Sjournals
Scientific Journals
Scientific Journal of Biological Sciences (2013) 2(7) 138-144
ISSN 2322-1968

Contents lists available at Sjournals
Scientific Journal of
Biological Sciences
Journal homepage: www.Sjournals.com

Original article

Phytotherapy of intermediate host snail by ferulic acid to kill the Fasciola gigantica larvae in different months of the year 2011-2012

K. Sunita, P. Kumar , D.K. Singh*
Malacology laboratory, Department of Zoology, DDU Gorakhpur University Gorakhpur 273009 (U.P), India.

*Corresponding author ; Malacology laboratory, Department of Zoology, DDU Gorakhpur University Gorakhpur 273009 (U.P), India.

ARTICLE INFO

Article history:
Received 25 June 2013
Accepted 18 July 2013
Available online 30 July 2013

Keywords:
Fasciolosis
Phytotherapy
Sporocyst
Redia
Cercaria
Ferulic acid

ABSTRACT

Fasciolosis is a parasitic helminthes disease caused by Fasciola gigantica. The different larval stage (sporocyst, redia and cercaria) are found in intermediate host snail Lymnaea acuminata. In the present study it has been tried to kill the F. gigantica larvae in vitro/ in vivo condition by the use of ferulic acid there is no mortality of snails. Mortality of larvae in in vivo and in vitro condition were observed at 2h, 4h, 6h and 8h, exposure of ferulic acid. Effect of seasonal variation on abiotic factors is one of the crucial phenomenon, which ultimately affect the toxicity of ferulic acid in different months of year 2011-2012. In in vitro treatment highest toxicity of ferulic acid against redia was noted in between July to August (8h LC50 0.37,-0.34 mg/ml), where as in case of cercaria highest toxicity was noted in month of September- October (8h LC50 0.10,-0.17 mg/ml). Highest toxicity in in vivo treatments against redia was noted in between July- August (8h LC50 0.43- 0.42 mg/L respectively). The lowest toxicity were observed in between the months of December to January. The highest temperature, free carbon dioxide, and lowest pH, dissolved oxygen was noted in the months of June to August.

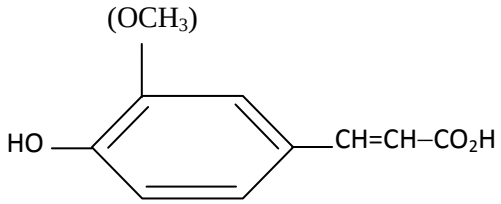
1. Introduction

Fasciolosis is an important helminthes disease caused by two trematode Fasciola hepatica and F. gigantica of Africa and Asia (WHO, 2007; Mas-Coma, 2009; Sunita and Singh, 2011). Fasciolosis is a common worldwide disease among cattle (WHO, 2007). Human fasciolosis is reported now in different part of world (Mas-Coma et al., 2005). According to WHO 2.4 million humans are infected with Fasciola and a further 180 million are at risk of infection (Anonymus, 1995). The fresh water snails are an intermediate host of the Fasciola species (Agarwal and Singh, 1988; Kumar and Singh, 2006; Kumar et al., 2012; Srivastava et al., 2013). Fasciolosis caused immense economic losses such as, lower production of meat, milk and wool, reduce weight gain, and impaired fertility (Agarwal and Singh, 1988; Parr and Gray, 2000) of infected animals. The freshwater snail L. acuminata is an intermediate host of the F. gigantica in northern parts of the India (Agarwal and Singh, 1988). One way to reduce the fasciolosis is to destroy the life cycle of fluke larval stage (sporocyst, redia and cercaria) inside the intermediate host snail L. acuminata at sublethal dose of molluscicides both in in vivo and in vitro conditions (Sunita and Singh, 2011). The aim of the present study is to explore the possibility that whether seasonal change in abiotic factors such as temperature, pH, dissolved oxygen and free carbon dioxide in test water can influence the in vitro and in vivo larvicidal activity of ferulic acid against different larva stages in infected snails. It is new approaches to reduce incidence of the fasciolosis without killing the snails.

2. Materials and methods

2.1. Test materials

Ferulic acid (4-Hydroxy-3-methoxycinnamic) is purchased for sigma chemical Co.USA.



2.2. Animals

Adult L. acuminata (2.6±0.20 cm in length) were collected locally. Cercaria shedding infected and uninfected snails were separated in two groups. The snails were allowed to acclimatize in laboratory condition. Each infected snail was dissected in a glass petridish containing 10 ml of dechlorinated water at 22oC-24oC. The pH of the water was 7.1-7.3 and dissolved oxygen, free carbon dioxide and bicarbonate alkalinity were 6.5-7.2 mg/L, 5.2- 6.3mg/L and 102.0- 105.0 mg/L, respectively. After dissection sporocyst, redia and cercaria were separated in different petridish containing 10 ml of dechlorinated water by the method of Sunita and Singh (2011). These larvae were kept in dechlorinated tap water where they survive up to 48h in laboratory condition.

2.3. Toxicity determination

2.3.1. *In vivo*

In vivo toxicity of ferulic acid against Fasciola larvae in infected L. acuminata was done by the method of Sunita and Singh (2011). Physical parameters of water such as temperature, pH, dissolve oxygen and free carbon dioxide were measured in each month of the year 2011- 2012. Dissolve oxygen and CO2 were estimated according to methods of APHA (2005). After 2h, 4h, 6h and 8h of treatment infected snails were dissected. Live and dead sporocyst, redia and cercaria were counted. Per cent mortality of larvae at 2h, 4h, 6h and 8h were used for the determination of LC50.

2.3.2. *In vitro*

In vitro toxicity of ferulic acid was performed in the petridish by the method of Sunita and Singh (2011). Ten sporocyst, redia and cercaria larva of Fasciola were separated in different petridish containing 10 ml dechlorinated

tap water. Treatment of ferulic acid was made directly in the petridish containing 10 sporocyst / redia / cercaria. Mortality and counting of larvae were observed with help of microscope after 2h, 4h, 6h and 8h of treatment.

Lethal value (LC50), lower and upper confidence limits (LCL and UCL), slop-values, t-ratio, g value and heterogeneity factors were calculated with the help of POLO computer programme of Robertson et al., (2007). One way ANOVA and product moment correlation coefficient were done by the method of Sokal and Rohlf (1996).

3. Results

In vitro and in vivo larvicidal activity of ferulic acid against the sporocyst, redia and cercaria larvae of F. gigantica was time and concentration dependent in each month of year 2011-2012. Figures 1-6 shows alteration in toxicity (LC50) against redia, cercaria and sporocyst larva among the different month. In in vitro treatment highest toxicity8h LC50 0.34mg/ml of ferulic acid against redia was noted in month of August at (August) and lowest (0.55mg/ml) in month of December (Figure-1). Highest toxicity of ferulic acid against cercaria (0.10mg/ml)/sporocyst (0.17mg/ml) was observed in the month of September and lowest in month of February (0.54mg/ml)/July (10.04mg/ml), respectively. In in vivo treatment ferulic acid caused highest mortality (8h LC50) of redia/cercaria and sporocyst larvae in month of August (0.42mg/L)/February (0.40mg/L) and July 0.10mg/L, respectively. The lowest mortality was observed in between November-January (redia 8h LC50 0.89-1.39mg/L, and cercaria 0.93-1.33mg/L, respectively), (Figure 4-6).

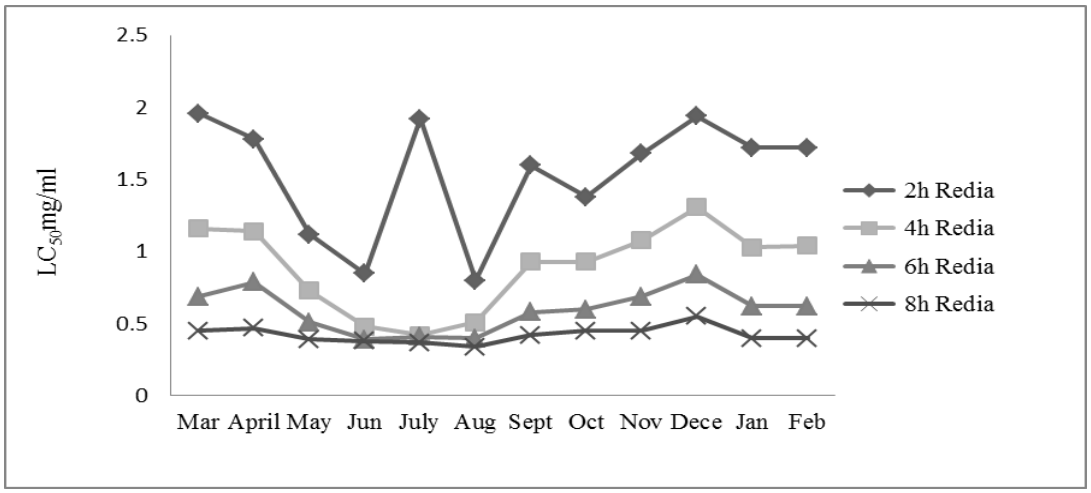


Fig. 1. In vitro alteration in toxicity (LC50 mg/ml) of Ferulic acid against redia larva in different months of year 2011-2012.

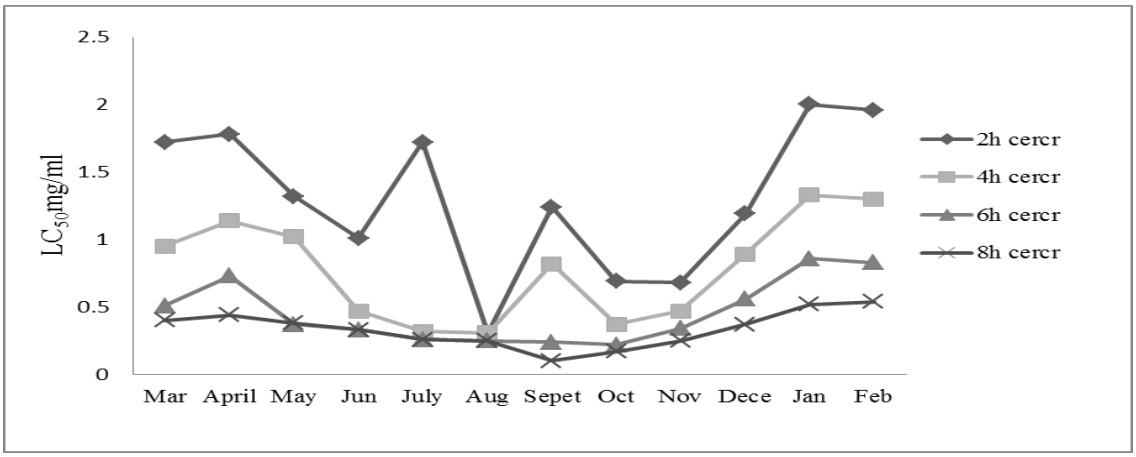


Fig. 2. In vitro alteration in toxicity (LC50 mg/ml) of Ferulic acid against cercaria larva in different months of year 2011-2012.

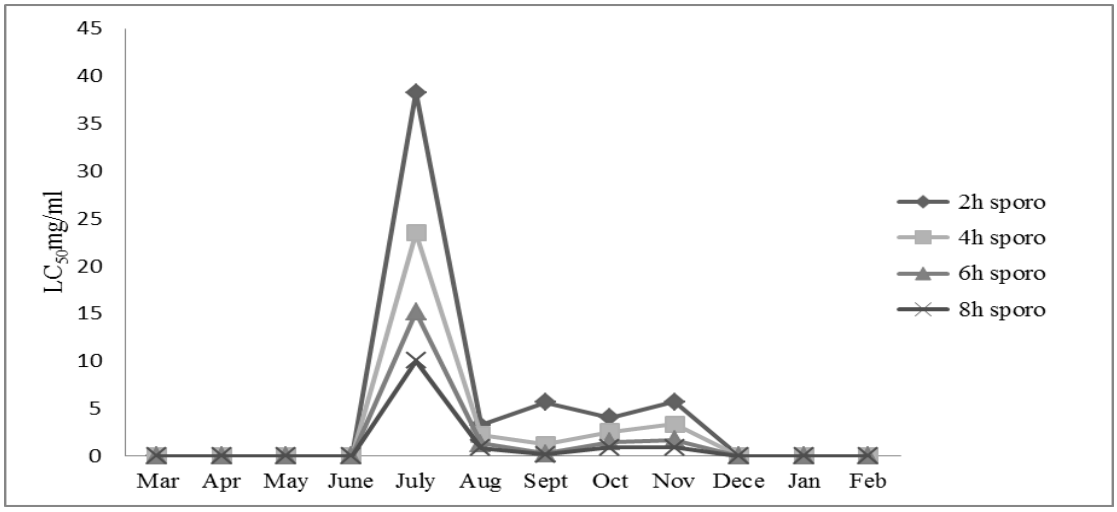


Fig. 3. In vitro alteration in toxicity (LC50 mg/ml) of Ferulic acid against sporocyst in different months of year 2011-2012.

The slope values were steep and separate estimation of LC50 based on each six replicate were found to be within the 95% confidence of LC50. The t-ratio was greater than 1.96 and the heterogeneity factor is less than 1.0. The g value was less than 0.5 at all probability levels (90, 95 and 99, respectively (Figure 1-6).

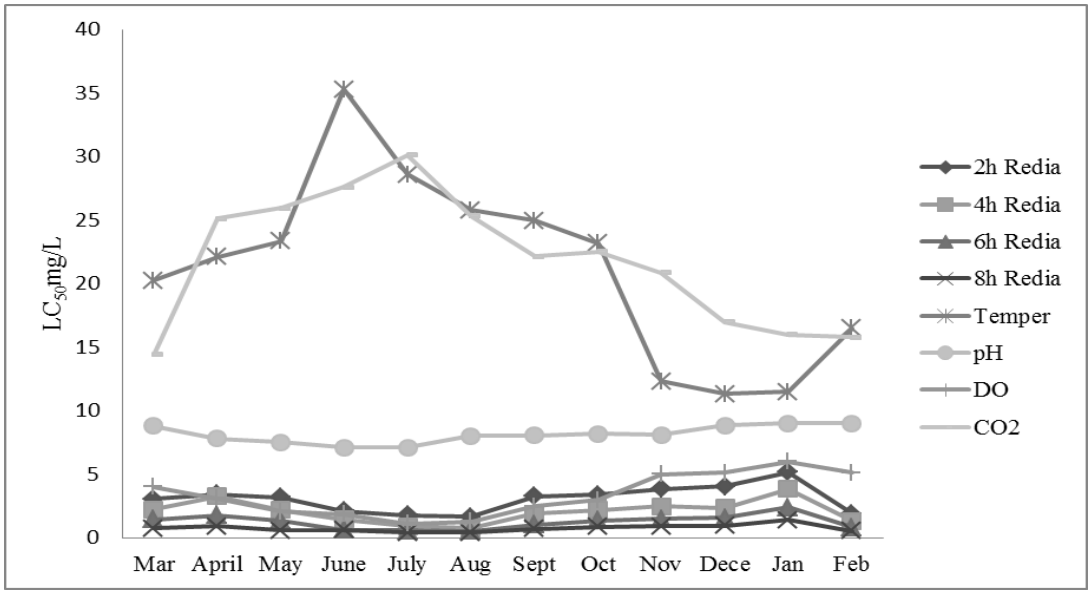


Fig. 4. In vivo alteration in toxicity (LC50 mg/L) of Ferulic acid against redia larva and different abiotic factor in different months of year 2011-2012.

4. Discussion

The active component ferulic acid is a potent molluscicide as well as larvicide against different larval stage of F. gigantica (Kumar and Singh 2006; Kumar et al., 2009; Sunita and Singh, 2011). The temperature of the water is a significant factor which alters the toxicity of ferulic acid in each month of the year 2011-2012. When the water temperature is higher in the season (July to August), in in vivo treatment the toxicity of ferulic acid was maximum. Contrarily, in the winter season the temperature of

water is low and the toxicity of ferulic acid is less, as evident by a higher LC50 value in both in vivo and in vitro treatments. Possibly ferulic acid become more soluble at higher temperature carbon dioxides, causing more larval (sporocyst, redia and cercaria) mortality inside the body of treated snails. However, dissolved oxygen is also one of the factors that alter the toxicity of ferulic acid. In winter, water holds more O2 as a result less mortality of larvae occurs during this period (Waterwatch Australia, 2002). The toxicity of ferulic acid against sporocyst, redia and cercaria in in vitro and in vivo condition was significantly ($p<0.005$) altered with respect to change in abiotic factors in each months of the year 2011-2012. Ferulic acid has larvicidal activity against different larva of F. gigantica in in vivo and in vitro treatments (Sunita and Singh 2011).

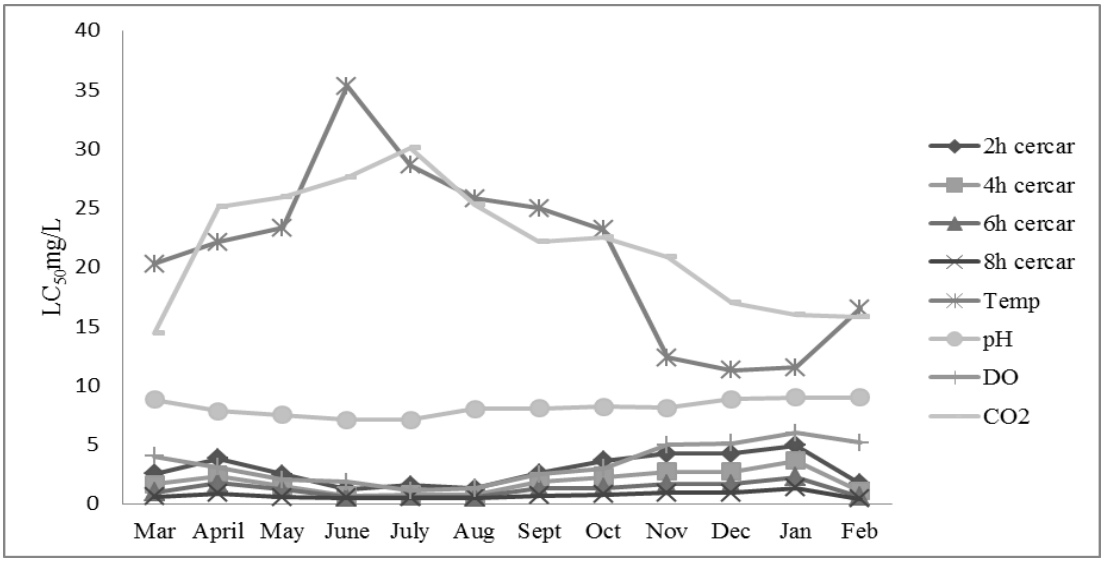


Fig. 5. In vivo alteration in toxicity (LC50 mg/L) of Ferulic acid against cercaria larva and different abiotic factor in different months of year 2011-2012.

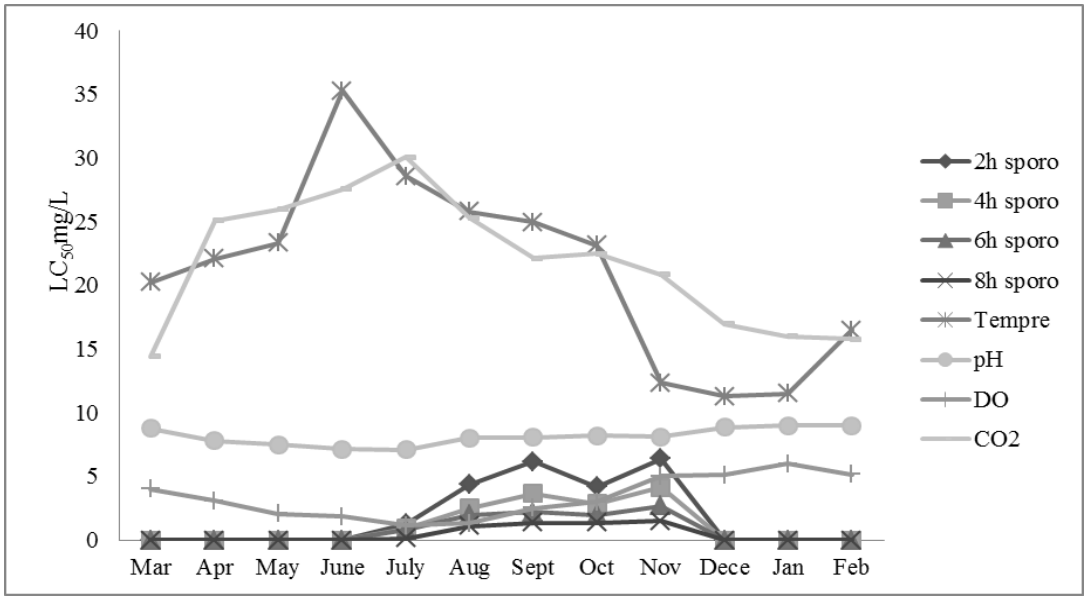


Fig. 6. In vivo alteration in toxicity (LC50 mg/L) of Ferulic acid against sporocyst and different abiotic factor in different months of year 2011-2012.

In Ayurveda and Greaco Arabic system of medicine, several spices are described as having medicinal effects (Srivastava and Mustafa, 1993). F. asafoetida are known to possess antimicrobial, antioxidant, anti carcinogenic antispasmodic, molluscicidal and antithelminthic activity (Bakker et al., 1995; Poolman et al., 1995; Hirotaka et al., 2003; Fatehi et al., 2004; Kumar and Singh, 2006; Sunita and Singh, 2011). The alcoholic extract of F. asafoetida and it active component (ferulic acid) has also shown moderate in vitro anthelmintic activity against F. gigantica larvae (Sunita and Singh 2011). Ferulic acid has been reported to have many physiological functions, including protection against coronary disease, lowers cholesterol and increases sperm viability (Shiyi and Kin- Chor, 2004). Ferulic acid has been shown to potentially exert several beneficial effect on health (Balasubashini et al., 2004), it significantly protect against UV- induced erythematic in human (Saija et al., 2000), act as a peroxyl radical scavenger and increased the resistance of LDL to oxidation. Ferulic acid is a potent molluscicides against L. acuminata, when released directly in aquatic system or bait formulation with snail attractant carbohydrate/amino acids (Kumar and Singh, 2006; Kumar et al., 2009; 2011; 2012). Trematode parasite is highly sensitive to abiotic factor. However, increasing problems of development of resistance in helminthes against anthelmintic drugs (Greek and Dorny, 1995) have led to the screening of medicinal plants for their anthelmintic activity. A number of medicinal plants have been used to treat parasitic infection in man and animals (Akhar et al., 2000). Present study clearly demonstrates that different larvae of F. gigantica inside as well as outside of snail body can be killed without killing the snails. Earlier it has been reported that ferulic acid (24h LC50- 2.21mg/L) is active molluscicides against L. acuminata (Singh et al., 1997). 8h LC50 of ferulic acid used against sporocyst, redia and cercaria larvae is several time lower than used to kill the L. acuminata (Kumar and Singh, 2006).

Concentrations used to kill sporocyst; redia and cercaria larva is not toxic to snails. In in vivo and in vitro killing of sporocyst, redia and cercaria of F. gigantica is beneficial as it kill directly target larvae of F. gigantica.

5. Conclusion

It can be concluded from the present study that mortality of sporocyst, redia and cercaria larvae of F. gigantica inside the body of vector snail L. acuminata by ferulic acid is significantly altered in different months of the year 2011-2012. Temperature, pH, free carbon dioxide and dissolved oxygen significantly alter the toxicity of ferulic acid in each month of year 2011-2012. Phytotherapy of infected snails by ferulic acid is one of the new approaches to control the fasciolosis.

Acknowledgement

One of the authors (Kumari Sunita) is thankful to Rajiv Gandhi National Fellowship (RGNF), University Grants Commission and New Delhi for financial assistance.

References

Agarwal, R.A., Singh, D.K., 1988. Harmful Gastropods and their control. Acta Hydrochim Hydrobiol., 16, 113-138.

Akhar, M.S., Zafar Iqbal khan, M.N., Lateef, M., 2000. Anthelmintic activity of medicinal plants with particular refence to their use in indo Pakistan sub continent., 38, 99-107.

Anonymus., 1995. Control of Foodborne Trematode Infections. WHO Technical Series No. 849. WHO, Geneva, 157 pp.

APHP., 2005. (American Public Health Association) standard methods for the examination of water and waste water. 21st edn. Washington, DC, APHA

Bakker, W., Hartmans, K.J., Diepenhorst, P., Gorris, L.G. M., 1995. The use of Carvone in agriculture: Suppression of potatoes and antifungal activity against potato tuber and other plant diseases. J. Indust. Crops prod., 4 (1), 3-13.

Balasubashini, M.S., Rukkumani, R., Viswanthan, P., Menon, V.P., 2004. Ferulic acid alleviates lipid peroxidation in diabetic rats. Phytotherp Res., 18, 310-314.

Fatehi, M., Farifteh, F., Hassanabad, Z.F., 2004. Antispasmodic and hypotensive effect Ferula asafoetida gum extract. J. Ethnopharmacol., 91, 321-32.

Greek, S., Dorny, P.P., 1995. Anthelmintic resistance in helminthes of animals of man in the tropics. Bulletin- des-Sciences Dutra- Mer, 3, 401-23.

Hirotaka, F., Shuhei, Yumiko I., Hiroshi, M., Toyokazw, Y., Taru, N., 2003. Ferulic acid production from clove oil by pseudomonas fluoresens E118. J. Biosci. Bioeng., 96, 404- 405.

Kumar, P., Singh, D.K., 2006. Molluscicidal activity of Ferula asafoetida, Syzygium aromaticum and Carum carvi and their active components against the snail Lymnaea acuminata. Chemosphere., 63, 1568-1574.

Kumar, P., Singh, V. K., Singh, D. K., 2012. Attractant Food Pellets Containing Molluscicides against the Fresh Water Snail Indoplanorbis exustus. Gobal Veterinaria., 8(2), 105-110.

Kumar, P., Singh, V.K., Singh, D.K., 2009. Kinetics of enzyme inhibition by active molluscicidal against ferulic acid, umbelliferone, eugenol and limonene in the nervous tissue of snail Lymnaea acuminata. Phytotherapy Research., 23, 172-177.

Kumar, P., Singh, V.K., Singh, D.K., 2011. Combination of molluscicides with attractant carbohydrates and amino acid in bait formulation against the Snail Lymnaea acuminata. European Review for Medical and Pharmacological Science., 15, 550-555.

Mas-Coma, S., Bargues, M.D., Valero, M.A., 2005. Fascioliasis and other plant borne trematode Zoonoses. Inter. J. Parasitol., 35, 1255-1278.

Mas-Coma, S., Valero, MA., Bargues, M.D., 2009. Climatic change effects on trematodiases, with emphasis on zoonotic fascioliasis and schistosomiosis. Vet. Parasitol., 163, 264-280.

Parr, S.L., Gray, J.S., 2000. A strategic dosing scheme for the control of fasciolosis in cattle and sheep in Ireland. Vet. Parasitol., 88 (3-4), 187-197.

Poolman, B., Oosterhaven, K., Smid, E.J., 1995. S-Carvone as a natural potato sprout inhibiting, fungistatic and bacteristatic compound. Indust. Crop Prod., 4, 23-31.

Robertson, J.L., Russell, R.M., Preciter, H.K., Savin, N.E., 2007. Bioassay with arthropods data, 2nd Eds Taylar and Francis, CRC press 1-224.

Saija, A., Tomaino, A., Trombetta, D., De, Pasquale, A., Uccella, N., Barbuzzi, T., Padino, D., Boonina, F., 2000. In votro and vivo evaluation of coffeic and ferulic acid as topical phtprotective agents. Int.J Pharm., 199, 39-47.

Shiyi, O.U., Kin-Chor, K., 2004. Ferulic acid: pharmaceutical functions, preparation and applications in foods. J. Sci Food. Agric., 84(11), 1261-1269.

Singh, S., Singh, V.K., Singh, D.K., 1997. Molluscicidal activity of some common spice plants. BAH., 14, 237- 249.

Sokal, R.R., Rohlf, F.J., 1996. Introduction of biostatistics, W.H. Freeman, San Francisco, Co, USA.

Srivastava, K.C., Mustafa, T., 1993. Pharmacological effect of spices Eicosnoid modulating activities and their significance in human health. J. Biochem. Revi., 2, 15-29.

Srivatava, A.K., Singh, D.K., Singh, V.K., 2013. Abiotic factors and anti-reproductive action of bait containing eugenol against Lymnaea acuminata. Scientific J. Biol. Sci., 2(4), 76-85.

Sunita, K., Singh, D.K., 2011. Fascioliasis control: In vivo and in vitro phytotherapy of vector snail to kill Fasciola larva. J. Parasitol. Res., 2011, 1-7.

Waterwatch Australia., 2002. National Technical Mammal. Module 4: Physical and Chemical parameters.

WHO., 2007. Report of the WHO informal Meeting on use of Triclabendazole in Fascioliosis control, WHO Headquarters, Geneva. Switzerland, 17-18 October. WHO/CDs/ NTD/PCT/ 2007.1. WHO, Geneva.