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# Black Cumin Seed Ethanolic Extract Decrease Motility and Shortening Mortality Time of *Ascaridia galli* Worm *In Vitro*

*( EKSTRAK ETANOL BIJI JINTAN HITAM MENURUNKAN MOTILITAS DAN MEMPERSINGKAT WAKTU MORTALITAS CACING ASCARIDIA GALLI SECARA IN VITRO)*

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## ABSTRACT

Ascaridiasis caused by roundworm *Ascaridia galli* is one of important diseases in poultry industry in Indonesia. This disease can affect economic losses in the form of death, growth retardation, reduced egg production as well as trigger for secondary viral or bacteria infections. Control of this nematode requires a good strategy to avoid the risk of anthelmintic resistance, one of which is the use of herbal medicine such as black cumin seed (*Nigella sativa*). This study was aimed to determine the level of motility and mortality time of *A. galli* after treated with black cumin seed extract *in vitro*. In this study, black cumin seed was extracted using ethanol 96%, and then diluted to 15% (P1), 25% (P2), and 45% (P3) concentration. Pyrantel pamoate preparation was used as positive control (C1), and carboxymethyl cellulose (CMC) solution as negative control (C0). Observations were carried out every 15 minutes until all the treatment worms died. The results revealed that the motility and mortality time of *A. galli* were significantly different in some treatment groups. The mortality time of group C0, C1 , P1, P2, and P3 were 2592, 801, 1557, 1350, and 612 minutes, respectively. 45% of black cumin seed extract had the ability to decrease worm motility, and it showed the shortest mortality time compared to other treatment groups. It can be concluded that ethanolic extract of black cumin seeds had potential anthelmintics property on *A. galli* worms.

Keywords: anthelmintic property; *Ascaridia galli; Nigella sativa;* mortality

## ABSTRAK

Ascaridiasis yang disebabkan oleh cacing gelang *Ascaridia galli* merupakan penyakit penting bagi industri perunggasan di Indonesia. Penyakit ascaridiasis ini dapat berdampak pada kerugian ekonomi berupa kematian, terhambatnya pertumbuhan, berkurangnya produksi telur serta menjadi pemicu infeksi virus atau bakteri sekunder. Pengendalian nematoda ini memerlukan strategi yang baik untuk menghindari risiko resistansi antelmintik, salah satu cara yang dapat ditempuh adalah dengan menggunakan obat herbal seperti biji jintan hitam (*Nigella sativa*). Penelitian ini bertujuan untuk mengetahui tingkat motilitas dan mortalitas cacing *A. galli* setelah diberikan ekstrak biji jintan hitam secara *in vitro*. Pada penelitian ini biji jintan hitam diekstrak menggunakan etanol 96%, kemudian dibuat menjadi konsentrasi 15% (P1), 25% (P2), dan 45% (P3). Sediaan pyrantel pamoat

digunakan sebagai kontrol positif (C1), dan larutan *carboxymethyl cellulose* (CMC) sebagai kontrol negatif (C0). Pengamatan terhadap cacing perlakuan dilakukan setiap 15 menit sampai semua cacing mati. Hasil penelitian menunjukkan bahwa motilitas dan mortalitas cacing *A. galli* berbeda nyata pada beberapa kelompok perlakuan. Waktu kematian kelompok C0, C1, P1, P2, dan P3 masing-masing adalah 2592, 801, 1557, 1350, dan 612 menit. Ekstrak biji jintan hitam 45% menunjukkan efek yang baik dalam menurunkan motilitas cacing, dan konsentrasi ini juga menunjukkan waktu kematian paling cepat dibandingkan dengan kelompok perlakuan lainnya. Dapat disimpulkan bahwa ekstrak etanol biji jintan hitam memiliki potensi sebagai anthelmintik pada cacing *A. galli*.

Kata-kata kunci: daya anthelmintic; *Ascaridia galli; Nigella sativa;* mortalitas

## INTRODUCTION

Poultry farming is one of the most demanding animal husbandry nowadays. The development has been tremendous worldwide, including in Indonesia. Chicken and ducks are reared for both meat and egg production in Indonesia, due to affordable prices and sources of high protein diet. In developing countries, demand for meat and egg increases rapidly and have positive impact in livestock production (Jaiswal *et al.,* 2020). However, poultry farming is constantly interfered with helminthic infections. There are large number of nematodes present in the gastrointestinal tract of poultry which can cause damages and inhibit growth, and eventually affects the economical value of the farm (Sharma *et al.,* 2018). One of the nematodes that found in poultry is *Ascaridia galli.*

*Ascaridia galli* (Schrank, 1788) belongs to genus Ascaridia Dujardin, 1845, is a primary intestinal parasite found in guinea fowl, turkey, geese, several wild birds, and the main host to chicken (Permin and Hansen, 2003). *Ascaridia galli* is a roundworm that attacks poultry with its target organ is the small intestine (Susanti *et al.,* 2015). *Ascaridia galli* is a pathogenic parasites by sucking nutrients from its host and the chickens will suffer from nutritional deficiencies (Winardiana, 2012). Free rearing chickens pose a risk of contracting parasitic infections in the digestive tract which can cause economic losses (Angel *et al*., 2018). Balqis *et al*. (2014) reported that the economic loss reached USD 2.5-3.5 million per year. These losses are caused by weight loss, as well as reduced productivity and quality of chicken eggs. To overcome this helminth infection, anthelmintic drugs are required.

Anthelmintics are drugs used to eradicate or reduce worms in the bodies of human and animals (Winardiana, 2012). Currently, anthelmintics with more specific and more effective action for *A. galli* are available, but these drugs have side effects on the host, so an alternative treatment is needed, especially using herbal medicine (Susanti *et al.,* 2015). Herbal medicine is widely used recently to replace convensional medicine mainly due to its medicinal property, lower price, and fewer side effects (Tiwow *et al.,* 2013). One of herbal medicines that has anthelmintics property is *Nigella sativa*, also known as black cumin seed or habbatusasauda.

Black cumin is an herbal plant that has long been used as an anthelmintic, anti-diarrheal, antibacterial, anti-inflammatory, appetite enhancer, analgesic, diuretic and treatment of rheumatism (Widjaja, 2020). Black cumin contains compounds such as alkaloids, tannins, saponins, terpenoids, and flavonoids (Rini *et al*., 2020). According to Susanti *et al*. (2015), compounds of tannins, saponins, triterpenoids, and glycosides are efficacious as anthelmintics. Several research have been conducted on the anthelmintic activity of black cumin seed on trematodes. Ullah *et al*. (2017) reported the bioactive compound of black cumin seed, especially thymoquinone, had the ability to disrupt tegument of *Fasciola gigantica*, and could interfere *F. gigantica* metabolism. Hambal *et al*. (2021) also reported the effects of black cumin seed extract on the motility and mortality of *Paramphistomum* sp., and the best dose of black cumin seed extract was at a concentration of 40%. However, not much information is available on the effect of black cumin seed extract as anthelmintics on nematodes. Thus, further research on the effect of black cumin seeds extract on the motility and mortality time of nematodes *A. galli* is substantial for variety of anthelmintics option. This study was aimed to determine the level of motility and mortality time of *A. galli* after treated with black cumin seed extract *in vitro*

## RESEARCH METHODS

### Preparation of Black Cumin Seed Extract

Black cumin seeds were obtained from the local market in Banda Aceh which was imported from India. As much as 500 g of black cumin seeds were washed, dried, and ground using a blender, and sieved with a size of 30 mesh. Then, black cumin seed powder was macerated with 96% ethanol for three days and stirred once every 24 hours according to the maceration method previously carried out by Hambal *et al*. (2021). After macerated, it was filtered using a funnel. The filtrate was then concentrated using vacuum rotary evaporator. The crude extract was stored in the refrigerator and then diluted into 15%, 25%, and 45% concentration.

### Collecting of *Ascaridia galli*

As many as 25 *A. galli* worms were collected from the small intestine of domestic chickens in Banda Aceh. The worms were selected as adult females without any anatomical defects, with an average size of 5-7 cm long, and still active. The worms were washed with phosphate buffer saline, and stored in a jar containing saline solution for further examination.

### Motility and Mortality Test

Motility and mortality tests were carried out using five groups which were divided into C0, C1, P1, P2, and P3. The P1 treatment was given 15%, P2 was given 25%, and P3 was given 45% of black cumin seed extract diluted with 0.5% carboxymethyl cellulose (CMC) solution. The CMC solution was used as negative control (C0) and pyrantel pamoate (375 mg) was used as positive control (C1). Each treatment group used five worms, observation was carried out every 15 minutes until the worms did not show any living signs. Scoring index was used to measure the motility level of the worms, according to Hu *et al*. (2013) and Weaver *et al*. (2017). Score 3 is given if the worm moved all over the body, score 2 is given if the worm moved only part of the body or is less active, score 1 is given if the worm is not moving on its own, but moved when touched with tweezers. Score 0 is given when the worms did not move even after stimulation, and considered dead.

## RESULTS AND DISCUSSION

This study was carried out to investigate the effect of ethanolic extract of black cumin seed on motility and mortality time of *A. galli*. The results indicated a strong anthelmintic property of black cumin seed extract against *A.*

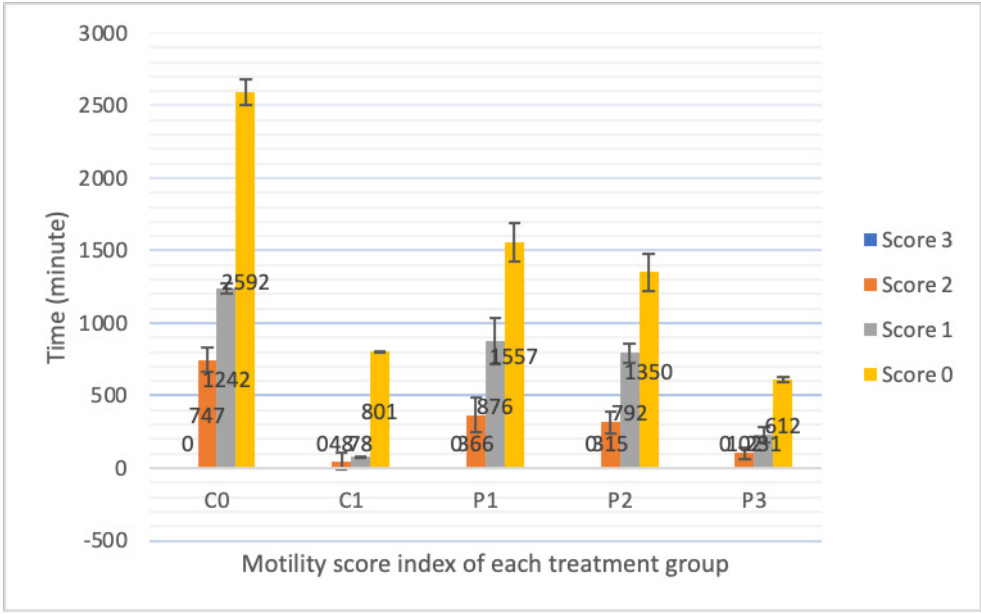


Figure 1. Motility score index of *Ascaridia galli* after treated with *Nigella sativa* seed extract (Notes: C0: negative control: C1: positive control: P1: 15% extract of *N. sativa:* P2: 25% extract of *N. sativa;* P3: 45% extract of *N. sativa)*

Table 1. Mortality time of *Ascaridia galli* after treated with *Nigella sativa* seed extract

| Group | Average Mortality time (minute) ± SD |
|---|---|
| C0 | 2592 ± 86.26 [d] |
| C1 | 801 ± 57.97 [a] |
| P1 | 1557 ± 122.64[c] |
| P2 | 1350 ± 72.11 [c] |
| P3 | 612 ± 37.95 [b] |

(Notes: C0: negative control: C1: positive control: P1: 15% extract of *N. sativa:* P2: 25% extract of *N. sativa;* P3: 45% extract of *N. sativa)*

*galli,* through decreasing motility and shorten mortality time of the worm. The data is presented in Figure 1.

Black cumin seed extract had the capacity to reduce the motility rate of *A. galli* worms. The average time of *A. galli* to show weak movement (score 2) in C1 (positive control) group was in 48 minutes, P3 was in 102 minutes, P2 was in 315 minutes, and P1 was in 366 minutes. The average time for *A. galli* to show faint condition (score 1) in C1 group was in 78 minutes, followed by P3 in 231 minutes, while in P2 and P1 were in 792 and 876 minutes, respectively. Based on this data, it was shown that P3 which had 45% of black cumin seed extract had the ability to reduce motility of *A. galli* in a shorter time compared to P1 and P2. This data indicated 45% of black cumin seed had the most effective impact on *A. galli*.

The effectiveness of anthelmintics can be measured by worm or fluke motility. The low level of worm motility affects the worm's ability to survive in the intestine. The more motile they are, the more they have opportunity to move freely in the intestine to get nutrients. If they are less motile or paralysed, it will be more difficult for them obtaining nutrients, which will cause metabolic disorder leading to paralysed and finally death. Paralysed worms will be easily removed from intestine through peristaltic movement (Novia *et al.,* 2020).

The concentration of 45% of black cumin seed had the ability to kill *A. galli* faster than the positive control, while 15% and 25% extract required longer time. The mortality time of *A. galli* (score 0) in P3 group was reached in 612 minutes, followed by C1 in 801 minutes, P2 in 1350 minutes, P1 in 1667 minutes, and C0 in 2592 minutes (Table 1). In this data, P3 had the shortest time to eliminate *A. galli*, and it was even faster than C1 as positive control ($p<0.05$). The comparison of mortality time of P2 and P1, although revealed a big different, but was not statistically different ($p>0.05$). The ability of black cumin seed extract to eliminate *A. galli* was mainly due to secondary metabolites contained in the seed. El Naggar *et al*. (2010) suggested that thymoquinone is one of secondary metabolites that was responsible to cause helminth and flukes paralysed leading to death.

Thymoquinone is the main compound of the terpenoid group in black cumin seed since this compound has the highest content compared to other compounds (Suryadi *et al*., 2017). Thymoquinone has an anthelmintic effect that damages the tegument of trematode worms which causes disruption of nutrient absorption and damages the defenses of trematode worms (Ullah *et al*., 2017; Hambal *et al*., 2021). Thymoquinone is also reported to cause oxidative damage to worm proteins and to increase host immunity as a defense against parasites (Ullah *et al*., 2017; Angel *et al*., 2018). Sen *et al*. (2021) reported that thymoquinone could inhibits non-enzymatic lipid peroxidation, thus it will affect the organism's lipid structures. Another study by El-Naggar *et al*. (2010) reported that black cumin had a sedative effect and the ability to accelerate the release of gamma-amino butyric acid (GABA). Therefore, the mechanism of action of black cumin seed as an anthelmintic is divided into two steps, namely the sedative effect which causes the worms to experience a decrease in activity (decrease motility) and the effect of accelerating the release of GABA by the worms which causes paralysis of the worm's muscles so that the worms die in a paralyzed state.

## CONCLUSION

The ethanolic extract of black cumin seed was effective as anthelmintic for *A. galli* by decreasing worm motility and reduce worm mortality time. The most recommended concentration in this study was 45% extract which could eliminate the worm within 612 minutes.

## SUGGESTIONS

It is necessary to carry out advance study regarding the anthelmintic property of black cumin seed extract using animal laboratory to discover its impact *in vivo*. Moreover, toxicity test is advised to study its effect on the animal's organs.

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## REFERENCES

Angel C, Akhter N, Arijo A, Qureshi TA, Gandahi JA, Qazi IH. 2018. Comparative efficacy of ivermectin and *Nigella sativa* against helminths in aseel chickens (*Gallus domesticus*). *J Helminthol* 93(5): 1-6.

Balqis U, Hambal M, Utami CS. 2014. Gambaran histopatologi usus halus ayam kampung (*Gallus domesticus*) yang terinfeksi *Ascaridia galli* secara alami. *Jurnal Medika Veterinaria* 8(2): 132-135.

El-Naggar T, Gomez-Seranillos MP, Palomino OM, Arce C, Cerretero ME. 2010. *Nigella sativa* L. seed extract modulates the neurotransmitter amino acids release in cultured neurons in vitro. *J Biomed Biotechnol* 2010. doi:10.1155/2010/398312

Hambal M, Vanda H, Ayuti SR. 2021. *Nigella sativa* seed extract affected tegument and internal organs of trematoda *Paramphistomum cervi*. *Advances in Animal and Veterinary Sciences* 9(4): 1-5.

Hu Y, Ellis BL, Yiu YY, Miller MM, Urban JF, Shi LZ, Aroian RV. 2013. An extensive comparison of the effect of anthelmintic classes on diverse nematodes. *PloS one 8*(7): p.e70702.

Jaiswal K, Mishra S, Bee A, 2020. Scanning electron microscopy of A scaridia galli in Gallus gallus domesticus in Lucknow, UP, India. *Indian Journal of Science and Technology* 13(19): 1944-1954.

Khoirunnisa S, Falyani SA, Damayanti DS. 2020. Efek ekstrak rimpang kunyit terhadap paralisis dan kematian cacing dewasa *Ascaridia suum* Goeza secara *in vitro*. *Jurnal Kedokteran Komunitas* 8(2): 48-58.

Novia D, Samudra AG, Camelia ZA. 2020. Pengaruh pemberian infusa daun jati (*Tectona grandis* LS) terhadap waktu kematian cacing *Ascaridia galli* sp secara *in vitro*. *Jurnal Ilmiah Pharmacy* 7(1): 79-88.

Permin A, Hansen JW. 2003. The Epidemiology, Diagnosis and Control of Poultry Parasites: An FAO Handbook. Rome, Italy. Food and Agriculture Organization of the United Nations. Pp. 24–29.

Rini YC, Susilowati F, Amal ASS. 2020. Uji aktivitas antioksidan ekstrak etanol dan ekstrak air biji habatussauda (*Nigella sativa*). *Pharmasipha* 4(1): 1-8.

Sen E, Ogut T, Olgun A, Kisa O. 2021. Anthelmintic activity of *Nigella sativa* against *Caenorhabditis elegans*. *Adv Pharmacol Phar* 9(4): 117-126.

Sharma N, Hunt PW, Hine BC, McNally J, Sharma NK, Iqbal Z, Normant C, Andronicos NM, Swick RA, Ruhnke I. 2018. Effect of an artificial *Ascaridia galli* infection on egg production, immune response, and liver lipid reserve of free-range laying hens. *Poult Sci* 97(2): 494-502.

Suryadi R, Ghulamahdi M, Kurniawati A. 2017. Pemupukan nitrogen dan fosfor untuk meningkatkan pertumbuhan, produksi biji dan kandungan thymoquinone jintan hitam. *Buletin Littro* 28(1): 15-28.

Susanti Y, Astuti I, Astuti AAD. 2015. Uji efektivitas antelmintik ekstrak rimpang bangle (*Zingiber purpureum* Roxb.) terhadap cacing *Ascaridia galli* secara *in vitro*. *Jurnal Ilmiah Manuntung* 1(2): 187-192.

Tiwow D, Bodhi W, Kojong NS. 2013. Uji antelmintik ekstrak etanol biji pinang (*Areca catechu*) terhadap cacing *Ascaridia lumbricoides* dan *Ascaridia galli* secara *in vitro*. *Jurnal Ilmiah Farmasi* 2(2)*:* 76-80.

Ullah R, Rehman A, Zafeer MF, Rehman L, Khan YA, Khan MAH, Khan SN, Khan AU, Abidi SMA. 2017. Anthelmintic potential of thymoquinone and curcumin on *Fasciola gigantica*. *Plos One* 12(2): 1-19.

Weaver KJ, May CJ, Ellis BL. 2017. Using a health-rating system to evaluate the usefulness of *Caenorhabditis elegans* as a model for anthelmintic study. *PLoS One* 12(6): p.e0179376.

Winardiana Y. 2012. Efektivitas ekstrak etanol biji labu merah (*Cucurbita moschata*) sebagai antelmintik terhadap cacing *Ascaridia galli* secara *in vitro*. *Ekologia* 12(1): 12-18.

Widjaja CH. 2020. Manfaat jintan hitam (*Nigella sativa*) sebagai antiinflamasi dan pereda nyeri pada osteoarthritis (OA). *Jurnal Ilmiah Kesehatan Sandi Husada* 12(2): 901-902.