

1 **Unlocking the Potential of Substrate Quality for Enhanced Antibacterial Activity of Black**
2 **Soldier Fly against Pathogens**

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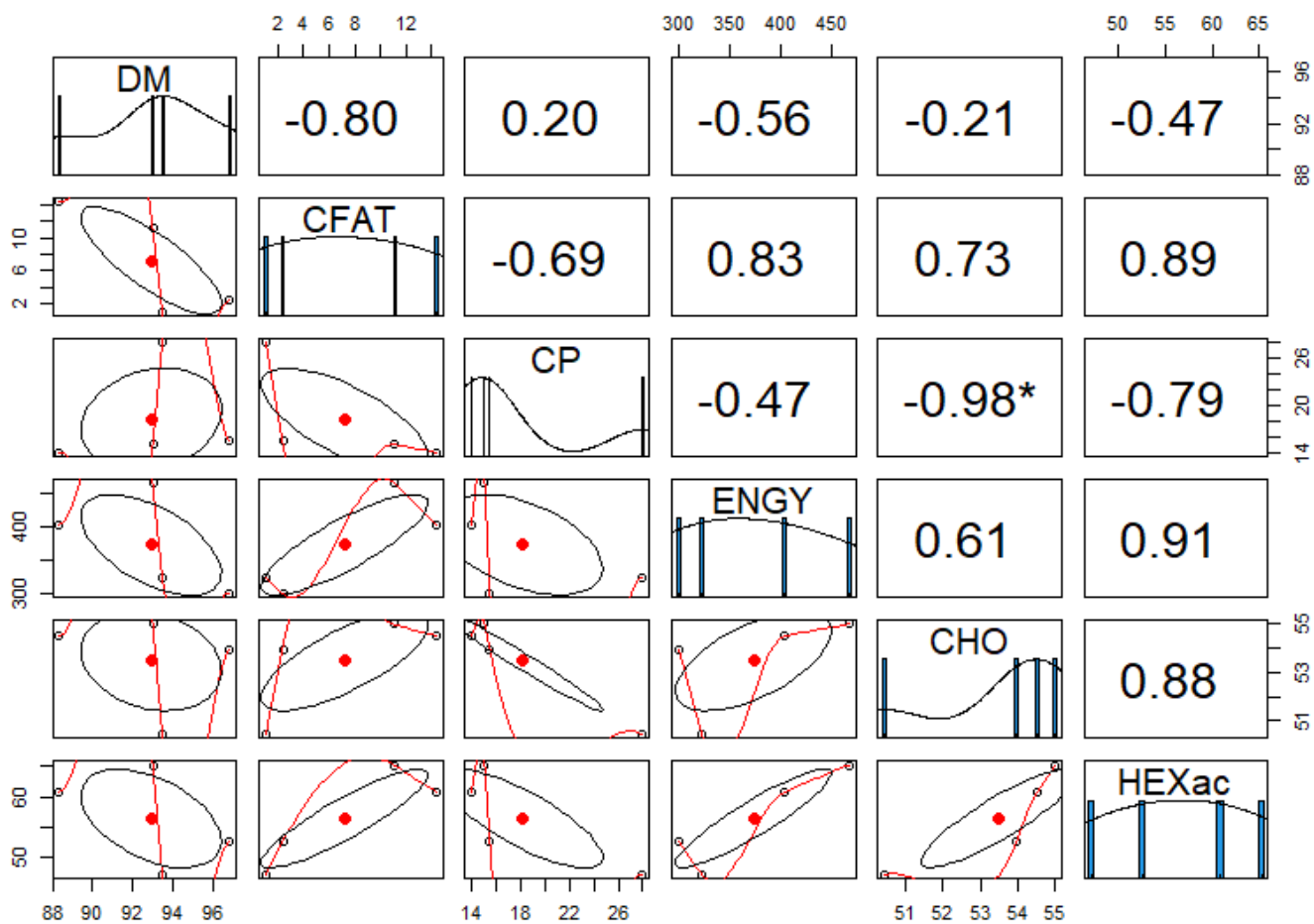
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Supporting Information



Supplementary Figure S1. Pearson's correlation coefficient (r) for proximate analysis and antibacterial activity of hexane extract.

Supplementary Table S1. Concentration of methylated fatty acids from hexane extracts of *H. illucens* larvae fed on CH, potato waste; MH, market waste; PH, pig manure and RH, rabbit manure.

S/NO	RT	Library/ID	Mean CH (µg/g)	Mean_MH (µg/g)	Mean PH (µg/g)	Mean_RH (µg/g)	F	DF	P- VALUE
1	14.82	Methyl nonanoate	14.06 ± 5.49 ^a	15.94 ± 12.94 ^a	8.34 ± 0.44 ^a	2.68 ± 0.57 ^a	2.18	3,8	> 0.05
2	16.25	Methyl decanoate	28.90 ± 9.73 ^a	30.89 ± 20.02 ^a	33.54 ± 3.17 ^a	-	0.1	2,6	> 0.05
3	16.97	Methyl 4,8-dimethylnonanoate	0.84 ± 0.13 ^{ab}	0.18 ± 0.09 ^a	1.53 ± 0.71 ^b	-	7.65	2,6	0.05
4	17.57	Methyl undecanoate	1.45 ± 0.66 ^a	0.53 ± 0.13 ^a	2.27 ± 0.64 ^{ab}	3.97 ± 1.20 ^b	11.11	3,8	0.01
5	18.92	Methyl dodecanoate	602.76 ± 88.94 ^c	318.17 ± 174.18 ^{ab}	214.42 ± 185.99 ^{ab}	27.71 ± 9.78 ^a	4.3	3,8	0.05
6	19.25	Methyl 2-methyl-dodecanoate	4.29 ± 0.79	-	-	-			
7	19.27	Methyl 2,6-dimethyl-undecanoate	3.870 ± 1.58	-	-	-			
8	19.30	Methyl dodecanoate	-	-	1.12 ± 0.21	-			
9	19.45	Methyl 4-methyl-dodecanoate	2.94 ± 0.77 ^a	2.35 ± 1.52 ^a	-	-	0.36	1,4	> 0.05
10	19.61	Methyl 11-methyl-dodecanoate	-	-	2.01 ± 1.28	-			
11	19.72	Methyl 10-methyl-dodecanoate	-	3.37 ± 2.11	-	-			
12	20.46	Methyl 3-methyl-tridecanoate	-	0.51 ± 0.09 ^a	0.51 ± 0.02 ^a	-	0.0002	1,4	> 0.05
13	21.16	Methyl tetradecanoate	-	-	214.91 ± 94.05 ^b	11.92 ± 1.94 ^a	13.97	1,4	0.05
14	21.51	Methyl 2-methyl-dodecanoate	3.31 ± 0.59 ^a	2.64 ± 0.45 ^a	-	-	2.38	1,4	> 0.05
15	21.72	Methyl 12-methyl-tetradecanoate	4.39 ± 1.43 ^a	26.00 ± 20.28 ^a	-	-	3.39	1,4	> 0.05
16	21.94	Methyl 9-methyltetradecanoate	3.99 ± 1.74 ^a	2.36 ± 2.24 ^a	-	-	0.99	1,4	> 0.05
17	21.94	Methyl pentadecanoate	22.46 ± 18.38	-	-	-			
18	22.23	Methyl dodecanoate	-	-	59.41 ± 46.81	-			
19	22.61	Methyl 3-methyl-pentadecanoate	-	-	-	0.38 ± 0.06			
20	23.37	Methyl hexadecanoate	738.28 ± 42.68 ^c	386.69 ± 123.30 ^b	31.29 ± 21.16 ^a	44.60 ± 6.72 ^a	76.96	3,8	0.001
21	23.93	Methyl 15-methyl-hexadecanoate	12.02 ± 1.20 ^b	4.04 ± 1.22 ^a	-	-	64.97	1,4	0.01
22	23.96	Methyl heptadecanoate	-	-	-	6.15 ± 0.19			
23	25.52	Methyl nonadecanoate	-	-	3.25 ± 1.03	-			
24	26.93	Methyl 18-methylnonadecanoate	-	-	11.94 ± 2.26 ^b	3.76 ± 1.16 ^a	30.99	1,4	0.01

25	27.74	Methyl 18-methylcosanoate	-	-	-	3.05 ± 1.43			
26	27.76	Methyl heneicosanoate	-	-	4.11 ± 0.96	-			
27	28.31	Methyl octadecanoate	2.92 ± 1.37 ^a	5.39 ± 0.20 ^a	1.56 ± 1.97 ^a	-	1.18	2,6	
28	28.55	Methyl docosanoate	8.43 ± 2.03 ^a	17.17 ± 6.85 ^a	17.33 ± 14.39 ^a	2.67 ± 0.51 ^a	2.38	3,8	> 0.05
29	29.31	Methyl tricosanoate	3.72 ± 1.34 ^a	4.29 ± 0.30 ^a	2.56 ± 2.05 ^a	-	1.13	2,6	> 0.05
30	30.09	Methyl tetracosanoate	4.07 ± 1.17 ^a	5.12 ± 1.33 ^a	3.67 ± 2.08 ^a	-	0.67	2,7	> 0.05
31	31.97	Methyl hexacosanoate	2.53 ± 0.85 ^a	2.63 ± 0.60 ^a	2.53 ± 0.19 ^a	-	0.03	2,8	> 0.05
		Σ SFA	1262.31	822.88	614.74	106.89			
32	17.46	Methyl 3Z-octenoate	-	-	-	0.42 ± 0.14			
33	18.56	Methyl 5Z-dodecenoate	2.78 ± 0.31 ^a	-	2.33 ± 0.91 ^a	-	0.64	1,4	> 0.05
34	20.89	Methyl 9Z-tetradecenoate	39.10 ± 17.77 ^b	18.22 ± 8.40 ^a ^b	31.07 ± 17.77 ^{ab}	1.11 ± 0.34 ^a	5.42	3,8	0.05
35	20.91	Methyl 5Z-dodecenoate	-	32.50 ± 27.02	-	-			
36	22.07	Methyl 7Z-hexadecenoate	-	-	-	1.09 ± 0.36			
37	23.22	Methyl 9Z-hexadecenoate	-	-	550.42 ± 124.99 ^b	50.58 ± 5.19 ^a	47.89	1,4	0.01
38	24.04	Methyl 10Z-heptadecenoate	21.72 ± 6.19 ^{ab}	30.33 ± 9.44 ^b	19.11 ± 18.20 ^{ab}	1.84 ± 1.15 ^a	3.72	3,8	> 0.05
39						115.25 ±			> 0.05
	25.07	Methyl 9E-octadecenoate	-	2.22 ± 2.30 ^a	268.67 ± 221.10 ^a	21.83 ^a	3.26	2,6	
40	25.45	Methyl 10-octadecenoate	-	21.04 ± 10.97	-	-			> 0.05
41	25.88	Methyl 10Z-nonadecenoate	4.22 ± 1.17 ^a	17.89 ± 9.67 ^a	19.15 ± 23.78	-	5.9	1,4	> 0.05
42	26.11	Methyl 2-heptenoate	-	18.75 ± 7.97	-	-			
43	26.96	Methyl 11-eicosenoate	-	19.58 ± 10.93	-	-			
		Σ MUFA	67.82	160.53	890.75	170.29			
44		Methyl (9Z,12Z)- octadecadienoate	853.24 ± 154.78 ^b	21.61 ± 4.56 ^a	25.97 ± 18.99 ^a	13.63 ± 5.09 ^a	85.41	3,8	0.001
45	25.61	Methyl 8,11,14-eicosatrienoate	3.48 ± 0.29	-	-	-			
46		Methyl (5Z,8Z,11Z,14Z)- eicosatetraenoate	10.68 ± 3.12 ^a	3.92 ± 1.01 ^a	27.53 ± 22.07 ^a	4.02 ± 1.55 ^a	2.97	3,8	> 0.05
47	26.53	Methyl 7,10,13-eicosatrienoate	10.23 ± 1.46	-	-	-			
		Σ PUFA	877.63	25.53	53.50	17.65			
		PUFA/SFA	0.70	0.31	0.09	0.16			

Supplementary Table S2. Mass of HIL (mg) in growth stages

Growth stages	Rabbit manure	Potato waste	Pig manure	Market waste	DF	F-Value	P-Value
Second instar	28.7 ^a ± 2.1	26.7 ^a ± 5.5	34.7 ^a ± 6.6	29.0 ^a ± 3.0	3, 8	1.61	0.262
Third instar	68.0 ^a ± 6.0	67.3 ^a ± 9.0	71.0 ^a ± 17.0	55.7 ^a ± 10.8	3, 8	1.043	0.425
Fourth instar	125.3 ^a ± 12.5	82.7 ^a ± 6.0	103.3 ^b ± 5.8	70.7 ^a ± 3.5	3, 8	28.96	0.00012
Fifth instar	172.0 ^a ± 5.3	151.0 ^a ± 18.2	165.7 ^a ± 6.7	116.0 ^b ± 9.5	3, 8	15.16	0.00116

Supplementary Table S3. Antibacterial activities of HIL Hexane extracts

Test pathogens	Rabbit manure	Potato waste	Pig manure	Market waste	Positive control	DF	F-Value	P-Value
<i>B. subtilis</i>	9.41 ^a ± 0.07	12.16 ^b ± 0.56	10.51 ^{ab} ± 0.84	13.04 ^b ± 0.63	17.47 ^c ± 1.50	4, 10	43.78	<0.001
<i>P. aeruginosa</i>	8.61 ^a ± 0.74	11.41 ^{bc} ± 1.05	9.92 ^{ab} ± 0.33	12.63 ^c ± 0.26	17.60 ^d ± 1.11	4, 10	58.67	<0.001
<i>E. coli</i>	8.95 ^a ± 0.81	10.20 ^a ± 0.44	8.66 ^a ± 0.16	10.56 ^a ± 0.52	17.53 ^b ± 1.19	4, 10	77.5	<0.001
<i>S. aureus</i>	8.62 ^a ± 0.39	11.34 ^{ab} ± 1.18	9.00 ^a ± 0.44	12.31 ^b ± 1.35	16.83 ^c ± 0.42	4, 10	89.35	<0.001

Supplementary Table S4. Antibacterial activities of HIL 20 % Acetic acid extracts

Test pathogens	Rabbit manure	Potato waste	Pig manure	Market waste	Positive control	DF	F-Value	P-Value
<i>B. subtilis</i>	9.10 ^a ± 0.03	13.85 ^b ± 1.07	8.73 ^a ± 0.27	14.66 ^b ± 1.53	17.52 ^c ± 0.89	4, 10	49.17	<0.001
<i>P. aeruginosa</i>	11.14 ^a ± 1.11	13.57 ^{ab} ± 0.93	10.32 ^a ± 1.38	13.96 ^b ± 1.30	17.82 ^c ± 1.99	4, 10	13.32	0.001
<i>E. coli</i>	10.07 ^a ± 0.76	12.35 ^{ab} ± 0.48	9.83 ^a ± 1.50	13.12 ^b ± 1.10	15.82 ^c ± 0.39	4, 10	20.45	<0.001
<i>S. aureus</i>	11.50 ^a ± 1.02	15.02 ^b ± 0.39	9.54 ^a ± 0.78	17.00 ^{bc} ± 0.35	18.33 ^c ± 1.62	4, 10	45.03	<0.001

Supplementary Table S5. Antibacterial activities of HIL 80 % methanol extracts

Test pathogens	Rabbit manure	Potato waste	Pig manure	Market waste	Positive control	DF	F-Value	P-Value
<i>B. subtilis</i>	6.80 ^a ± 0.23	8.21 ^b ± 0.60	6.87 ^a ± 0.12	9.25 ^c ± 0.21	16.43 ^d ± 1.19	4, 10	588.2	<0.001
<i>P. aeruginosa</i>	6.91 ^a ± 0.30	9.58 ^b ± 0.38	6.91 ^a ± 0.31	9.07 ^b ± 0.31	15.90 ^c ± 0.43	4, 10	602.1	<0.001
<i>E. coli</i>	6.95 ^a ± 0.86	8.52 ^b ± 0.47	6.84 ^a ± 0.32	9.40 ^b ± 0.43	16.37 ^c ± 0.60	4, 10	142.6	<0.001
<i>S. aureus</i>	6.87 ^a ± 0.07	8.40 ^b ± 0.44	7.09 ^a ± 0.19	9.34 ^b ± 0.20	16.21 ^c ± 0.65	4, 10	311.9	<0.001