis. The data was subjected to appropriate statistical tool, AGRES and meaningful conclusions were derived.

RESULTS AND DISCUSSION

The purpose of this investigation was to study the effect of eight antimicrobial agents in different combination on developmental parameters of the *S. litura*. The control diet used in the study consists of four antimicrobial agents, methyl paraben (1800 ppm), sorbic acid (1300 ppm), streptomycin sulphate (250 ppm) and formaldehyde (2000 ppm). The diet without antimicrobial agents was also studied but a visual contamination of both fungi and bacteria was observed after 3 and 5 days under rearing room temperature (27±1°C) and fridge (5°C) respectively. All the larvae died within 8 to 10 days because of the poor quality of diet. Hence it was excluded from the experiment.

The diets (D1 and D2) with low methyl paraben and high sorbic acid combinations and diets (D3 and D4) with high methyl paraben and low sorbic acid combinations did not work well for the insect. The per cent survival, fecundity and growth index were low on diets of other combinations. The high concentrations (>2500 and >2000 respectively) of these agents in diet have detrimental effects on the development of insect probably either due to reduced palatability or repellent effect and deleterious effect on symbionts as reported by Dunkel and Read (1991). They discussed that higher concentration of sorbic acid (2000 ppm) interfere with microbial symbionts of insects as in case of cigarette beetle where yeast fungi which supplies necessary B vitamins were significantly declined.

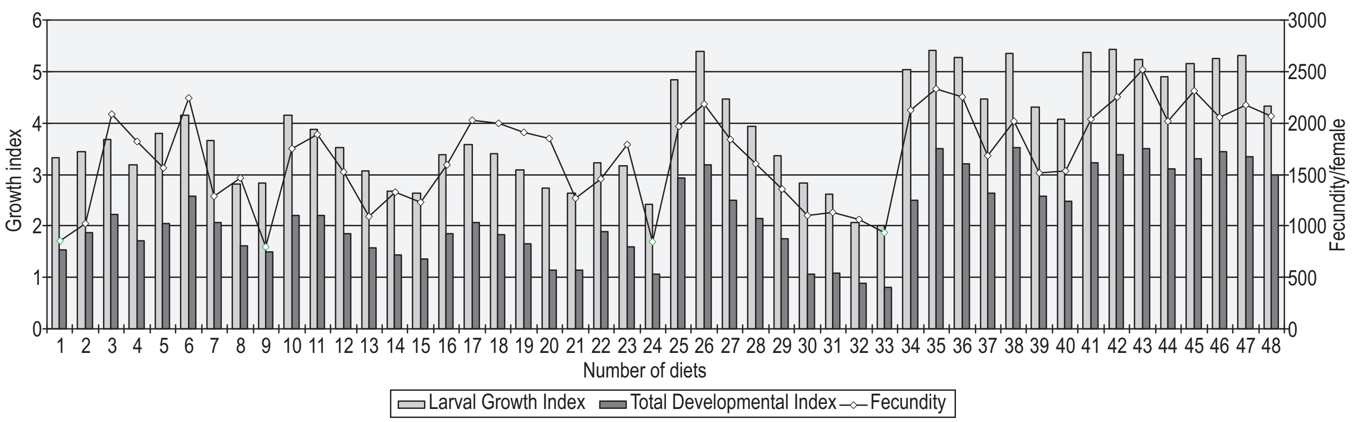

Fig 1 Effect of diet antimicrobial agents on growth index and fecundity of *S. litura*

Table 1 The treatment details of experiments

Diets	Diet antimicrobial agents (ppm)							
	MP	SA	SS	FOR	PA	BA	Chlor	Oxyt
D1	1000	2000	250	2000				
D2	1300	1600	250	2000				
D3	2500	800	250	2000				
D4	1800	800	250	2000				
D5	1800	1600	250	2000				
D6	2500	1600	250	2000				
D7	1800	2000	250	2000				
D8	1000	1300	250	2000				
D9	1500	1300	250	2000				
D10	2500	1300	250	2000				
D11	2000	1300	250	2000				
D12	1800	1300						
D13		2000	250	2000	800			
D14		1600	250	2000	1200			
D15		800	250	2000	1600			
D16	2500		250	2000	800			
D17	1500		250	2000	1200			
D18	1000		250	2000	1600			
D19	1500			1500				
D20	2000			2000				
D21		800		2500				
D22		1200		2500				
D23	2500		250	2500				
D24	1800	1300	250	3500				
D25	1800	1300	200	2000				
D26	1800	1300	400	2000				
D27	1800	1300	600	2000				
D28	1800	1300		2000				400
D29	1800	1300		2000				600
D30	1800	1300		2000				800
D31	1800	1300	250				300	
D32	1800	1300	250				500	
D33	1800	1300	250				700	
D34	1800	1300		2000		100		
D35	1800	1300		2000		200		
D36	1800	1300		2000		300		
D37	1800	1300	200			200		
D38	1800	1300	400			200		
D39	1800	1300	600			200		
D40	1800	1300		1000		200		
D41	1800	1300		1500		200		
D42	1800	1300		2500		200		
D43	2500	1300	400	2500				
D44	2500		400	2500		200		
D45	1800	1300		2000		250		
D46	1800	1300	400			250		
D47	1800	1300		2500		250		
Control*	1800	1300	250	2000				

*Standard diet was considered as a control

MP, Methyl paraben; SA, sorbic acid; SS, streptomycin sulphate; FOR, formaldehyde; PA, propionic acid; BA, boric acid; Oxy, oxytetracyclin; Chlor, chloramphenicol

Sorbic acid when incorporated at high concentration (0.2-0.4%) in the diet has significantly lengthened the larval period. The possible effect of high concentrations of methyl paraben and sorbic acid on gut microbial population of *S. litura* is a question and need to be investigated. Diet 6 composed of methyl paraben, sorbic acid, streptomycin sulphate and formaldehyde at 2500, 1600, 250 and 2000 ppm, respectively had significantly positive effect on biological parameters like shortened developmental period, good percent survival, fecundity, larval growth index and total developmental index as compared to other treatments. The pupal weight and fecundity were increased by 35 mg and 5% respectively over control. Similar results were also found by Kishaba *et al.* (1968) that the combination of methyl paraben (2000 ppm) and sorbic acid (1500 ppm) was safe to the larval development of *Trichplusiani*. Sofos (1989) discovered that sorbic acid added at levels of 0.13% was most effective in suppressing growth of *Streptomyces aureus* over 14 days of refrigerated storage. The other combinations of methyl paraben and sorbic acid negatively affected the performance of insect. Diet (D12) with only antifungal agents, methyl paraben and sorbic acid too afforded lower survival, adult emergence, fecundity and growth index indicating that they are not enough to inhibit both bacterial and fungal contamination in diet (Table 2; Fig 1) as pupal weight and fecundity were also declined. Among the various concentrations of methyl paraben and sorbic acid tried, 2500 and 1300 or 1600 ppm respectively improved diet quality and biological attributes of test insect.

Diets with propionic acid as one component (D13-D18) did not work well, when tried in combination with various concentrations of methyl paraben and sorbic acid and yielded low larval weights (40-63 mg), and increased developmental period by 4-7 days besides poor survival. However high pupal weights were recorded on these diets though there was no significant difference between treatments. Though propionic acid at 1200 ppm in combination with methyl paraben at 1500 ppm (D17) increased the per cent survival but it was significantly lower than control. It is possible that a little increase in propionic acid in combination with methyl paraben might substitute sorbic acid. In diets (D16-18) with propionic acid and methyl paraben, fecundity was reduced by nearly 40 per cent as compared to the D43 which gave maximum egg laying. The low adult survival and further low fecundity will have significant negative effect on population build up of subsequent generations. The TDI of other combinations in this experiment was also poor ranging from 1.3 to 1.5 as compared to 2.99 in control diet (Fig 1). The probable reason could be that propionic acid possesses minimal antibacterial properties and is primarily active against molds and possesses limited activity against yeasts (Block 1991). In a study by Inglis and Cohen (2004), they discovered that the presence of antibacterial agents like streptomycin sulphate and chlortetracyclin decreases the fungistatic activity of the propionic acid (Table 2; Fig 1).

Table 2 Effect of diet antimicrobial agents in different combinations on growth and development of *S. litura*

Treatments (Diet antimicrobial agents ppm)	LW–8th day (mg)	LW–14th day (mg)*	LP (days)	Pupation (%)**	PW (mg)*	DP (days)	AE (%)**	Days (%)**	AL (days)
Experiment 1									
D1 - MP 1000 + SA 2000 + SS 250 + FOR 2000	19.38^{e}	270.86^{c} (16.45)	22.41	74.67^{cd} (59.89)	385.25^{ab} (19.62)	34.08	69.46^{c} (56.49)	52.00^{d} (46.19)	7.67^{c}
D2 - MP 1300 + SA 1600 + SS 250 + FOR 2000	24.48^{cd}	346.68^{b} (18.61)	22.4	77.33^{bc} (61.59)	362.78^{c} (19.04)	34.99	85.94^{b} (68.04)	65.33^{ab} (53.96)	7.83^{b}
D3 - MP 2500 + SA 800 + SS 250 + FOR 2000	27.54^{ab}	381.03^{b} (19.51)	22.49	82.67^{abc} (65.43)	390.21^{abc} (19.75)	31.68	85.48^{b} (67.60)	70.67^{b} (57.22)	8.12^{b}
D4 - MP 1800 + SA 800 + SS 250 + FOR 2000	25.50^{bc}	352.00^{b} (18.73)	22.6	72.00^{d} (58.29)	363.44^{c} (19.06)	34.99	83.63^{b} (66.20)	60.00^{cd} (50.80)	8.17^{b}
D5 - MP 1800 + SA 1600 + SS 250 + FOR 2000	22.44^{d}	331.28^{b} (18.20)	22.13	84.00^{ab} (66.53)	402.64^{ab} (20.06)	33.98	82.51^{b} (65.33)	69.33^{b} (56.41)	7.83^{b}
D6 - MP 2000 + SA 1600 + SS 250 + FOR 2000	28.56^{a}	459.35^{a} (21.43)	21.24	88.00^{a} (69.91)	410.83^{a} (20.41)	31.6	92.41^{a} (74.19)	81.33^{a} (64.51)	8.02^{bc}
Control- MP 1800 + SA 1300 + SS 250 + FOR 2000	24.99^{c}	457.50^{a} (21.39)	21.12	86.67^{a} (68.63)	387.50^{ab} (19.63)	31.68	93.87^{a} (75.85)	81.33^{a} (64.43)	8.67^{a}
CD (P=0.05)	2.14	1.35	NS	6.35	0.86	NS	4.43	5.29	0.42
Experiment 2									
D7 - MP 1800 + SA 2000 + SS 250 + FOR 2000	15.47^{b}	246.41^{cd} (15.69)	22.98	84.00^{b} (66.53)	336.47^{bc} (18.34)	34.94	85.54 (67.53)	72.00^{ab} (58.21)	7.67
D8 - MP 1000 + SA 1300 + SS 250 + FOR 2000	21.80^{a}	286.04^{c} (16.91)	22.81	64.00^{c} (53.15)	323.72^{c} (17.99)	34.63	87.21 (69.57)	56.00^{c} (48.88)	7.83
D9 - MP 1500 + SA 1300 + SS 250 + FOR 2000	17.32^{b}	235.87^{d} (15.35)	23	65.33^{c} (53.94)	340.71^{bc} (18.45)	34.57	79.53 (63.17)	52.00^{c} (46.15)	8.5
D10 - MP 2500 + SA 1300 + SS 250 + FOR 2000	21.63^{a}	358.00^{b} (18.92)	20.21	84.00^{b} (66.53)	396.46^{a} (19.91)	32.81	85.54 (67.89)	72.00^{ab} (58.21)	8.67
D11 - MP 2000 + SA 1300 + SS 250 + FOR 2000	22.28^{a}	435.84^{a} (20.87)	20.61	80.00^{b} (63.51)	369.54^{ab} (19.22)	33.14	91.4 (75.86)	73.33^{ab} (59.20)	8.67
D12 - MP 1800 + SA 1300 only	15.23^{b}	231.40^{d} (15.21)	22.7	80.00^{b} (63.51)	330.95^{c} (18.19)	34.75	79.8 (63.45)	64.00^{bc} (53.21)	8.89
Control- MP 1800 + SA 1300 + SS 250 + FOR 2000	21.28^{a}	413.00^{a} (20.33)	21.61	90.67^{a} (72.29)	349.54^{bc} (18.69)	34.28	91.17 (73.08)	82.67^{a} (65.54)	8.67
CD (P=0.05)	3.41	1.53	NS	4.63	0.98	NS	NS	8.4	NS
Experiment 3									
D13 - PA 800 +SA 2000 + SS 250 + FOR 2000	6.85^{c}	63.02^{c} (7.90)	25.2^{b}	77.33^{c} (61.59)	405.74 (20.14)	37.17^{bc}	75.88^{c} (60.80)	58.67^{cd} (50.020)	7.00^{b}
D14 - PA 1200 SA 1600 + SS 250 + FOR 2000	6.23^{c}	57.63^{c} (7.59)	24.8^{b}	66.67^{d} (54.79)	345.46 (18.48)	36.38^{bc}	78.19^{c} (62.26)	52.00^{d} (46.15)	7.00^{b}
D15 - PA 1600 + SA 800 + SS 250 + FOR 2000	5.36^{c}	40.82^{c} (6.34)	24.8^{b}	65.33^{d} (53.94)	395.6 (19.89)	36.37^{bc}	75.49^{c} (60.33)	49.33^{d} (44.62)	6.00^{b}
D16 - PA 800 +MP 2500 + SS 250 + FOR 2000	14.86^{ab}	284.00^{b} (15.04)	24.8^{b}	84.44^{b} (66.53)	405.92 (20.15)	37.43^{c}	82.36^{bc} (65.38)	69.33^{b} (56.50)	8.00^{b}
D17 - PA 1200 MP 1500 + SS 250 + FOR 2000	15.99^{ab}	299.31^{a} (17.27)	22.3^{a}	80.00^{bc} (63.51)	425.53 (20.62)	34.86^{b}	90.07^{ab} (71.99)	72.00^{b} (58.09)	9.50^{a}
D18 - PA 1600 + MP 1000 + SS 250 + FOR 2000	14.44^{b}	219.40^{b} (14.72)	23.5^{ab}	88.00^{bc} (63.51)	458.61 (21.39)	35.55^{bc}	81.64^{bc} (64.79)	65.00^{c} (53.99)	8.50^{a}
Control- MP 1800 + SA 1300 + SS 250 + FOR 2000	21.26^{a}	402.24^{a} (20.07)	21.6^{a}	90.67^{a} (72.29)	349.54 (18.69)	30.28^{a}	92.56^{a} (74.76)	84.00^{a} (66.71)	8.66^{a}
CD (P=0.05)	6.66	2.06	2.06	4.66	NS	2.41	7.71	6.4	1.14

(*Continued*)

Table 2 (*Continued*)

Treatments (Diet antimicrobial agents ppm)	LW–8th day (mg)	LW–14th day (mg)*	LP (days)	Pupation (%)**	PW (mg)*	DP (days)	AE (%)**	Days (%)**	AL (days)
Experiment 4									
D19 - FOR 1500 + MP 1500	13.30^{b}	237.00^{b} (15.13)	24.10^{b}	74.67^{bc} (60.01)	425.00^{ab} (20.62)	36.22^{b}	80.89^{bc} (64.46)	60.00^{c} (50.78)	7.16^{bcd}
D20 - FOR 2000 + MP 2000	8.60^{c}	149.00^{cd} (12.15)	24.86^{b}	68.00^{cd} (55.58)	410.00^{ab} (20.24)	37.12^{b}	62.66^{cd} (52.35)	42.67^{d} (40.77)	6.66^{c}
D21- FOR 2500 + SA 800	8.10^{d}	94.00^{d} (9.70)	25.30^{b}	66.67^{cd} (54.75)	400.00^{bc} (19.99)	37.47^{b}	64.09^{cd} (53.22)	42.67^{d} (40.78)	6.83^{c}
D22 - FOR 2500 + SA 1200	10.10^{cd}	156.00^{bc} (12.48)	24.82^{b}	80.00^{ab} (63.51)	420.00^{ab} (20.50)	36.58^{b}	86.73^{ab} (68.69)	69.33^{b} (56.39)	7.50^{abc}
D23 - FOR 2500 + MP 2500 + SS 250	10.80^{bc}	211.00^{bc} (14.53)	23.98^{b}	76.33^{bc} (60.72)	433.00^{a} (20.82)	36.87^{b}	77.15^{bc} (61.48)	58.67^{c} (50.01)	7.66^{ab}
D24 - FOR 3500 + MP 1800 + SA 1300 + SS 250	8.10^{d}	185.00^{bc} (13.59)	25.44^{b}	61.33^{d} (52.11)	406.00^{ab} (20.16)	37.83^{b}	65.48^{d} (54.04)	40.00^{d} (39.22)	6.83^{cd}
Control - MP 1800 + SA 1300 + SS 250 + FOR 2000	21.80^{a}	415.25^{a} (20.37)	21.34^{a}	86.67^{a} (68.63)	382.00^{c} (19.54)	31.96^{a}	95.38^{a} (79.76)	82.67^{a} (65.54)	8.00^{a}
CD (P=0.05)	2.63	2.75	1.68	6.04	0.67	2.05	6.04	4.31	0.78
Experiment 5									
D25 - SS 200 + MP 1800 + SA 1300 + FOR 2000	29.00^{ab}	766.00^{a} (27.69)	18.15^{ab}	88.00^{b} (69.91)	409.00^{a} (20.21)	26.76^{a}	89.38^{ab} (71.44)	81.33^{a} (64.33)	7.83^{a}
D26 - SS 400 + MP 1800 + SA 1300 + FOR 2000	30.00^{a}	799.00^{a} (28.11)	17.30^{a}	93.33^{a} (75.20)	425.00^{a} (20.25)	26.29^{a}	90.10^{ab} (72.24)	84.00^{a} (66.53)	8.00^{a}
D27 - SS 600 + MP 1800 + SA 1300 + FOR 2000	26.00$^{b.}$	776.00^{a} (27.79)	17.91^{ab}	80.00^{c} (63.51)	368.00^{b} (19.18)	27.21^{ab}	85.06^{ab} (67.43)	68.00^{b} (55.58)	8.33^{a}
D28 - Oxy 400 + MP 1800 + SA 1300 + FOR 2000	26.00^{b}	571.00^{b} (23.90)	19.30^{b}	76.00^{cd} (60.72)	362.00^{b} (19.02)	28.71^{bc}	80.66^{bc} (63.97)	61.33^{bc} (51.57)	8.16^{a}
D29 - Oxy 600 + MP 1800 + SA 1300 + FOR 2000	21.00^{c}	412.00^{c} (20.30)	21.84^{c}	73.33^{d} (58.93)	357.00^{b} (18.88)	30.42^{c}	72.71^{bc} (58.51)	53.33^{c} (46.91)	7.66^{ab}
D30 - Oxy 800 + MP 1800 + SA 1300 + FOR 2000	20.00^{c}	176.00^{d} (13.22)	22.12^{c}	62.67^{e} (52.34)	347.00^{b} (18.62)	31.30^{c}	53.19^{c} (46.83)	33.33^{d} (35.26)	7.00^{b}
Control- MP 1800 + SA 1300 + SS 250 + FOR 2000	27.00^{ab}	686.00^{ab} (26.16)	19.11^{b}	89.33^{ab} (71.01)	375.00^{ab} (19.36)	28.20^{bc}	94.14^{a} (78.65)	84.00^{a} (66.53)	7.90^{a}
CD (P=0.05)	3.68	2.45	1.62	4.45	1.02	1.77	9.28	4.9	0.72
Experiment 6									
D31- Chlor 300 + MP 1800 + SA 1300 + SS 250	12.00^{c}	248.00^{d} (15.74)	20.44^{bc}	53.33^{c} (46.91)	319^{c} (17.86)	30.74^{c}	62.64^{c} (52.36)	33.33^{d} (35.26)	7.16^{ab}
D32 - Chlor 500 + MP 1800 + SA 1300 + SS 250	10.00^{c}	167.00^{e} (12.95)	23.27^{d}	48.00^{d} (43.81)	233^{e} (15.24)	32.77^{c}	61.11^{c} (51.44)	29.33^{de} (32.78)	6.00^{c}
D33 - Chlor 700 + MP 1800 + SA 1300 + SS 250	8.00^{c}	127.00^{f} (11.29)	22.58^{c}	45.33^{d} (42.31)	277^{d} (16.60)	31.67^{c}	55.56^{c} (48.20)	25.33^{e} (30.15)	7.00^{b}
D34 - BA 100 + MP 1800 + SA 1300 + FOR 2000	17.00^{b}	748.00^{a} (27.34)	18.03^{a}	90.67^{b} (72.29)	362^{b} (19.01)	27.30^{ab}	75.03^{b} (60.09)	68.00^{c} (55.58)	7.83^{ab}
D35 - BA 200 + MP 1800 + SA 1300 + FOR 1000	25.00^{a}	773.58^{a} (27.81)	17.25^{a}	93.33^{a} (75.23)	413^{a} (20.31)	25.12^{a}	94.32^{a} (76.38)	88.00^{a} (69.73)	8.00^{a}
D36 - BA 300 + MP 1800 + SA 1300 + FOR 2000	24.00^{a}	765.67^{a} (27.67)	17.43^{a}	92.00^{ab} (73.57)	409^{a} (20.20)	26.93^{ab}	94.20^{a} (76.26)	86.67^{ab} (68.63)	7.50^{ab}
Control- MP 1800 + SA 1300 + SS 250 + FOR 2000	16.00^{b}	490.00^{c} (22.38)	20.36^{b}	89.33^{b} (71.01)	395.07^{ab} (19.87)	29.86^{bc}	92.56^{a} (74.33)	82.67^{b} (65.43)	7.44^{ab}
CD (P=0.05)	3.78	1.33	2.15	3.41	1.1	3.15	5.27	3.4	0.96

(*Continued*)

Table 2 (*Concluded*)

Treatments (Diet antimicrobial agents ppm)	LW–8th day (mg)	LW–14th day (mg)*	LP (days)	Pupation (%)**	PW (mg)*	DP (days)	AE (%)**	Days (%)**	AL (days)
Experiment 7									
D37 - BA 200 + SS 200 + MP 1800 +SA 1300	16	490.00^{c} (22.13)	18.53^{c}	82.67^{de} (65.43)	377.00^{bc} (19.40)	27.78^{b}	88.73 (70.46)	73.33^{c} (58.93)	7.00^{c}
D38 - BA 200 + SS 400 + MP 1800 +SA 1300	16.8	751.00^{a} (27.40)	17.33^{a}	93.33^{bc} (75.20)	427.00^{a} (20.65)	25.80^{a}	97.1 (81.79)	90.67^{a} (72.64)	8.33^{ab}
D39 - BA 200 + SS 600 + MP 1800 +SA 1300	15.5	432.00^{d} (20.78)	18.53^{c}	80.00^{e} (63.51)	373.00^{c} (19.29)	28.46^{bc}	91.74 (73.45)	73.33^{c} (58.93)	7.66^{bc}
D40 -BA 200+ FOR 1000 + MP 1800 +SA 1300	15.2	622.00^{b} (24.91)	19.00^{d}	77.33^{e} (61.59)	384.00^{bc} (19.59)	28.40^{c}	91.4 (73.13)	70.67^{c} (57.22)	6.66^{c}
D41 - BA 200 + FOR 1500 + MP 1800 +SA 1300	13	404.00^{d} (20.09)	17.64^{ab}	94.67^{ab} (76.83)	423.00^{ab} (20.56)	25.99^{a}	88.71 (70.45)	84.00^{b} (66.53)	8.33^{ab}
D42 -BA 200+ FOR 1000 + MP 1800 +SA 1300	18.4	576.00^{bc} (23.98)	17.93^{b}	97.33^{a} (82.12)	443.00^{a} (21.04)	26.03^{a}	90.39 (72.03)	88.00^{ab} (69.91)	9.00^{a}
Control- MP 1800 + SA 1600 + SS 250 + FOR 2000	15.5	513.00^{c} (22.74)	19.58^{e}	88.00^{cd} (69.91)	400.00^{bc} (19.99)	29.28^{c}	94.06 (78.55)	82.67^{b} (65.54)	8.00^{ab}
CD (P=0.05)	NS	1.57	0.47	6.01	0.66	0.91	NS	5.45	1.01
Experiment 8									
D43- MP 2000 + SA 1300 + SS 400 + FOR 1000	26.70^{a}	743.26^{a} (27.26)	17.83^{a}	93.33^{ab} (75.20)	427.19 (20.67)	26.29^{a}	98.61 (85.69)	92.00^{a} (73.57)	8.33
D44- MP 2000 + BA 200 + SS 400 + FOR 1000	25.72^{ab}	679.19^{c} (26.05)	18.26^{a}	89.33^{bc} (71.01)	439.99 (20.97)	26.56^{a}	92.62 (74.74)	82.67^{c} (65.43)	8.43
D45- BA 250 + MP 1800 + SA 1300 + FOR 1000	25.15^{ab}	765.67^{a} (27.67)	17.59^{a}	90.67^{bc} (72.29)	414.31 (20.35)	25.75^{a}	94.14 (76.19)	85.33^{bc} (67.53)	8.34
D46- BA 250 + SS 400 + MP 1800 +SA 1300	17.34^{c}	742.71^{a} (27.25)	17.53^{a}	92.00^{bc} (73.92)	431 (20.76)	25.94^{a}	97.04 (81.69)	89.33^{ab} (71.54)	8.44
D47-BA 250+ FOR 1000 + MP 1800 +SA 1300	20.04^{c}	596.94^{d} (24.43)	18.10^{a}	96.00^{a} (78.47)	451.68 (21.25)	26.37^{a}	91.67 (72.58)	88.00^{abc} (69.91)	8.67
D48- Control- MP 1800 + SA 1300 +SS 250 + FOR 2000	23.80^{b}	513.00^{e} (22.65)	19.72^{b}	88.00^{c} (69.91)	400 (20.00)	29.43^{b}	94.07 (78.24)	82.67^{c} (65.43)	8
CD (P=0.05)	2.88	1	1.21	5.12	NS	1.79	NS	5.67	NS

* Values in the parenthesis are square root transformed; ** values in the parenthesis are arc sin transformed; NS, non-significant

D followed by number in the treatments represents diet number; MP, Methyl paraben; SA, sorbic acid; SS, streptomycin sulphate; FOR, formaldehyde; PA, propionic acid; BA, boric acid; Oxy, oxytetracyclin; Chlor, chloramphenicol; LW, Larval weight; LP, larval period; PW, pupal weight; PP, pupal period; DP, developmental period; AE, adult emergence; AL, adult longevity

The diet D34 (100 ppm boric acid) though initially supported the larval growth and development but it significantly affected the post larval development of insect. Boric acid substituting streptomycin sulphate at 200 and 300 ppm (D35 and D36) significantly shortened the larval period and total developmental period by 2.75 and 3-4.5 days respectively, besides recording higher larval weights (765.67 and 733.58 mg). The survival was increased by 4-6 per cent. The fecundity (2334 and 2247), larval growth index (5.33 and 5.35) and total developmental index (3.45 and 3.26) of insect were improved over the control (1920, 4.38 and 2.76 respectively) as shown in Fig 1. But the survival and TDI were reduced by 1.5 per cent and 0.30 in D36 than D35. Hence a middle concentration of 250 ppm (D45, D46 and D47) was studied further.

Diets 19 to 24 were studied with various combinations of formaldehyde either with methyl paraben or with sorbic acid. The larval weights both on 8^{th} and 14^{th} day were drastically reduced almost to half which affected the post larval developmental stages. The larval period was longer to compensate the nutrition which resulted in accumulation of high pupal biomass. The total developmental period was prolonged by almost 20 per cent than normal duration. Longer time was taken by adults to emerge from pupae (Table 2). In diet 24, the fecundity was minimum that is almost one third as compared to diet D43. It may be because of high concentration of formaldehyde (3500 ppm) which may be imparting direct toxicity or might have impaired the nutritional quality of diet. Similar findings were also observed by Siddiqui and Sarup (1982) when formaldehyde concentration increased (3 to 4 ml) in the artificial diet of *Chilo partellus* has completely checked the larval growth apart from controlling the microbes. The larvae need high quality and well balanced diet to produce high yields in post larval developmental stages. It has been observed that some times high concentrations of these agents may deter or reduce the feeding activity of the larvae.

The antimicrobial combinations of diets containing streptomycin sulphate (D25-D27) were studied. At 400 ppm (D26) it improved biological attributes like larval period, larval and pupal weight, apart from diet quality although per cent survival was on par with control. Developmental periods were accelerated by 2 days, highest larval weight on 14th day was recorded in D26 among the 47 diets tested, larval growth index (5.39) and developmental index (3.19) were statistically better than control diet (Fig 1). The fecundity in D26 (1.2%) was little lower than control. However, higher concentration (600 ppm in D27) exerted significant negative effects on the post larval developmental parameters like fecundity, per cent survival despite good initial support to the growing larvae. Our results are in accordance with Buyukguzel and Kalender (2009) who found that streptomycin sulphate supported the larval development but at higher concentration decreased the survivorship, altered bodymass, chemical composition and retarded the development of *Galleria mellonella*.

The antimicrobial combinations of diets containing oxytetracyclin (D28-D30) showed significant inhibitory effect on post larval development at all concentrations tested. As the concentration of oxytetracyclin increases from 400 to 800 ppm, per cent survival declined from 61.33 to 33.33. Our findings are in accordance with Singh and House (1970) who found that oxytetracyclin showed direct toxic effect to *Agria affinis* at concentration tested (50-110 mg/100 ml). Most antimicrobial agents ionize under physiological conditions; the degree of ionization is one of the important factors determining the nutrient absorption. Chloramphenicol when tested as a replacement to formaldehyde (D31, D32 and D33) had lengthened the larval and total developmental period by 2-3 days. The survival was drastically declined by 42-47 per cent over control. Furthermore, it was also observed that concentrations were negatively correlated with the growth and development of test insect (Table 2; Experiment 6). Buyukguzel and Yazgan (2002) suggested that the antimicrobial agents like chloramphenicol accumulated and distened midgut and thereby decreased feeding rate of insects. Catnach (1994) has explained that osmotic pressure of insects fed on diet with chloramphenicol and oxytetracyclin is much lower than other parts of alimentary canal, hence the absorption of nutrients may be impaired. Most antimicrobial agents ionize under physiological conditions; the degree of ionization is one of the important factors determining the nutrient absorption. Therefore these antimicrobial agents, oxytetracyclin and chloramphenicol may not be suitable substitutes to either streptomycin sulphate or formaldehyde at any concentration tested.

The various concentrations of diet antimicrobial agents which proved effective in the basic diet individually were combined to study their cumulative effect on the biological attributes of *S. litura*. The diet antimicrobial combination of D43 (MP 2500 + SA 1300 + SS 400 + FOR 2500 ppm) improved the diet quality and enhanced the adult emergence, survival and fecundity by 4.54, 9.33 and 12.8 per cent respectively as compared to control. The diet 42 had significant positive effect on larval period and total developmental period as these were shortened by 2-2.3 and 3.2-3.5 days respectively. The larval growth index and total developmental index were remarkably increased (Table 2 and Fig 1).

In Diet 38 boric acid substituted formaldehyde survived the insect up to 90.67 per cent (8 per cent increase) high larval and pupal weights (751.00 and 427.00 mg) and shortened the developmental period by 3.48 days as compared to control. Boric acid at 250 ppm was also studied but no significant difference between the effects of 200 and 250 ppm was observed. Boric acid at 300 ppm in D36 was slightly affected larvae as it reduced the per cent survival and lengthened developmental period. The results in our experiments show its potential as a diet antimicrobial agent. Boric acid was observed as an effective antifungal and antibacterial agent improved the biological attributes of *S. litura* without any negative effect and is needed in low concentration and can be economic substitute to formaldehyde where the latter is required in high quantity (2500 ppm), human carcinogenic and cause nasopharyngeal cancer reported by International Agency for Research on Cancer (IARC 2004).

The formaldehyde was considered as a probable human carcinogen by US environmental protection agency in 1987 under the conditions of high and prolonged exposure. The International Agency for Research on Cancer (IARC) also classifies formaldehyde as human carcinogen (IARC 2004; NTP 2011). Hence suitable alternative antimicrobial agent which can substitute formaldehyde in the diet is needed. In this process, boric acid was tested to substitute either formaldehyde or streptomycin sulphate. Though the literature on boric acid as an antimicrobial agent of insect diet is lacking, it is being widely used in the field of medicine in ophthalmology at lower concentrations indicating it harmless. Haesebrouck *et al.* (2009) found that boric acid along with acetic acid at 2% each successfully inactivated the *Staphylococcus pseudintermedius* under laboratory *in vitro* condition. They also noted the pronounced bacteriostatic effects of boric acid at a concentration of 0.2% against common microbial contaminants and pathogens. In another *in vitro* study conducted by Houlsby *et al.* (1986), boric acid buffered solutions at high concentrations exhibited significant antimicrobial activity against 15 Pseudomonas strains, 12 strains of enteric bacteria and seven strains of Staphylococcus bacteria.

The effect of antimicrobial combinations of diets D45, D46 and D47 on biological attributes of test insect was also significantly better than control. However there was no significant difference between the diet treatments with respect to pupal weight, adult emergence and adult longevity. It was also found that no significant difference between the effects of boric acid at 200 and 250 ppm. Hence boric acid can be used to substitute the formalhehyde in the diet. Moreover the LD_{50} of boric acid is 5 to 20 g/kg for oral dosage given to mammals (Murray 2004) which is safer than formaldehyde where the tolerant level is 3 ppm for an exposure period of 8 hours (OSHA 1991).

These diets (D26, D35, D38, D42, D43 and D46) selected out of 47 combinations have long shelf-life to support culturing of *S. litura* in individual vials as cannibalism was reported in this insect. Many workers have reported that cannibalism is mainly induced by often shortage of food resources, high population density in a colony, and developmental and physiological states of insects (Trabalone *et al.* 1998). Ryuda *et al.* (2008) found the inheritance of cannibalistic behavior in larvae of *S. litura* and identified a novel gene (779 bp) under starvation. They explained that increase in the concentration of juvenile hormone (JH) under starvation makes the insect more aggressive.

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