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IN VITRO EFFICACY OF SOME INDIGENOUS PLANTS ON THE INHIBITION OF DEVELOPMENT OF EGGS OF *ASCARIDIA GALLI* (DIGENIA: NEMATODA)

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ABSTRACT

In vitro efficacy of five indigenous plants namely Bishkatali (*Polygonum hydropiper*), Neem (*Azadirachta indica*), Papaya (*Carica papaya*), Korolla (*Momordica charantia*) and Mahogany (*Swietenia macrophylla*) were studied against the development of *Ascaridia galli* eggs from July 2007 to May 2008. Fresh juice, extracts and dust of leaves were tested. Fresh juice of leaves were trialed at 5%, 10% and 20% concentrations; aqueous, ethanol and methanol extracts were used at 1%, 2% and 4% concentrations and dusts of leaves were applied at 10% and 20% concentration. Among the trials, 4% of methanol extracts of papaya showed the highest efficacy (92.86%) followed by 4% ethanol extract of papaya (92%). Among the selected plants and in all three concentrations of fresh juice of leave, Bishkatali (88.46% at 20% conc.) was the highest effective plant against the development of *A. galli* eggs. Papaya showed the highest efficacy (71.42%) in 1% aqueous solution, but bishkatali was found as the best (73.33% and 83.33% respectively) in 2% and 4% concentration of ethanol extract of the five selected plants. *In vitro* screening of 5 plants with ethanol extract revealed that papaya was the highest efficacious plant (92% at 4% conc.) against development of *A. galli* eggs. Among the plants, in all concentrations of methanol extract of leaves, papaya was observed as best plant (92.86%, 88% and 78.95% at 4%, 2% and 1% of conc. respectively) followed by bishkatali (80% and 75% at 4% and 2% of conc. respectively) and neem (78.57% and 73.08% at 4% and 2% conc. respectively). In two concentrations of dust of leaves, bishkatali was observed as the effective plant (75% at 20% and 73.33% at 10% conc.) among the five plants. The present study suggests that dust of bishkatali leaves can be used with litter for inhibition of development of *A. galli* eggs and fresh juice and extract of bishkatali, neem and papaya may be impregnated in litter and used after sun dry.

Key words: *In vitro* efficacy, indigenous plants, development, *Ascaridia galli* eggs

INTRODUCTION

Ascaridia galli is one of the most common parasitic roundworms of poultry (Soulsby, 1982; Anderson, 1992; Permin, 1997). Females of this parasite lay thick heavy-shelled eggs in the intestine that pass in the feces (Soulsby, 1982; Urquhart 1996) and embryonated eggs are very hardy and under laboratory conditions may live for two years and under ordinary conditions, however, few probably live more than one year (Cruthers *et al*, 1974; Matter and Oester, 1989). Disinfectants and other cleaning agents do not kill eggs under farm conditions and birds become infected by eating infective eggs containing L3 (Permin, 1997). Chemical anthelmintics have long been considered the only effective way of controlling this parasitic infection. Available drugs remove only the adult parasite. However, the drug is expensive and often unavailable to farmers in rural areas, furthermore, some serious disadvantages of using manufactured drugs have become evident in the world, such as drug resistance, food residues and environmental pollution. So, poultry producers have continued to use indigenous plants as dewormers, drawing upon centuries of knowledge of herbal medicine. *In vitro* and *in vivo* screening of plant materials as anthelmintic against adult *Ascaridia galli* has done throughout the world with the aim of controlling *A. galli* in poultry. But in Bangladesh, very few works (Ali, 2006) has done yet, which was done against adult *A. galli*. Though, routine anthelmintic treatments or medicinal plants are usually applied in chickens in order to control adult ascarids, but eggs generally have a long survival rate in the environment and thus a high infection potential. So, the present study will have a great importance in controlling *A. galli* in chickens, as this work will be a stand point to control *A. galli* by inhibiting the development of eggs using available indigenous plants and thus reducing the getting infection. Therefore, the present study was designed to evaluate and compare the *in vitro* efficacy of some indigenous plant materials on the inhibition of development of *A. galli* eggs.

MATERIALS AND METHODS

The present study was conducted during the period from July 2007 to May 2008 in the laboratory of the Department of Parasitology, Bangladesh Agricultural University (BAU), Mymensingh.

Preparation of plant materials

Five plants namely Bishkatali (*Polygonum hydropiper*), Neem (*Azadirachta indica*), Papaya (*Carica papaya*), Korolla (*Momordica charantia*) and Mahogany (*Swietenia macrophylla*) were selected on the basis of their ethnomedical uses for screening. Only leaves of these plants were selected and were collected from the surrounding areas of BAU Campus, Mymensingh.

Preparation of fresh juice

After washing, the fresh leaves were cut into small pieces and water was added at 1:1 ratio in a kitchen blender. Then juice were made by blending the leaves for 2-3 minutes and stored in a refrigerator at 4° C to maintain the active ingredients of juice.

Preparation of dust of leaves

For the preparation of dust, the leaves were dried in the shade at room temperature and then in the oven at 55-60°C. The dried leaves were cut into small pieces and pulverized with a blender, then sieved and preserved in airtight plastic container.

Preparation of extract of leaves

To prepare aqueous extract, firstly, 10 gm of dust were mixed with 100 ml of distilled water and then the mixture was stirred with a magnetic stirrer at 600 rpm for an hour and then left for overnight. The mixture was then filtered, and was condensed into 10 ml by the evaporation of solvent in a water bath at 50-60°C. This condensed extract was preserved as a stock solution in refrigerator at 4°C until their use. Ethanol and methanol extract were prepared following the same procedure by mixing ethanol and methanol respectively instead of distilled water.

Preparation of different concentration of extract

Three different concentrations such as 1%, 2% and 4 % solution of different extract were used for screening. The solutions of these concentrations were prepared using Phosphate Buffer Saline (PBS) as a base.

Collection of eggs of A. galli

At first, intestines were collected from chickens slaughtered at Kamal-Ranjit Market in BAU campus and brought to the laboratory; and then adult *A. galli* were collected following a standard method (Fowler, 1990). Female parasites were identified under microscope, eggs were recovered by grinding the female parasites by adding 5 ml PBS.

Treatment of eggs with fresh leave juice

Petri dishes were properly washed, dried and then labeled. PBS was used as media for this trial and 10 ml of total volume were made for each trial. Upper meniscus of total 10 ml volume of suspension in all petri dishes was marked by permanent ink. Fresh leave juices were used as 5%, 10% and 20% and for control, one petri dish with egg-PBS suspension retained without treatment. The petri dishes were kept at room temperature for 20 days at a large tray and moist cotton was used under the petri dishes to prevent desiccation. About half of the petri dishes were kept open to allow aeration for development of eggs and continuous monitoring of petri dishes had been done and the upper meniscus of fluid was maintained by adding PBS if necessary.

Treatment of eggs with aqueous, ethanol and methanol extract

Prepared 1%, 2%, 4% concentration of aqueous, ethanol and methanol extracts were used. For making the total volume of 10 ml for each trial, 1 ml of egg-PBS suspension was added to 9 ml of extract. All the petri dishes were prepared, maintained and monitored on the same way as with the fresh juice trial. In every cases,

control petri dishes were kept and the control solution were prepared by adding 1 ml, 2 ml, 4 ml of distilled water, ethanol and methanol to 99 ml, 98 ml and 96 ml of PBS, respectively. For the preparation of control petri dishes, 1 ml of egg-PBS suspension was added to 9 ml of control solution to make 10 ml of final volume for trial.

Treatment of litter with dust

For preparing 10% and 20% concentrations, 8 gm and 9 gm of litter were mixed with 2 gm and 1 gm of dust respectively. For each trial, total 10 gm of litter-dust mixture were kept in petri dishes and 1 ml of egg-PBS suspensions was sprayed. For the control, only 10 gm of litter were sprayed with 1 ml of egg-PBS suspension. All petri dishes were kept at room temperature for 20 days and after then water was added with the mixture and sieved to remove litter. The filtrate was allowed to stand for 30 minutes for sedimentation of eggs. The supernatant was poured off and the sediment was washed for several times to make it clear. Finally, the filtrate was centrifuged at 1500 rpm for five minutes and the sediment was taken on a clear slide to examine the eggs under microscope.

Examination of eggs for development of larva

Fresh juice, extract and dust treated eggs were examined at 10^{th} day, 15^{th} day and 20^{th} day for the development of larva within the egg. Developed eggs were identified with the presence of larva within egg and the movement of larva.

Determination of efficacy of plants

In vitro screening of fresh leaves juice, different extracts and dust of leaves of selected plants on the inhibition of development of *A. galli* eggs were done for their efficacy; and the plant/preparations were considered as effective having at least 70% efficacy.

Statistical analysis

In vitro effects of different preparations of plants leaves were statistically analyzed with ANOVA technique to obtain the level of significance using MSTAT-C package programme developed by Russell (1986). The mean differences were compared by Duncan's New Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

In vitro screening of fresh juice of leaves

During *in vitro* screening of fresh leave juice of five selected plants at 5% concentration, the highest efficacy in terms of inhibition of development of growth of larvae was found in bishkatali (77.27%) followed by papaya (71.43%), neem (63.16%), mahogany (62.5%) and korolla (46.67%). Investigation of efficacy of fresh leave juice at 10% concentration, bishkatali was found as highest efficacious plant (82.35%) followed by papaya (77.78%), neem (68.18%), mahogany (66.67%) and korolla (47.36%). At 20% concentration of fresh leave juice, highest efficacy was found in bishkatali (88.46%) followed by papaya (80%), neem (72.73%), mahogany (68.18%) and korolla (57.89%) (Table 1).

Among the selected plants and in all three concentrations of fresh juice of leave, bishkatali was the highest effective plant against the development of *A. galli* eggs. Papaya was the second highest followed by neem and mahogany; whereas korolla was the least efficacious. The present result indicates that bishkatali leaves are more efficacious as fresh juice than papaya leaves and neem leaves. This result is partially as an agreement with Chakroborty (2007), where fresh juice of bishakatali leaves found the highest efficacious against sporulation of *E. tenella* oocysts and better than that of papaya, but fresh juice of neem leaves was the second in position and was better than papaya. The agreement of these studies can be explained that ingredients of bishkatali leaves are more active in fresh juice form than that of papaya. Whether, active principles of papaya might be more active in fresh juice against development of *A. galli* eggs than neem but might be less active against sporulation of *E. tenella* eggs than neem.

Table 1. *In Vitro* efficacy of fresh leave juice of some indigenous plants on the inhibition of development of *Ascaridia galli* eggs

Name of plants	Concentration (%)	No. of eggs counted	No. of developed eggs	No. of undeveloped eggs	Effects on development of eggs (% of undeveloped eggs)
Bishkatali	5	22	5	17	77.27a
	10	17	3	14	82.35a
	20	26	3	23	88.46a
Neem	5	19	7	12	63.16c
	10	22	7	15	68.18c
	20	22	6	16	72.73c
Papaya	5	21	6	15	71.43b
	10	18	4	14	77.78b
	20	15	3	12	80.00b
Korolla	5	15	8	7	46.67d
	10	19	10	9	47.36d
	20	19	8	11	57.89e
Mehogany	5	16	6	10	62.50c
	10	21	7	14	66.67c
	20	22	7	15	68.18d
Control	5	20	18	2	10.00e
	10	23	20	3	13.04e
	20	25	22	3	12.00e
Level of significance					**

In a column figures with same letter or without letter do not differ significantly whereas figures with dissimilar letter differ significantly (as per DMRT) in same concentration, ** = Significant at 1% level of probability.

In vitro screening of different extracts of leaves

In vitro screening of aqueous extract

At 1% aqueous solution, the highest efficacy was found in papaya (71.42%) followed by bishkatali (68.18%), neem (60.87%), mahogany (60%) and korolla (52.94%). Treatment of *A. galli* eggs with 2% aqueous solution of leave extract, bishkatali showed the highest efficacy (73.33%) followed by the papaya (72.22%), neem (66.67%), mahogany (64.29%) and korolla (59.09%). At 4% aqueous solution of leave extract, the highest efficacy (83.33%) was found in case of bishkatali. Second highest efficacy was found in papaya (72.73%) followed by neem (68.42%), mahogany (66.67%) and korolla (63.16%) (Table 2). Though papaya showed the highest efficacy in 1% aqueous solution while bishkatali was in the second position but bishkatali was found as the best in 2% and 4% concentration, followed by the papaya, neem, mahogany and korolla. These findings also establish the hypothesis that active principles of bishkatali are more soluble in water than papaya. On the other hand, efficacy of fresh juice of bishkatali, papaya and almost all other plants showed better efficacy in fresh juice than aqueous extract which can be explain by the fact that all fresh juices were prepared from fresh leaves and stock solution of juice were made at 1:1 ratio which were used at 5%, 10% and 20%. So, the concentration of active ingredients may be were more in fresh juice than aqueous extract.

In vitro screening of ethanol extract

Among 5 plants, the highest efficacy was found in papaya (79.17%) followed by neem (69.57%), bishkatali (66.67%), korolla (57.89%) and mahogany (55%) in 1% concentration of ethanol extract. Screening with 2% ethanol extract, the highest efficacy was found in papaya (82.35%) followed by the neem (75%), bishkatali (68.18%), korolla (69.57%) and mahogany (57.89%). At 4% concentration of ethanol extract, the highest efficacy was found in papaya (92%) followed by neem (89.66%), bishkatali (78.57%), korolla (69.56%) and mahogany (68.42%) (Table 2). *In vitro* screening of 5 plants with ethanol extract revealed that papaya was the highest efficacious plant against development of *A. galli* eggs. Second highest efficacy was showed by the

neem followed by bishkatali, korolla and mahogany. This result reveals that papaya leaves are more efficacious in ethanol than neem and bishkatali. It is difficult to compare this result due to lack of relevant literature. It can be explain that the active ingredients of neem and bishkatali were less extracted with ethanol than that of papaya.

Table 2. *In vitro* efficacy of different extracts of leaves of some indigenous plants on the inhibition of development of *Ascaridia galli* eggs

Name of plants	Preparation	Concentration (%)	No. of eggs counted	No. of developed eggs	No. of undeveloped eggs	Effects on development of eggs (% of undeveloped eggs)
		1	22	7	15	68.18b
	Aqueous	2	15	4	11	73.33a
		4	24	4	20	83.33a
		1	27	9	18	66.67c
Bishkatali	Ethanol	2	22	7	15	68.18c
		4	28	6	22	78.57c
		1	17	6	11	64.70b
	Methanol	2	24	6	18	75b
		4	20	4	16	80b
		1	23	9	14	60.87c
	Aqueous	2	18	6	12	66.67b
		4	19	6	13	68.42c
		1	23	7	16	69.57b
Neem	Ethanol	2	16	4	12	75b
		4	29	3	26	89.66b
		1	23	9	14	60.87c
	Methanol	2	26	7	19	73.08c
		4	28	6	22	78.57b
		1	21	6	15	71.42a
	Aqueous	2	18	5	13	72.22a
		4	22	6	16	72.73b
		1	24	5	19	79.17a
Papaya	Ethanol	2	17	3	14	82.35a
		4	25	2	23	92a
		1	19	4	15	78.95a
	Methanol	2	25	3	22	88a
		4	28	2	26	92.86a
		1	17	8	9	52.94d
	Aqueous	2	22	9	13	59.09c
		4	19	7	12	63.16d
		1	19	8	11	57.89d
Korolla	Ethanol	2	23	7	16	69.57c
		4	23	7	16	69.57d
		1	21	9	12	57.14d
	Methanol	2	21	8	13	61.90d
		4	23	8	15	65.21d
		1	20	8	12	60c
	Aqueous	2	14	5	9	64.29b
		4	21	7	14	66.67c
		1	20	9	11	55d
Mehogany	Ethanol	2	19	8	11	57.89d
		4	19	6	13	68.42d
		1	18	7	11	61.11c
	Methanol	2	21	7	14	66.67d
		4	27	9	18	66.67c
Level of significance						**

In a column figures with same letter or without letter do not differ significantly whereas figures with dissimilar letter differ significantly (as per DMRT) in same concentration of same extract, ** = Significant at 1% level of probability.

In vitro screening of methanol extract

At 1% concentration of methanol extract of 5 plants, the highest efficacy was observed in papaya (78.95%) followed by bishkatali (64.70%), neem (60.87%), mahogany (61.11%) and korolla (57.14%). At 2% ethanol extract, the highest efficacy was found in papaya (88%) followed by the bishkatali (75%), neem (73.08%), mahogany (66.67%) and korolla (65.21%). Treatment with 4% concentration of ethanol extract, the highest efficacy was found in papaya (92.86%) followed by bishkatali (80%), neem (78.57%), mahogany (66.67%) and korolla (61.90%) (Table 2). Among the plants, in all concentrations of methanol extract of leaves, papaya was observed as best plant followed by bishkatali, neem, mahogany and korolla. *In vitro* study using methanol extracts of leaves showed that papaya was the best plant and this result indicates that methanol extracted well the active components of papaya than that of bishkatali and neem.

In vitro screening of dust of leaves

At 10% concentration of dry powder of leaves, the highest efficacy was observed in bishkatali (73.33%), followed by papaya (66.67%), neem (61.53%), mahogany (60%) and korolla (50%). In case of dust preparation of leaves at 20% concentration, the highest efficacy was found in the bishkatali (75%), followed by papaya (69.23%), neem (68.75%), mahogany (63.64%) and korolla (53.33%) (Table 3). Among the plants, in all concentrations of dust of leaves, bishkatali was observed as the best plant followed by papaya, neem, mahogany and korolla. Among dust form of leaves of five plants, bishkatali found only the effective plant against development of *A. galli* eggs. Chakraborty (2007) found the same result against sporulation of *E. tenella* oocysts. This might be due to active ingredients of bishkatali, which may be active in dry form also, but of course less active than moist forms of preparations.

Table 3. *In Vitro* efficacy of dust of leaves of some indigenous plants on the inhibition of development of *Ascaridia galli* eggs

Name of plants	Concentration (%)	No. of eggs counted	No. of developed eggs	No. of undeveloped eggs	Effects on development of eggs (% of undeveloped eggs)
Bishkatali	10	15	4	11	73.33a
	20	12	3	9	75a
Neem	10	13	5	8	61.53c
	20	16	5	11	68.75b
Papaya	10	12	4	8	66.67b
	20	13	4	9	69.23b
Korolla	10	14	7	7	50d
	20	15	7	8	53.33d
Mehogany	10	10	4	6	60c
	20	11	4	7	63.64c
Control	10	14	11	3	21.43e
	20	15	12	3	20e
Level of Significance					**

In a column figures with same letter or without letter do not differ significantly whereas figures with dissimilar letter differ significantly (as per DMRT) in same concentration, ** = Significant at 1% level of probability.

In vitro screening of different preparations of bishkatali leaves showed the best efficacy in both fresh juice (88.46% at 20% conc.) and aqueous extract of leaves (83.33% at 4% aqueous extract) against the development of *A. galli* eggs. Rahman (2002) recorded the same findings against gastro intestinal nematodes where aqueous extract of bishkatali showed the best (100%) efficacy than ethanol extract (92%). Plants are known to synthesis many chemical compounds that possess as many biological activities (Klocke, 1989). The anthelmintic efficacy of bishkatali might be described by the presence of active ingredients in it. Some of these compounds especially sesquiterpene (Bizimana and Scherecke, 1996; Robinson, 1975; Klocke, 1989) have been reported elsewhere to

have biological activities against helminth. The more efficacy of bishkatali leaves in fresh juice and aqueous extract can be explain by the presence of the active ingredients responsible for inhibition of *A. galli* eggs are more soluble in water than alcohol. In addition, the highest efficacy of fresh juice may be due to the reason that the juice were made with fresh leaves at high concentration (1:1 ratio) which later used at 5%, 10% and 20% concentration.

In this experiment, neem leaves showed better efficacy on the inhibition of development of *A. galli* eggs in alcoholic preparation than fresh juice or aqueous solution. Rahman (2002) recorded the highest efficacy (100%) of neem leaves in alcoholic extract whereas aqueous extract have the lower efficacy (92%) than alcohol against gastro intestinal nematodes in goats. Akhter and Riffat (1985) proved the highest efficacy of neem seeds in ethanol extract followed by methanol and water extract in *A. galli* infected chickens *in vivo*. Kumar *et al.* (2007) stated that contents of the alcoholic extract are different from aqueous extract and contain substances which have better anthelmintic effect. One of the active ingredients of neem leaves is Azadirectin which proved as an effective nematocidal compound (Sharma *et al*, 2003). Besides, neem leaves contain quercetin , a polyphenolic flavonoid, acts as anti-oxidant (Ghani, 2003) and vitamin C which also a anti-oxidant (Kayser, 2002). These antioxidant substances may inhibit the development of *A. galli* eggs.

Papaya showed its anthelmintic efficacy against *A. galli* for many times. Different parts of papaya tree proved effective against development of infective eggs of *A. galli* (Purwati and He, 1991**)** and against adult *A. galli* (Satyanarayana and Krishnaiah, 1982; Purwati and He, 1991; Mursof and He, 1991; Singh and Nagaich, 1999; Satrija *et al.*, 2001; Adu and Akingboye, 2002; Ali, 2006). Papaya also found as an anthelmintic against other helminths (Satrija *et al*., 1994; Murdiati *et al*., 1997; Rahman, 2002) rather than *A. galli*. The anthelmintic efficacy of papaya might be due to presence of proteolytic enzymes such as papain, chymopapain and lysozymes in the latex as well as in leaves (Dakpogan, 2005). All parasites and their developmental stages of course the protein substance that can be ingested by papain. Kumar *et al.* (1991) compared *in vitro* effects of BITC (benzylisothiocyanate), an anthelmintic principle of *Carica papaya* with mebendazole against *A. galli* and found effective. The present study reflects that in almost all studied preparation, papaya leaves showed its efficacy against development of *A. galli* eggs. This study also indicates that in same concentration papaya leaves have better efficacy in methanol extract than ethanol extract and found lower efficacious in aqueous extract and fresh juice. This may be explained that the active ingredients of papaya leaves are more soluble in alcoholic extract than water. Among the alcoholic solutions, active principles of papaya leaves are relatively more soluble in methanol than ethanol. It may be suggested that papaya leaves have strong efficacy against the development of *A. galli* eggs in methanol extracts, similarly in ethanol extracts; and also have efficacy in fresh juice form and aqueous extract.

There are several literatures which describes the anthelmintic efficacy of Korolla against adult *A. galli* (Ali, 2006) as well as other helminths (Rahman, 2002; Beloin *et al.*, 2005; Das *et al*., 2006), but in this study, korolla observed as lower efficacious on the inhibition of development of *A. galli* eggs, as it showed its efficacy up to 69.57%. Though Ali (2006) recorded korolla as best plant against adult *A. galli*, when korolla was found 67% effective. Rahman (2002) recorded the efficacy of korolla in aqueous (67%) and ethanol extract (79%) against gastro intestinal nematodes *in vivo*. These findings are partially agreed with percentage of efficacy of present study, though present study recommends the plants as effective which have at least 70% efficacy. However, the anthelmintic efficacy of korolla may be due to the presence of alkaloids (momordicine) and glycosides (a saponon like substance), because secondary metabolites of herbal remedies such as alkaloids, glycosides and tannins have shown dose-dependent anti-parasitic properties (Githiori *et al.*, 2006).

Mahogany leaves was found more than 60% effective in all preparations except 1% and 2% ethanol extract, where the highest efficacy was recorded 68.42% in 4% ethanol extract. It is very difficult to compare the anthelmintic property of mahogany because of paucity of literature. Chakraborty (2007) recorded the efficacy (77%) of mahogany leaves against sporulation of *Eimeria tenella* oocysts. Though mahogany leaves possess more than 60% against development of *A. galli* eggs, but this study does not recommend mahogany as its efficacy below 70%. However, the efficacy of mahogany leaves can be described that mahogany contains saponin which is a glycoside and herbal remedies as glycosides have anti-parasitic property (Githiori *et al.*, 2006).

So, the present study suggests that dust of bishkatali leaves can be used with litter for inhibition of development of *A. galli* eggs. Although fresh juice and extract of bishkatali, neem and papaya found efficacious but they can not be used directly, as many microbes multiply in moist litter. However, juice or extract may be impregnated in litter and used after sun dry.

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