INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2001/03–4–451–453
http://www.ijab.org

In Vitro Inhibitory Effects of *Sorghum bicolor* on Hatching and Moulting of *Haemonchus contortus* Eggs

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ABSTRACT

Studies were conducted to evaluate the aqueous extract of sorghum (*Sorghum bicolor*) for its *in vitro* inhibitory effects on the hatching and moulting of eggs of *Haemonchus (H) contortus.* The eggs were recovered from adult female *H. contortus* and cultured on faecal medium. The cultures were sprayed with aqueous extracts of sorghum derived from dried field grown sorghum plants in the ratio of 1 g herbage : 20 mL water. Hatching of *H. contortus* eggs on day 2 post treatment in sorghum extract treated cultures was 5.8%; whereas, it was 69.8% in cultures treated with distilled water. However, only rare (≤ 4%) L_1 moulted to L_3 in sorghum treated cultures in contrast to control (distilled water) where, 45% eggs developed to L_3. It was concluded that Sorghum possesses inhibitory effects on the hatching and moulting of eggs of *H. contortus.*

Key Words: *Sorghum bicolor*; Inhibitory effects; *Haemonchus contortus;* Hatching; Moulting

INTRODUCTION

Gastrointestinal nematode parasitism is a major problem of grazing livestock in tropical countries (Maqsood *et al*., 1996) resulting in heavy economic losses (Iqbal *et al*., 1993). Control of gastrointestinal (GI) nematodes for the past 30 years has relied heavily on the use of anthelmintics. These compounds have been very successful but the development of anthelmintic resistance in GI nematodes in a number of countries (van Wyk *et al*., 1997) gives a clear indication that control programs based exclusively on their use are not sustainable. The development of integrated programs to control GI nematodes is vital, but such control programs require viable alternatives to the use of anthelmintics (Waller, 1999). Numerous plants from a number of countries have been listed as having anthelmintic activity and many are currently used as part of traditional veterinary practices. The main use of these plants have been reported in animals (Hammond *et al*., 1997; Akhtar *et al*., 2000). Since, nematodes are a major problem of grazing livestock, therefore, efforts may also be focused on the control of developmental forms (eggs to larvae) on pastures. There are many epidemiological factors which play role in the perpetuation of life cycle of *H. contortus* in the host, and of free living stages on the soil and pasture. The development and survival of nematode eggs has been reported to be affected with the change in texture and composition of soil (Singhal *et al*., 1983; Samad, 1995; Iqbal *et al*., 2000; Munir *et al*., 2001). The chemical composition of soils undergoes drastic changes depending on the species of plants cultivated because of variations in physiological and biochemical requirements and reactions resulting in release of allelochemicals by the leaves by rain water, secreted by roots or produced from decaying plant matter (Ting, 1982). Therefore, it was expected that allelopathic effects reported for plants against weeds (Putnam & Defrank, 1983; Cheema, 1988) can also be exploited for changing soil and pasture environment and thus creating unfavorable conditions for the development of free living stages of nematodes.

In this experiment, effects of water extracts of sorghum (*Sorghum bicolor*) on hatching and moulting of *H. contortus* eggs and larvae were investigated with an objective to cultivate sorghum as a rotational crop on pastures, if its allelochemical content inhibits the development of eggs and larvae.

MATERIALS AND METHODS

Collection of adult *H. contortus* and extraction of eggs. Abomasa of sheep, slaughtered at Faisalabad abattoir were collected, incised longitudinally and examined for the presence of adult *H. contortus* (Maqsood *et al*., 1996). The worms present in ingesta or attached to the abomasal epithelium were picked manually using artery forceps and placed in a bottle containing PBS (pH 7.2). Female worms were separated from males by grossly witnessing the blood filled intestine spirally coiled around white ovary giving an appearance of barber's pole worm. The female worms were washed thrice in PBS (pH 7.2), transferred to 0.9% normal saline solution and incubated at 37ºC for 24 h. Thereafter, worms were removed from normal saline solution and eggs laid by them were collected by centrifugation at 500 RPM for 10 min. The eggs were counted in one drop of normal saline (a dropper calibrated to have 15 drops of normal saline per mL), and hence, inoculi containing 25,000 eggs in 10 mL of normal saline were prepared for further use.

Preparation of aqueous sorghum extracts. Field grown sorghum plants (uprooted at maturity) were dried at 22ºC for a few days and were chopped into 5 cm pieces with an electric fodder cutter. The dried material was soaked in distilled water for 24 h at room temperature (20–22ºC) in the ratio of 1 g herbage : 20 mL water (Cheema, 1988). The water extract was obtained by filtering the mixture (herbage and water) through a Whatman # 42 filter paper.

Experiment. Eggs of *H. contortus* were cultured in fresh faeces obtained from parasite free sheep. The pH of faecal medium was controlled at 7.2-8.0 and periodically measured electrometrically. Sheep faeces (50 g) was added to 10 dishes to a depth of 3 cm. Each of the culture dishes was inoculated with 25,000 eggs of *H. contortus.* The cultures in the five dishes were sprayed with sorghum extracts, while the other five cultures were used as control and sprayed with distilled water. It was ensured that relative humidity of the cultures ranges between 70-75%, and the cultures are not too wet or dry. The cultures were stirred gently daily, covered with lids and incubated at 27ºC (relative humidity 70-75%) for eight days. Three aliquots each of one gram medium from different places of each culture dish were taken randomly and mixed to make a homogenous suspension in 45 mL normal saline. The eggs and/or larvae were counted using microscope in five drops placed separately on a glass slide under low power and calculated for 1 mL (5 x 3= 15 drops) of saline solution. These counts were made for all the treated and control cultures at day 0, 2, 4, 6 and 8 of experiment. The data thus obtained on number of eggs hatched to L_1, L_2 and L_3 in sorghum treated and control groups (five replicates of each group) were subjected to analysis of variance technique using Microsoft Excel 2000 program.

RESULTS AND DISCUSSION

Hatching of eggs of *H. contortus* started on day 2 PT in both sorghum treated and control groups. However, per cent hatching was lower in sorghum treated cultures compared with control (distilled water treated) cultures. Results (Table I) revealed that 5.8 and 11.8% eggs hatched to L1 in sorghum treated cultures on day 2 and 4 PT, respectively; whereas, 69.8, 46.2 and 23.6% eggs hatched to L_1 on day 2, 4 and 6 PT, respectively. It was found that only rare (< 20) L_1 moulted to L_2 and L_3 in sorghum treated cultures. In contrast to it, 9, 40.6 and 25.8%; and 27.6, 34.4 and 45% L_1 moulted to L_2 and L_3 on day 2, 4, 6 or 8 PT, respectively in control group. It is evident from these results that sorghum adversely affected the hatching, moulting and survival of developmental forms of *H. contortus*.

The exact mechanism with which sorghum inhibited hatching and moulting of *H. contortus* eggs has not been so far investigated. However, it is speculated that the allelochemicals of sorghum like benzoic acid, p–hydroxybenzoic acid, vanillic acid, m–coumaric acid, p–coumaric acid, gallic acid, caffeic acid, ferulic acid and chlorogenic acid (Cheema, 1988) changed the pH of the media, which disturbed metabolic processes required for the hatching of eggs and moulting of larvae.

Sorghum has been reported to possess allelopathic effects due to its allelochemicals content (Evenari, 1949; Putnam & Defrank, 1983; Cheema, 1988). Allelopathy is a biochemical interaction between plants (Anderson, 1983) or biochemical interactions (both inhibitory or stimulatory reciprocal) between all types of plants including microorganisms (Rice, 1974) or any direct or indirect harmful effect by one plant (including micro–organism) on another through production of chemical compounds that enter into the environment (Rice, 1974) or biochemical interactions between plants, both inhibitory and stimulatory, through the release of secondary chemical substances into the environment by decomposition of plant residues, root exudates or leachates of leaves by rain water (Muller *et al*., 1969; Ting, 1982; Rice, 1984).

The inhibitory effects may have been mediated through ingestion of condensed tannins (CT) of sorghum like catechin tannin, procyanidins, anthocyanidin (Gupta & Haslam, 1980), luteoforol (Bate–Smith, 1962; 1969), apiforol (Watterson & Butler, 1983) and flavon–4–ols (Jambunathan *et al*., 1986). These effects may also be due to the interactions of these tannins with the external surface of larvae of nematodes. Some evidence in support of the anthelmintic activity of CT was provided by Lorimer *et al.* (1996) when they demonstrated that the inhibitory effects on migration of exsheathed *T. colubriformis* L_3 by a plant extract known to contain polyphenolics were greatly reduced after the polyphenolics were removed. Further evidence for an anthelmintic effect of CT was reported from a preliminary project (Duncan, 1996), which evaluated the potential of CT to inhibit the viability of sheep nematode parasites. Various bioassays were used including larval migration through screening sieves and larval development where development of nematode eggs was quantified after exposure to CT. Migration of sheathed and exsheathed L_3 nematode larvae (unspecified species) was determined after exposure to various concentrations of CT (stock solution in water of 24 mg/mL) extracted from *Acacia aneura* (mulga),

Table I. Effect of Sorghum extract on hatching, moulting and survival of eggs and larvae of *Haemonchus contortus*

Days PT	Sorghum				Control			
	Eggs/mL	L_1	L_2	L_3	Eggs/mL	L_1	L_2	L_3
0	500	–	–	–	500	–	–	–
2	190	29* (5.8)	–	–	113	349 (69.8)	45 (9.0)	–
4	120	59* (11.8)	R**	–	R	231 (46.2)	203 (40.6)	138 (27.6)
6	R	R**	R**	R**	–	118 (23.6)	129 (25.8)	172 (34.4)
8	R	R	R	R**	–	R	R	225 (45.0)

PT= Post–treatment; R ≤ 20 (4%); **indicates significant (P < 0.01) difference compared with the same parameter in control group; Values in parenthesis indicate per cent hatching or moulting out of 500.

Acacia saligna, *Leucaena leucocephala*, and *Acacia harpophylla* (brigalow). Condensed tannins were subsequently diluted with a salt solution containing 12% fatty acids and 0.5% sodium carbonate. Relative to a CT–free control, migration of sheathed larvae was unaffected but migration of exsheathed larvae was reduced by CT. Condensed tannin from *L. leucocephala* was most effective at reducing migration of exsheathed larvae (–60%) at high CT concentrations but at concentrations of CT from 6–12 mg/mL the CT from *A. aneura* and *A. saligna* were most effective. Development of nematode eggs to the third larval stage was found to be reduced in the presence of CT from all test species with reductions of the magnitude 70% to 40% recorded for CT from *A. aneura* and *A. saligna*. In addition, CT extracted from a number of woody plants has been shown to reduce *in vitro* nematode viability.

There are a number of plants releasing allelochemicals like sorghum, soybeans, sunflower, oilseed rape, wheat and field pea (Putnam & Defrank, 1983; Cheema, 1988) and yet there are also many forages rich in tannin content like crownvetch, lespedeza, lotus, sainfolin and trefoil (Sanderson *et al*., 1975; Jones & Mangan, 1977). It is obviously better if plants having both the qualities of having allelochemicals and significant tannin component be used as rotational crops and also fed to animals.

CONCLUSIONS

Sorghum extracts delayed hatching and development of eggs of *Haemonchus contortus* at lower concentrations and proved lethal at higher (>5–10 %) concentrations. Sorghum may be used as an alternate crop as a pasture management approach for the control of free living stages of *H. contortus*. *In vivo* studies are suggested for evaluation of anthelmintic activity of sorghum as grain and whole plant because of its tannin content and other chemicals.

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(Received 11 August 2001; Accepted 18 September 2001)