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Studies on the anthelmintic activity of *Syzygium aromaticum* oil oncommon poultry worms *Ascaridia galli* and *Heterakis gallinae*

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ABSTRACT

Dried flower buds of cloves (Family myrtaceae) commonly known as Lavangaha are grind with the help of grinder. Fine powder of cloves is taken and oil was extracted using petroleum ether as solvent in soxhlet extractor for 20 to 24 hr. The greasy mass obtained after the complete evaporation of solvent is suspended in ethylalcohol. Different conc. of test solution i.e. 2, 4 and 6% were prepared by diluting the test solution and test for in vitro anthelmintic activity. Different concentration 2%, 4% and 6% caused mortality in A. galli and H. gallinae after a maximum exposure of 11 and 16 hr. Clove oil significantly reduces glucose uptake, glycogen content, oxygen consumption and relatively activity of acid and alkaline phosphomonoesterase in both the parasites. The possible mode ofaction is discussed.

Key words: *Syzygium aromaticum, Anthelmintic, Ascaridia galli, Heterakis gallinae.*

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Introduction:

The present investigation aims at evaluating the efficacy of *Syzygium aromaticum* (Clove) against two avian nematode *Ascaridiagalli* and *Heterakis gallinae.*

Materials and methods :

The cloves are dried, highly aromatic unexpanded flower buds of *S. aromaticum*, a small evergreen tree measuring 12-15 m in height. It is a member of Family Myrtaceae and is locally known as “Lavangaha”. It is mainly found in India and Ceylon. It is economically important because of considerable quantity of volatile oil (oil of cloves), it contains Parts which are commonly used are fruit, dried flower buds and oil. Oil consists of caryophylin or

cugenin, a crystalline substance (which is converted to eugenic acid), tannin (converted to gallotannic acid) and woody fibres etc. Cloves are stomachic, carminative, stimulant, aromatic and antispasmodic. Oil is antiseptic. These cloves (unopened flower buds) are generally used as spices. Medicinally, they are used as purgative to relieve flatulence, gastric irritability, colic dyspepsia and to increase the flow of saliva. It is reported that internally it increases circulation, raises blood heat, promotes digestion of fatty and crude food, promoters nutrition and relieves gastric and intestinal pains and spasms.

The parasites *A. galli* and *H. gallinae* were obtained from the intestine and caecum respectively, of the common fowl *(Gallus gallus)* slaughtered in local poultry farms. After several washings in normal saline they were transferred saline (pH 7.2) to which 1 g of glucose/100ml was added. The requisite quantity of the extract was added to the incubation medium to obtain the required concentration and its effect was compared with untreated controls. Worms were incubated at 38^0C. Death was assumed to have occurred when all signs of movement had ceased.

Glucose uptake was determined by the method of Ahmad and Nizami (1987). Glycogen was estimated in the homogenates (20% w/v) of these worms according to the method of Good *et al.* (1933) as modified by Montgomery (1957). Rate of oxygen consumption was measured manometrically by the method of Warburg as described by Umbreit *et al.* (1964). Lactic acid production was measured by the method of Baker and Summerson (1941). Acid and alkaline phosphomonoesterase activity was also determined in homogenates, according to Bergmeyer (1971), whereas cholinesterase activity was measured by the method of Huerga *et al.* (1952), using acetylcholine as substrate. The chemicals used were of analytical grade.

In the present studies, *S. aromaticum* oil is used after triturating with tween 20.

Results:

A. Effect of *S. aromaticum* oil on the parasites incubated *in vitro*

S. aromaticum oil caused mortality of both *A. galli* and *H. gallinae* after an incubation of 11, 9 and 7 hrs. and 10, 8 and 6hrs. at concentrations of 2, 4 and 6% respectively.

B. Effect of *S. aromaticum* oil on some biochemical activities of the parasites

(i) Glucose uptake : The glucose uptake of *A. galli* and *H. gallinae* was inhibited by 72 and 65% respectively, on *in vitro* treatment with *S. aromaticum* (6%) oil (Table-1).

Table-1
Changes in glucose uptake (mg/g wet weight) and glycogen contents(% wet wt.) in *A. galli* and *H. gallinae* after *in vitro* incubation with different concentrations of *S. aromaticum* oil.

Parasites	Concentration			
	Control	2%	4%	6%
Glucose uptake *A. galli*	6.0±0.26[a]	3.0±0.1 (50.0)	2.4±0.22 (60.0)	1.7±0.21 (71.6)
H. gallinae	6.0±0.22	3.4±0.14 (43.3)	2.7±0.13 (55.00)	2.1±0.12 (65.0)
Glycogen contents				
A. galli	7.2±0.37	6.7±0.32 (6.94)	5.6±0.17 (22.2)	4.8±0.32 (33.33)
H. gallinae	6.8±0.28	5.6±0.17 (17.64)	4.3±0.17 (36.76)	3.4±0.14 (50.0)

a. Mean ± S.D. Value in parentheses are percent change of control values.

Table-2
Changes in the rate of oxygen consumption (µl/mg weight/hour) andlactic acid production (µ mol/gm wet weight) in *A. galli* and *H. gallinae* exposed to different concentrations of *S. aromaticum* oil.

Parasites	Concentration			
	Control	2%	4%	6%
Rate of oxygen Consumption *A. galli*	5.4±0.14[a]	4.2±0.12 (22.22)	3.9±0.17 (27.77)	3.5±0.13 (35.18)
H. gallinae	4.7±0.34	3.7±0.17 (21.27)	3.1±0.2 (34.04)	3.6±0.12 (44.68)
Lactic acid production				
A. galli	4.3±0.14	5.5±0.17 (27.90)	5.9±0.17 (37.20)	6.4±0.14 (48.83)
H. gallinae	3.7±0.28	5.3±0.24 (43.24)	5.7±0.14 (54.15)	6.2±0.17 (67.56)

a. Mean ± S.D. Value in parentheses are percent change of control values.

Table-3

Changes in acid and alkaline phosphomonoesterase (phosphatase units) and cholinesterase activity (µ moles acetylcholine/hour) in *A. galli* and *H. gallinae* following *in vitro* incubation with different concentrations of *S. aromaticum* oil.

Parasites	Concentration					
	Control	2%	4%	6%	I_{50}[a]	r[b]
Acid Phosphomonoesterase						
A. galli	7.9±0.02[c]	6.2±0.17 (21.51)	5.5±0.17 (30.37)	4.3±0.1 (45.56)	6.58	0.9995
H. gallinae	6.6±0.1	5.2±0.28 (21.21)	4.4±0.31 (33.33)	3.7±0.28 (43.93)	6.83	0.9935
Alkaline Phosphomonoesterase						
A. galli	8.4±0.14[c]	7.3±0.36 (13.09)	6.5±0.17 (22.61)	5.8±0.14 (30.95)	9.69	0.9916
H. gallinae	7.7±0.02	6.4±0.14 (16.88)	5.5±0.26 (28.57)	4.7±0.14 (38.96)	7.70	1.199
Cholinesterase						
A. galli	7.3±0.47	6.3±0.18 (13.69)	5.3±0.24 (27.39)	4.2±0.13 (44.46)	7.06	0.9799
H. gallinae	6.5±0.17	5.1±0.52 (21.53)	4.1±17 (36.92)	3.0±0.12 (53.84)	5.57	0.9919

a. Concentration required for 50% inhibition.
b. r = correlation coefficient of the activity of control and treated samples.
c. Mean ± S.D.
Values in parentheses are percent change of control values.

Table-4

The effect of different concentrations of *S. aromaticum* oil on hosttissues (intestine and caecum) *in vitro*

	Concentration		
	2%	4%	6%
Glucose uptake	-	-	6.93*
Glycogen content	-	-	4.35
Rate of oxygen consumption	-	-	4.12
Lactic acid	-	-	6.88
Acid phosphomonoesterase	-	-	2.33
Alkaline phosphomonoesterase	-	-	1.84
Cholinesterases	-	-	0.43

a. % reduction/enhancement of control values ($n \geq 10$)

(i) Glycogen contents: Glycogen contents of both the parasites were reduced significantly (P<0.05), when exposed to different concentrations of *S. aromaticum* oil.

(iii) Rate of oxygen consumption: As shown in Table 2, *S. aromaticum* (6%) oil reduced the rate of oxygen consumption by 35 and 45% in *A. galli* and *H. gallinae,* respectively. This reduction in the values of oxygen consumption was significant (P<0.05) statistically.

(iv) Lactic acid production: The level of lactic acid in *A. galli* and *H. gallinae* was enhanced by 49 and 68%, respectively when exposed to 6% of *S. aromaticum* oil (Table 2).

(v) Acid phosphomonoesterase activity: When treated *in vitro* with 6% *S. aromaticum* oil, the activity of acid phosphomonoesterase was diminuted by 46 and 44%, in *A. galli* and *H. gallinae*, respectively (Table 3).

(vi) Alkaline phosphomonoesterase activity: Activity of alkaline phosphomonoesterase was also diminuted significantly. The oil (6%) caused an inhibition of 31 and 39% in *A. galli* and *H. gallinae,* respectively (Table 3).

(vii) Cholinesterase activity : As shown in the Table 3, the activity of cholinesterase was reduced significantly (P<0.05) in both *A. galli* and *H. gallinae* when exposed to 6% oil of *S. aromaticum.*

C. Effect of *S. aromaticum* oil on host tissues

No significant change was observed in the biochemical activities of host tissues, when incubated with different concentrations of *S. aromaticum* oil (Table 4).

DISCUSSION

The anthelmintic effect of an Ayurvedic recipe Kuberakshadi yoga containing clove oil was first reported by Amar Singhe et al. (1993) against intestinal worms in children. In the present investigations involving *in vitro* incubation of *A. galli* and *H. gallinae,* the mortality was observed after a maximum period of 11 hours with 6% clove oil.

Data collected on biochemical activities of treated worms indicated that *S.*

aromaticum oil mainly altered the carbohydrate metabolism of both *A. galli* and *H. gallinae.* This was reflected through suppression of oxygen consumption (Table 2), reduction in glycogen contents (Table 1) and enhancement of lactic acid production (Table 2). This was further supported by the inhibitory action of the oil on the activity of non-specific acid and alkaline phosphomonoesterases (Table 3) in both *A. galli* and *H. gallinae,* since these phosphatases are reported (Pappas) and Read, 1975) to play a significant role in the transport of Carbohydrates. Cholinesterase activity was however not affected significantly in either of the parasite (Table 3). *S. aromaticum* oil was found to be non-toxic at all concentrations (Table 4) used for the host as no significant change was observed in biochemical activities of host tissues incubated with *S. aromaticum* oil. In view of its non-toxic effects on the host tissues, it appears that *S. aromaticum* may be used as an effective anthelmintic against *A. galli* and *H. gallinae.*

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