Plant Archives Vol. 20, Supplement 1, 2020 pp. 106-109 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

GARLIC (*ALLIUM SATIVUM*) AND CLOVE (*SYZYGIUM AROMATICUM*) AS ALTERNATIVE TREATMENTS FOR THE CONTROL OF *HAEMONCHUS CONTORTUS* IN SHEEP

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Abstract

Garlic (*Allium sativum*) and clove (*Syzygium aromaticum*) plants have always played a major role in the treatment of human and animal diseases and it has main role in the popular medicine. The aim of this study was to explore the potential activities of the alcoholic and aqueous extracts of the two plants on The larva of *Haemonchus contortus* and evaluate the antiparasital effect(s). The several dilutions of the alcoholic and aqueous extracts from 50, 75 and 100 mg/ml were used on *Haemonchus contortus* larvae in vitro. The mortality rate of larvae was count until 11 days. In present study, it was found that the alcoholic extract of garlic had greater inhibitory effect and killed all the larvae during the fourth and fifth days, while 50µg/ml dilution and more of the alcoholic and aqueous extract of *S. aromaticum* are effective on larvae stage and the mortality rate was 100 % on eleventh day

***Keywords* :** *Haemonchus contortus*, garlic (*Allium sativum*), clove (*Syzygium aromaticum*)

Introduction

Helminthiases in sheep are caused by parasites belonging to Nematoda, Cestoda and Trematoda classes, and the main genera are: *Haemonchus, Trichostrongylus, Strongyloides, Moniezia, Cooperia, Oesophagostomum, Trichuris* and *Cysticercus* (Rodrigues *et al.*, 2007). The ruminants Trichostrongylids are nematodes parasites of the digestive tract, which have direct life cycle. The parasite eggs or larvae are excreted along with the feces, and they are ingested orally when animals are grazing (Melhorn *et al.*, 2011), thus completing the cycle. The disease improves in temperate climates and affects all ages specially the young animals (Issa *et al.*, 2004, Altaif, 2009). Trichostrongylids responsible of large economic loose throughout the global, reduced growth rate, with low quality wool and decrease milk production (Levine, 2011, Anderson and Hall, 2015). The most common genera that infect sheep is *Haemonchus* spp. Haemonchosis results in accentuated economic losses due to a decrease in animal productivity caused by damage to the gastric system. This damage causes decreased forage consumption as well as alterations to the absorption of protein, energy and minerals from feed (Maciel *et al.*, 2006). The clinical signs associated with Haemonchus contortus infection, known as haemonchosis, are mostly related to anemia, and include pallor, exercise intolerance, ventral oedema and frequently, death (Taylor *et al.*, 2007).

Garlic (*Allium sativum*) has been reported to be a parasiticide, amebicide, acarifuge, vermifuge, larvicide, fungicide and immunostimulant, besides other properties (Tsai *et al.*, 2012). The unique flavor and health-promoting functions of garlic are generally attributed to its rich content of sulfur-containing compounds, i.e., alliin, g-glutamylcysteine, and their derivatives. According to Mehlhorn *et al.* (2011), garlic is also described as an anthelmintic agent. However its efficacy against endoparasites may be associated with the action of herbal plant agents or the stimulation of high passage rate of feed in the gastrointestinal tract, due to the amount of oil contained in this phytotherapy.

Syzygium aromaticum popularly known as clove is a well known spice of southern India. Its oil is traditionally applied as a remedy for bronchitis, common cold, cough, dyspepsia, flatulence, stomach distension and gastrointestinal spasms (Bankar and Sandhna, 2011). Clove bud oil has biological activities, like anthelmintic, antibacterial, antifungal, analgesic, antispasmodic, anticancerous, anticarminative and antioxidant properties. The predominant constituents in clove bud oil are eugenol and B-caryophyllene (Park *et al.*, 2007). *S. aromaticump* possess saponins, tannins, phenols, cardiac glycoside, flavonoids, alkaloids and anthracene (Emmanuel, 2015).

The present study was used to test aqueous and alcoholic effects of Garlic (*Allium sativum*) and clove (*Syzygium aromaticum*) plants on the larva of *H. contortus* cultivated in culture media.

Materials and Methods

Preparing of plant extracts

Methods of Riose *et al.* (1987) and Prabhakar et al (2009) were followed to obtain the plant extraction of garlic (*A. sativum*) and clove (*S.aromaticum*) were collected; isolated; washed and left to dry at room temperature. Fifty grams of each plant were separately crushed to a powder form by using sterilized mortar and pestle. These grind materials were extracted sequentially into 200ml of ethanol and sterilized water. The mixture was continuously stirred at room temperature for 2 hr and filtered through filter paper. Resulting extracts in two solvents were evaporated and concentrated to dryness using the rotary evaporator at 45 ºC and stored at 4 ºC. Serial double fold dilution was done to each stock aqueous and alcoholic extracts solutions in sterile labeled tubes and the concentration tested were (50, 75 and 100) mg/ml.

Egg isolation and larva Culturing

The anthelmintic activity of the plants extracts was tested on the larvae stage of *Haemonchus contortus* local isolate. The sheep were acquired from College of Veterinary medicine/ animal house. Adult worms of *H. contortus* were collected from the abomasums of the local sheep. Immediately after slaughtering, the abomasums were collected and transported to the laboratory. Adult female parasites were then selected, washed, and crushed to liberate the eggs. The eggs were then cultured in a glass jar filled with charcoal and wood sawdust powder for 10 days at room temperature. At the end of the 10th day, infective larvae were harvested by sedimentation using Biermann's devices and kept at 4 °C until use.

Larvae activity inhibition

The anthelmintic effect of the alcoholic and aqueous extract of plants used in this study on larva activity was evaluated according to Hounzangbe-Adote (2005) Solutions of the aqueous and alcoholic extracts were prepared with PBS at three different concentrations (50, 75 and 100) mg/ml., and 1 mL of each of these solutions was deposited in titration plate wells. Actively moving larva were then placed into each well (5 larvae/well). PBS and levamisole (250 µg.mL^{-1} in PBS) solution was also prepared and used as negative and positive controls, respectively. The test was repeated three times for each concentration and for controls. The inhibition of motility and activity of larva was used as the criterion for anthelmintic activity. After exposing larva to the extracts, activity was observed every 24h using a light microscope. Activity inhibition of larva was evaluated as the following ratio: The observations ended when all the larva in PBS died.

Data analysis

Statistical analyses were performed with the statistical program for the social sciences SPSS version 16.0. The significance of herbal drugs were assessed using analysis of variance (ANOVA) for different concentrations of extracts. The term significant had been used to indicate difference for which P≤0.05.

Results

The study reveals that the aqueous and alcoholic extracts of *A. sativum* showed 100% killing effect on larva stage at 50 mg/ml concentrations, and all the worms were found completely immotile after the fourth and fifth days post-exposure for alcoholic and aqueous extracts respectively.

As shown in Table (2), the concentration of the aqueous and alcoholic extracts of *S. aromaticum* (50, 75 and 100) mg/ml showed significant anthelmintic activity on the larva of *H. contorts.* The extracts were associated with a reduced larvae activity after 24 h post-exposure, and all the larvae were found immotile or dead in the wells containing plants extracts on the tenth day (for alcoholic extract) and eleventh day (for aqueous extract) of treatment. By this time, levamisole exhibited 100% inhibition after eight h post-exposure.

Table 1 : Effects of various concentrations of aqueous and alcoholic extract of *Allium sativum* on larvae of *Haemonchus contortus*

Extract	Days / Concentrations	1	2	3	4	5	Average concentration
Aqueous	50	69	49	37	19	0	43.5a
	75	57	44	32	13	0	36.5b
	100	55	40	28	9	0	33b
Average days * aqueous extract		60.33a	44.33b	32.33c	16.66d		
Alcoholic	50	59	42	22	0		41a
	75	55	39	17	0		37b
	100	53	37	12	0		34c
Average days * Alcohol extract		55.66a	39.33b	17c			
Control		78	67	55	49	44	

Table 2 : Effects of various concentrations of aqueous and alcoholic extract of *Syzygium aromaticum* on larvae of *Haemonchus contortus*

Extract	Days / Concentrations	1	2	3	4	5	6	7	8	9	10	11	Average concentration
Aqueous	50	73	57	48	40	33	27	19	13	6	1	0	31.7a
	75	69	54	46	38	31	25	17	10	4	1	0	29.5b
	100	61	50	43	35	29	21	15	8	2	0	0	29.3B
Average days * aqueous extract		67.6 a	53.6 b	45.6 c	37.6 d	31 e	24.3 f	17 g	10.3 h	4 i	0.6 i		
Alcoholic	50	77	73	59	54	49	46	38	27	18	0		49a
	75	75	70	57	52	45	42	35	25	15	0		46.2a
	100	72	67	55	50	41	39	33	21	10	0		43.1a
Average days * Alcohol extract		74.6 a	70 b	57 b	52 b	45 c	42.3 c	35.3 d	24.3 e	14.3 f			
control		78	67	55	49	44	40	36	33	30	26	22	

Different letters refer to significant differences at (P ≥ 0.05)

Discussion

The control of *H. contortus* with anthelmintics is being challenged by the development of resistance in the worms. Anthelmintic resistance to H. contortus has been reported in South Africa, Australia, New Zealand, Malaysia, Spain, France, Denmark, the United Kingdom, Brazil and the United States (Fleming *et al.*, 2006).

The mode of action of the garlic varies depending on the parasite species, and can include the alteration of intracellular membranous structures, inhibitions of macrophage nitric oxide production, cystein proteinase, phosphatidylcholine biosynthesis, parasitic synthesis of coenzyme Q and cell lysis, interaction with thiol-containing enzymes, interference with protein and lipid trafficking in parasite and host cell membranes, alcohol dehydrogenase and the alterations of intracellular membranous structures (Anthony *et al.*, 2005; Williams, Lamprecht, 2007). In garlic, the bioactive compound is allicin (diallylthiosulfinate). This compound is responsible for garlic's pungent odour and the many medicinal health benefits associated with the consumption of garlic (Amagase *et al.*, 2001). Masamha *et al.* (2010) found that the administration of garlic reduced the EPGs of Trichostrongylus and Strongylus in sheep. These authors believe that the antihelmintic effects of garlic can be attributed to the high tannin content, which may have a direct effect on the resident worm population, disrupting the normal physiological functions. This statement agrees with Bastidas (1969) who asserted that *Allium sativum* does not prevent the egg production, but may prevent the eggs of certain parasites from developing into larvae.

The present study showed that *Syzygium aromaticum* had higher potential anthelmintic activity against *H. contortus* worms of sheep.

The major constituents in bud oil are eugenol and â-carophyllene (Srivastava *et al.*, 2003). Bioactive constituent of clove oil is eugenol, but its exact mechanism of action on nematodes is unknown (Yang *et al.*, 2003) but, wormicidal activity of clove extracts could be attributed to its strong corrosive action on cuticle and tegument of helminthes which needs further evaluation.This result agreed with Pessoa *et al.* (2002) who reported the anthelmintic activity of eugenol against *H. contortus*. Kumar and Singh (2014) recorded in vitro toxicity of eugenol against adult *Fasciola gigantic*. Likewise, Dhanraj and Veerakumari (2015) tested ethanolic extract of *S. aromaticum* on the motility of *Cotylophoron cotylophorum* .

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