Effect of papaya and neem seeds on *Ascaridia galli* infection in broiler chicken

S. Feroza¹, A. G. Arijo†¹ and I. R. Zahid²

¹Department of Parasitology, Sindh Agriculture University, Tandojam, Pakistan
²Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand, Pakistan

†Corresponding author: abdullaharijo@gmail.com

**Abstract**

Experiment was carried out to assess the efficacy of ethanolic extract of papaya (*Carica papaya*) and neem (*Azadirachta indica*) plant seeds on *Ascaridia galli* infectivity in broiler chicken. A total of eighteen broiler birds were randomly selected that were divided into three groups (A, B and C) with 6 birds in each group. The birds were then artificially infected with *Ascaridia galli* @ 2000 eggs/bird. Ethanolic extracts of papaya and neem were applied to Group B and C, respectively while Group A was left untreated that served as control. The fecal egg count (FEC) was conducted on weekly basis. The pre-treatment values of FEC in all three groups found negative from day 0 to 14 after artificially inducing infection. On day 21, the FEC pretreatment values in group A, B and C were recorded as 1424.5, 1346.3 and 1806.4, respectively. The FEC post treatment values of groups B and C were significantly (P>0.05) decreased as compared to the control (group A). However, no significant difference was observed between group B and C. It was concluded that the ethanolic extract of both the papaya and neem was effective in controlling the *Ascaridia galli* infection in chicken. However, papaya extract was found more effective than neem.

**Keywords:** Anthelmintics, *Ascaridia galli*, neem, papaya, broiler chicken.

The intestinal parasitic nematodes cause severe diseases of poultry birds in developing countries including Pakistan (Basit *et al.*, 2014). *Ascaridia galli* is the most common and important nematode that causes sustains economic losses to the poultry birds. Birds infected with *A. galli* usually suffer from severe diarrhea, anemia leading to the loss of body weight resulting onto heavy economic losses in poultry farming (Permin & Raving, 2001) therefore their timely control may help bear better economic benefits. Mostly the synthetic compounds are used for controlling the internal parasites. The use of these compounds affect the drawbacks of negative impact on health of bird itself as well as lead to public health issues with residues of drugs in poultry meat leading to carcinogenesis (Butaye *et al.*, 2003). These compounds also develop resistance against the causative agents. However, high cost of antihelmintics has diverted the interest towards alternative control practices, including the usage of medicinal plants in traditional remedies. World Health Organization reported that 80% of the rural population in the world has confidence on the herbal remedies for their basic health care. Since the days of human civilization, numerous plants are being used in conventional medicine. The data on usefulness of majority of plants is unavailable. However, the research is going on to confirm the efficacy of various plants (Nayak *et al.*, 2011; Sofowora *et al.*, 2013). The herbal remedies are cost effective, having minimum toxicity with reduced health hazards and easily available in market as compared to synthetic medicines (Khandaker *et al.*, 2016). In the current research study, the efficiency of seed of traditional remedial plants viz., neem (*Azadirachta indica*) and papaya (*Carica*
papaya) was evaluated for controlling the A. galli parasite in broiler chicken. Papaya fruit has the tremendous health benefits and the leaf and seeds are used against the various health disorders including liver cirrhosis, parasitic infections and digestive disorders. Its anticancer properties have also been reported (Aruoma et al., 2014; Gajowik & Dobrzyńska, 2014). Previously, the effects of leaf on growth performance of chicken have well been documented (Adeyemo & Akanmu, 2012; Kamal et al., 2015; Sorwar et al., 2016).

The medicinal properties of neem tree have been recognized since thousands of years in sub-continent where people are using its bark, root, leaf, seed, oil and kernel for the treatment and control of different parasites of human and animals (El-Tahir et al., 1998; Biswas et al., 2002; Parida et al., 2002; Udeinya et al., 2004; Yanes et al., 2004; Udeinya et al., 2008). The leaf extract as growth promoter has also been reported by many researchers (Adeyemo & Akanmu, 2012; Kharde & Soujanya, 2014; Sarker et al., 2014; Kamal et al., 2015). The antifungal properties of neem seed have been reported by Moslem & El-Kholie (2009), Ospina-Salazar et al., (2015). However, the effectiveness of neem seeds against red mites in poultry has also been observed (Abdel-Ghaffar et al., 2008). In present investigation controlling potential of papaya and neem seed's ethanolic extracts have been evaluated against the A. galli in broiler chicken.

**Materials and Methods**

The study was carried out at Department of Veterinary Parasitology, Sindh Agriculture University, Tandojam during 2015.

**Sample size and experimental birds:** Three weeks old, 18 commercial broiler chicks were bought from market and placed them into 3 groups, viz., A, B and C, each comprising of 6 birds. The birds were reared in experimental sheds of Parasitology Department with equal management environment. The birds were vaccinated against Coccidiosis, Gumboro, New Castle Disease, E. coli, Salmonella and other bacterial infections.

**Collection of the A. galli eggs:** The eggs were obtained from infected birds, processed for direct fecal examination, identified with published keys. Briefly, a small drop of equal amount of water and feces was thoroughly mixed to obtain a fairly homogenous and adequately clear preparation and placed on a microscope slide. A cover slip was placed on fluid, allowed to settle for few minutes and was then observed under lower magnification (10x) of compound microscope. A. galli eggs were identified by size, shape and colour of the shell using the key provided by Soulsby (1983).

**Ethanolic extraction of the plant seeds:** The seeds of papaya and neem were collected from field. The collected seeds were dried under shade at existing air temperature of the surrounding area, minced to powder by electrical blender and stored in air tight glass bottles.

Extraction from seeds powder was carried out by following the procedure given before (AOAC, 2000; Wang & Waller, 2006). Briefly, 4g of a moisture free sample was measured in extraction thimble, plugged with absorbent cotton wool and then placed in an extractor that was fixed under the condenser of the extraction apparatus. Receiving flask contained 150 ml of the solvent was connected to the apparatus and placed on water bath at 40 °C with the rate of condensation at 3-4 drops per second. After 10 hours, thimbles from extractors were removed and just before drying the solvent, extract was transferred into a clean tarred evaporating basin with ether washing. Basin was placed in oven at 105°C for 2 hrs and then cooled in desiccators for 30 min. Extraction was measured as follows:
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Inoculation of experimental birds: All the experimental birds were artificially infected orally with 2000 *A. galli* eggs. The fecal samples of all birds were collected and checked on a regular basis for confirmation of *A. galli* eggs. On day 21 (after confirmation of infection) the groups B and C were treated with extraction of papaya and neem seeds @ 20 ml/kg, respectively. While group A received no treatment and served as control. The fecal examination was continued up to 42 days and the Fecal Eggs Counts - FEC (quantitative examination) was carried out by McMaster Technique according to the method given by Urquhart *et al.*, (1996).

Statistical analyses: Data analysis was done through statistical program Graphed Prism (Graphed Software Incorporated 2000) by one-way analysis of variance (ANOVA) and Chi-square analysis (x-analysis) t-test.

**Results**

Effect of medicinal plants on *Ascarida galli* infection: In present study the seeds of two plants viz., neem and papaya were used against *A. galli* infection in chicken. The aim of the present study was to explore the potential of these plants to control the *A. galli* infection in chicken.

In vivo study: Table 1 represents the data of all experimental birds shading *A. galli* eggs from day 1 to 42 recorded on weekly basis. The groups A (control), B (infected-papaya treated) and C (infected-neem treated) showed no egg shedding from day 1 to 14 after inoculation. However, the egg shedding was started from day 21 and the pretreatment fecal egg count (FEC) of groups A, B and C was recorded as 1424.5, 1346.3 and 1806.4, respectively. It was further revealed that the egg shedding was significantly (*P*>0.05) reduced after the treatment of seed extracts and lower FEC was recorded on days 28, 35 and 42 in group B and C while group A showed continued increase in FEC up to the end of trial. The egg per gram was recorded on day 28 (A, 1705.2; B, 1013; C, 1416.3); 35 (A, 2166.2; B, 233; C, 399.75) and 42 (A, 2606.4; B, 6.6; C, 41.5). The FEC of group B (99.5%) and C (97.7%) was significantly reduced as compared to the group A. The comparisons of groups A vs B and A vs C was very significant (*P*<0.0001) but group B vs C didn’t show any significant difference (*P*>0.05).

Discussion

The use of medicinal plants against various diseases of humans and animals is widely documented. Various parts of medicinal plants have shown anti parasitic characteristics and have usually been applied as a remedy against internal and external parasites (Bauri *et al.*, 2015). Many workers demonstrated the medicinal effect of numerous plants, including papaya and neem, against *A. galli* infection in poultry and some other animals (Akhtar & Riffat, 1984; Muhamd & Riffat, 1985; Hafeez & Venkataratnam, 1989; Hammond *et al.*, 1997; Purwati & He, 1991; Taylor *et al.*, 2001; Lateef, 2002; Rahman, 2002; Adu *et al.*, 2002; Hordegen *et al.*, 2003; Beloin *et al.*, 2005; Iqbal *et al.*, 2005; Hordegen *et al.*,2006; Ali, 2006; Das *et al.*, 2006; Fakim, 2006; Siamba *et al.*, 2007; Thomas *et al.*, 2007; Shahadat *et al.*, 2008; Islam *et al.*, 2008; Satish *et al.*, 2009; Adu *et al.*, 2009; Ayub *et al.*, 2011; Adeyemo & Akanmu, 2012; Kamal *et al.*, 2015). The results of present study, revealed that on day 21 of artificial inoculation of *A. galli*, the infection established and EPG of groups A, B & C was 1424.5, 1346.3 and 1806.4, respectively. On the same day, the treatment of papaya and neem seed extract was given to group B and C, respectively and significant (*P*<0.001) decrease in parasitic burden was recorded in the following days while in group A (control-without treatment), the significant increase in infection was observed. On day 42, the EPG in group B (papaya) and C (neem) was found to
Table 1. Screening of A. galli in experimental chickens.

<table>
<thead>
<tr>
<th>Day</th>
<th>Group A (EPG) Control</th>
<th>Group B (EPG) Papaya treated</th>
<th>Group C (EPG) Neem treated</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>21</td>
<td>1424.5 ± 367.46</td>
<td>1346.3 ± 45.88</td>
<td>1806.4 ± 249.96</td>
</tr>
<tr>
<td>28</td>
<td>1705.2 ± 357.50**</td>
<td>1013.0 ± 215.50**</td>
<td>1416.3 ± 38.13</td>
</tr>
<tr>
<td>35</td>
<td>2166.2 ± 228.39***</td>
<td>233.00 ± 52.81***</td>
<td>399.75 ± 118.38***</td>
</tr>
<tr>
<td>42</td>
<td>2606.4 ± 244.39***</td>
<td>6.600 ± 14.75***</td>
<td>41.500 ± 49.89***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(99.5% decreased)</td>
<td>(97.7% decreased)</td>
</tr>
</tbody>
</table>

decrease 99.5 and 97.7%, respectively (Table 1). It was shown that the ethanolic extraction of papaya and neem seeds were effective against A. galli in chicken although papaya was more effective than neem. Adu et al., (2009) used extraction from latex of papaya and reported 77.7% decrease in EPG of A. galli in the feces of chicken. Present observations revealed strong effectiveness of papaya seeds against A. galli infection. Our observation is in-agreement with the study of Ameen et al., (2012) who demonstrated successful control of helminths including A. galli through extraction of papaya seeds. A successive control of A. galli was also reported (in-vitro) by papaya seed extract (Alam et al., 2014).

Previous studies have shown the effectiveness of neem leaves extract to control the A. galli infection in poultry (Khokan et al., 2014) and also act as growth promoter (Adeyemo & Akanmu, 2012; Kamal et al., 2015), antifungal (Moslem & El-Kholie, 2009; Ospina-Salazar et al., 2015). Subapriya & Nagini (2005) reviewed the activities of neem leaf extract as antiviral, antibacterial, anti-hypertensive, antifungal, anti-malarial, anti-fertility, antipyretic, analgesic, anti-inflammatory and anti-ulcerogenic. Neem leaves extract proved 22-30% more effective than commercial anthelmintic for the control of gastrointestinal parasites in sheep and goat also (Thomas et al., 2007). In present study, papaya was proved to be more effective as compared to neem for controlling the A. galli infection. These findings are thus in conformity with Alam et al., (2014) who testified the various medicinal plants against A. galli and demonstrated the best control with papaya seed extract followed by neem. Islam et al., (2008) also reported that the papaya leaf extract is more effective than neem against A. galli eggs.

Conclusion

It is concluded that ethanolic seed extracts of Azadirachta indica (neem) and Carica papaya (papaya) are effective against the A. galli infection in chicken. However, the papaya extract was found to be more effective. However, more research towards particular active compound against the gastrointestinal parasites in poultry is needed.
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(Accepted: Dec. 12, 2016)