Phytotherapeutics: As anticipating substitutes to synthetic drugs in combating antinematicidal-resistant gastrointestinal nematodes of small ruminants

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ABSTRACT

The plant kingdom embraces a plethora of various phytomedicines which have been exploited for thousands of years by conventional pastoralists, herbalists and healers as a part of ethno-veterinary medicine against livestock nematodiasis. The role of medicinal plants has been diminished after the marketing of synthetic thiabendazole (first generation of benzimidazole group) in 1961. Virtually, the magnitude of herbal medicines has been substantiated again in recent decades due to the rapid emergence of antinematicidal resistance among the parasitic nematode populations as a result of discriminate use of the synthetic chemotherapeutics worldwide. Furthermore, other promoted alternatives viz. biological control, immunization, grazing management, nutritional supplements and genetic approaches have not been commercialized yet due to some practical bottlenecks. In this article, the significance of medicinal botanicals and the performed studies in the field of phytotherapy toward antinematicidal-resistant alimentary tract nematodes parasitized ovine and caprine have been reviewed elaborately.

1. INTRODUCTION

It has been narrated and postulated that various plants were utilized as therapeutic agents for the first time by the distinct primate ‘Neanderthal’ (*Homo neanderthalensis*) between 60,000-80,000 years BC in the Kurdistan Region of Iraq (Agelarakis, 1993; Sommer, 1999; Hamad, 2012). Records attribute the use of medicinal plants to prehistoric Mesopotamian Civilizations (land between the two rivers of Tigris and Euphrates), in particular the Sumerians who dwelt modern over 5,000 years ago (Levetin and McMahon, 2003). It is noteworthy to mention that exploitation of herbal remedies was practiced by Chinese herbalists (Ergil et al., 2002) and Indian botanists (Aggarwal et al., 2007) around 2700 BC and 1900 BC, respectively. Ancient Greeks and Romans as well as Muslim scientists have also contributed to the use of phytomedicines (Iqbal et al., 2001). The recent decades have witnessed a distinct development in herbology by employing modern pharmacological techniques.
in the extraction of crude materials and active constituents of several plants (Niezen et al., 1998). For this purpose, the adulticidal, larvicidal and ovicidal efficacy of different herbal medicines have been validated and documented by investigators in Asia, Africa and Latin America to fight gastrointestinal nematodes of small ruminants (Iqbal et al., 2001; Githiori, 2004; Carvalho et al., 2011; Hamad, 2012). Evidently, justifications behind the use of botanical medicines are attributed to rapid development of antinematicidal resistance among alimentary tract nematodes against renowned synthetic dewormers around the world (Kaplan, 2004; Jabbar et al., 2006). Moreover, the presence of some drawbacks and side effects including environmental pollution, toxicity and residual impacts have reckoned other reasons to employ botanical dewormers in place of allopathic drugs (Waller et al., 2001). Researchers in the field of phytotherapy have accomplished significant levels of success. However, more sophisticated phytopharmacological, phytotoxicological and biochemical assays are needed for the commercialization of herbal remedies (Hammond et al., 1997).

2. Antinematicidal magnitude of medicinal plants

Phytotherapy is a traditional medicinal practice relying on the utilization of ethnobotanicals and their extracts to treat various human and animal diseases. Conventionally, thousands of indigenous plants are being used by herbalists and livestock raisers in developing countries to cure infections with gastrointestinal (GI) nematodes in small ruminants (Akhtar et al., 2000; Waller et al., 2001). On the other hand, scientific validations and documentations of indigenous medicinal plants against GI nematodes have witnessed a considerable progress, particularly in the southern hemisphere (Waller, 2006). When compared to orthodox medicinal practices, botanical medicines possess some unique properties viz. low toxicity, less residual effects, very slow evolution of resistance as well as being environmentally friendly (Waller et al., 2001). Moreover, the plant kingdom is rich with phytomedicines in addition to their relatively low costs, accessibility and acceptability by farmers mainly in developing countries. Routinely, different parts of the plants are being used including the whole plant, leaves, barks, fruit, root, rhizomes, vinegar, aerial parts, cake, bulb, pulp, latex, wood, stigmas, stem, oil, wine, gum, shoots, buds, seeds, flowers, inflorescence…etc (Iwu, 1993; Van Wyk et al., 1997; Akhtar et al., 2000; Hamad et al., 2012; Chagas, 2015 ). It is noteworthy to mention that medicinal botanicals contain various active phytocconstituents such as phenols, flavonoids, tannins, alkaloids (approximately present in 158 botanical families), terpenoids, glycosides, exudates…etc. (Cowan, 1999; Chagas, 2015). Regarding the current problem which is associated with the development of antinematicidal resistance (AR) among GI nematodes in a wide range of countries, herbal medicines could be the apposite substitute to overcoming this dilemma. Expectantly, these phytochemicals will occupy their places in the inventory of effective antinematicidals in the near future (Jabbar et al., 2006).

3. Development of resistance against synthetic drugs

Resistance among parasitic nematodes has emerged against the renowned broad-spectrum anthelmintics which include benzimidazoles/pro-benzimidazole, tetrahydropyrimidines and macrocyclic lactones (avermectin/milbemycins) in almost every country especially in areas where small
ruminants are intensively being raised (Kaplan, 2004; Beech and Silvestre, 2010). There are some reports about field detection of AR against monepantel (a recent dewormer produced by Novartis Animal Health INC. under the commercial name Zolfix which marketed in 2009) in New Zealand (Scott et al., 2013) and Uruguay (América et al., 2014). In this regard, investigators in the field of parasitology have laid blame on some factors that contribute to the escalation of AR. These enhancing factors may include the indiscriminate use of drugs, recurrent deworming, sub-standard dosing (prophylactic doses), mono-drug strategy and poor quality drugs in developing countries (Dorny et al., 1994; Hoekstra et al., 1997). Dissemination of AR against commonly used dewormers has been presented in table 1.

4. Rampancy of resistance (selected countries)

AR has been evidently rooted in almost every country in the world. Undoubtedly, this problem is most serious in areas where small ruminants are being reared intensively such as Australia, Uruguay, South Africa, New Zealand and Pakistan. The dissemination of AR in some countries is shown in table 2.

5. Phytopharmacology and Phytotoxicology of medicinal plants

Several phytopharmacological and phytotoxicological considerations should be elaborated regarding the utilization of ethnobotanical extracts for treatment of parasitic and non-parasitic ailments. While ethical medicinal products (pharmaceuticals) have been widely investigated pharmacologically and toxicologically by commercial companies, the studies on medicinal plants are still rare (Couatre, 2004), particularly in the field of veterinary medicine. Nevertheless, documentation and validation of home-grown phytomedicines (as a substitute to orthodox drugs) against GI nematodes of small ruminants are being executed extensively by South Latin American, Asian and African researchers, yet their trials mostly encompass clinical studies without comprehensive phytochemical investigations (Chagas et al., 2014).

In order to be more reliable and safer for clinical pharmacology use, phytotherapeutic substances should undergo pharmacokinetic and pharmacodynamic studies. In this regard, in vitro and in vivo assays to assess antinematicidal (adulticidal) activity of trialed plant extracts should be performed properly (Hamad, 2012). Moreover, herbal extracts or their active ingredients should be evaluated for their potency to prevent egg hatching and larval development (in vitro techniques) of nematodes (Max, 2010; Tayo et al., 2014). It’s worth mentioning that medicinal plants can impair egg hatching and not egg embryonation (Hamad, 2012). Pragmatically, likely positive impacts of plant extracts take place such as reduction of parasite burdens. This, in turn, leads to less egg contamination of pastures, minimizing the likelihood of re-infection with infective stages of nematodes (Max, 2010; Tayo et al., 2014). Needless to say, the adulticidal, larvicidal and ovicidal potency of a plant extract in vitro are not a reflection of its efficacy in vivo owing to some pharmacological considerations such as, ruminal pH, destruction of active constituents and biodegradation by rumen flora, bioavailability, absorption, metabolism and excretion (Pervez et al., 1994; Peneluc et al., 2009; Katiki et al., 2012).

In spite of the importance of botanical medicines to control antinematicidal-resistant nematodes, the toxicological aspects and safety concerns need not be ignored when any medicinal plant extract is prepared for animal treatment. In this regard, we should remember the words of Paracelsus, the father of modern toxicology, who said “All substances are toxic with the dose making the difference” (Borzelleca, 2000). Certainly, this statement...
goes through with the herbal medicine extracts because most of the in vivo herbological experiments have revealed that the majority of phytomedicines are dose-dependent in their toxic effects and antinematicidal efficacy especially when they are used as crude extracts (Hamad, 2012).

While no mortality cases were recorded, sheep exposed to 4 g kg⁻¹ (high dose) crude aqueous-methanol extract (CAME) of Nicotiana (N.) tabacum had showed clinical signs of central nervous system (CNS) intoxication including, restlessness, staggering gait, slight salivation, recurrent recumbency and slight nasal discharges, while those exposed to a low dose (2 g kg⁻¹) of the same plant has not conduced to develop any symptom of CNS intoxication. Statistically speaking, no significant difference (P > 0.05) was observed between both therapeutic doses (Hamad, 2012; Hamad et al., 2012; Hamad et al., 2014).

In this regard, further initial trials have been advocated such as, determining acute, sub-chronic and chronic toxicity in experimental laboratory animals (rats and mice). Moreover, teratogenic, carcinogenic, neurotoxic and mutagenic influences have been suggested to be investigated in targeted animals (Camurça-Vasconcelos et al., 2005).

6. Are phytomedicines inevitable alternatives?

The adoption and popularity of medicinal plants are increasing promptly among developing nations and even in highly industrialized countries particularly after the utilization of state-of-the-art techniques to conduct contemporary scientific investigations in the field of herbology. This technological revolution in the last few decades has enhanced sufficiently in recognizing a plethora of different phytochemicals and analyzing their active constituents to familiarize and convince people to use them in the domain of human and veterinary medicine (McGaw and Eloff, 2008). Habitually, poor pastoralists in most remote rural areas of developing countries have been adapted to deworm their livestock with herbal remedies as a result of either high expenses of allopathic antinematicids (western pharmaceuticals) or unavailability of these vermifuges in their territories (Mathias, 2004). Fortunately, these ethnoparasitic practices have conduced to a much reduced emergence of antinematicidal-resistant individuals among GI nematode populations which reflects an increase in the number of parasites in refugia (those that are not exposed to synthetic drugs such as larvae in pasture).

It is noteworthy to mention that the main reason for the slow development of AR in those remote rural areas may be due to the presence of a number of active constituents in one plant which, in turn, can act in various mechanisms (Athanasiadou et al., 2007). Most probably, the AR will develop rapidly as a consequence of the wide use of an active ingredient isolated from a given medicinal plant, particularly, if its antinematicidal potency has a single pharmacodynamic (Chagas, 2015). Moreover, the efficacy of some medicinal herbs as effective agents to control antinematicidal-resistant nematodes could be attributed to the occurrence of a sort of additive or synergistic action between active chemical compounds (working at single or several targets) within a particular plant and not between different medicinal plants. On the other hand, as pointed out by Tyler (1999), this synergistic or additive pharmacological impact can be useful by eliminating the problem of unfavorable side effects associated with the predominance of a sole xenobiotic chemical compound in the body. In this respect, the study of Hamad (2012) did not reveal any difference (P > 0.05) in the synergistic or additive effects of antinematicidal potency of some combined medicinal plants (details in the next subtitle).

In order to maintain drug susceptibility and minimize the percentage of resistance among antinematicidal-resistant GI nematodes, it will be valuable to add or replace 5-10% of a flock (harboring resistant nematode worms) with small ruminants from the rural areas where ethno-veterinary botanical medicine is
being practiced where access to synthetic antinematicidals is impossible (Sindhu et al., 2010). This process can dilute resistant roundworm populations and reduce the speed of AR evolution. However, this hypothesis is almost unfeasible in developing countries due to several bottlenecks and constraints associated with culture, education, costs and the likelihood of transmission of other non-parasitic infectious diseases.

On the other hand, the idea of “preserving in refugia nematodes” to sustain susceptible nematodes on a farm may be done by another way that has been suggested by some researchers. Basically, the policy depends on leaving 10% of the stock untreated leading to the production of susceptible eggs by unexposed worms to synthetic antinematicidals. This strategy, while difficult to be conducted, but using of FAMACHA Anaemia Guide Chart (developed by Faffa Malan in South Africa) can facilitate the selection of animals carrying the most parasitic burden for deworming purposes. In light of this chart, small ruminants in stage 3, 4 and 5 of anaemia should be treated and those in stage 1 and 2 will be left untreated because the parasitic load is low (Macedo et al., 2010). Unfortunately, some of the drawbacks of FAMACHA Chart include its applicability for haematophagous nematodes such as Haemonchus contortus (also for trematodes like liver fluke) and the fact that it may not be available everywhere especially in developing countries.

Nevertheless, for non-blood sucking GI nematodes, the five- point check (an extension of FAMACHA system), which has been developed in South Africa as well, could be applied for determining the deworming strategy to retard AR. Another policy that has been suggested by some researchers is associated with the use of combined synthetic drugs and phytomedicines (Dupuy et al., 2003). This recipe, however, has not been tried yet (Cala et al., 2014).

AR become a phenomenon on all the continents especially in areas where tamed small ruminants are being raised intensively, and therefore, any attempt to avoid development and prevalence of resistance among GI nematodes is too late (Waller, 1997). Hence, the utilization of ethnobotanicals to overcome the problem of resistance dissemination among antinematicidal-resistant GI nematodes might be an anticipated substitute in the near future.

Comprehensive studies on the effectiveness of medicinal plants to control the prevalence of antinematicidal-resistant GI nematodes of livestock are very rare (Hamad, 2012). The reasons behind the ignorance of employing ethnobotanicals may be owing to the focus on other strategies (non-chemical methods) such as, biological control, grazing management, genetic approaches, nutritional supplementation, and immunization particularly in the northern hemisphere. Unfortunately, these strategies have achieved a limited degree of success and they are still far from the ambitions of livestock raisers. On the other hand, while the popularity of medicinal plants is increasing in human medicine, especially in Germany (about 67000 medicinal plant extracts and products are being used) (Foster, 2009).

It is noteworthy to mention that antinematicidal-resistant pathogenic nematodes are more prolific, more pathogenic, have enhanced settlement rates in the host, and have raised longevity of the free-living stages in pasture (Kelly et al., 1977). It has also been reported that eggs of resistant strains will embryonate and hatch in higher concentrations of antinematicidals than those of susceptible parasites (Le Jambre, 1976).

Thus, these defence properties of resistant pathogenic helminths have emboldened investigators in the field of nematology to adopt the aforesaid alternative strategies. Until now however, unfavorable results have been obtained in field. Additionally, some of these policies including vaccination, biological control using nematophagous fungi, and genetic approaches remain under investigation (Stear et al., 2007).

In contrast, under the umbrella of non-chemotherapeutic approaches, phytotherapy is
presently a motivating area of research anticipated to be a promising reliable alternative to restrict rampanty of ecto-endo parasites in the near future. Researchers, especially in the Indo-Pakistan subcontinent and in other Asian, African and South Latin American countries, have conducted several studies on the potency and validation of indigenous medicinal plants to mitigate the parasitic burden (Akhtar et al., 2000; Waller et al., 2001; Carvalho et al., 2011). Obviously, their trials were done randomly without differentiation between antinematicidal-resistant and susceptible parasites.

The study executed by Hamad (2012) is perhaps the first attempt to utilize extracts of some native medicinal plants in Pakistan against antinematicidal-resistant Haemonchus contortus in sheep. His study has revealed the efficacy of CAMEs of N. tabacum leaves, Azadirachta (A.) indica seed kernels and combined N. tabacum leaves and A. indica seed kernels through performing the fecal egg count reduction test (FECRT), egg hatch assay (EHA), and adult motility test (AMT).

Concerning the mean fecal egg count reduction percentage [FECR (%)], the results exhibited 88.6, 85.14, 94.59 for N. tabacum leaves, A. indica seed kernels and combined N. tabacum leaves and A. indica seed kernels, respectively. In this regard, we have to point out the recommendations of W.A.A.V.P (second edition) edited by Wood et al. (1995) that suggest that any dewormer with FECR% (98) is deemed highly effective; FECR% (80) and above is effective; whilst FECR% less than (80) is not commended for use. So based on the study carried out by Hamad (2012), Hamad et al. (2012) and W.A.A.V.P recommendations, the aforementioned phytomedicines are effective against antinematicidal-resistant nematodes.

Moreover, the in vitro assay (EHA) was also an indicator for potency of the aforesaid medicinal botanicals for their ovicidal efficacy through the calculation of LC50 values and 95% fiducial confidence interval (Lower-Upper). The ranks of the tested plants in this study were determined as shown in table 3. Regarding the other in vitro assay (AMT), the adulticidal activity of the abovementioned herbs was determined depending on the comparison between the three factors of time, concentration, mortality where LC50 values were calculated as well. The analysis is demonstrated in table 3.

It should be mentioned that the effectiveness of CAMEs of N. tabacum leaves, A. indica seed kernels and combined N. tabacum leaves and A. indica seed kernels on antinematicidal-resistant H. contortus through utilizing FECRT, EHA and AMT was significant. These botanicals contain detrimental chemicals against adult worms and their ova. Furthermore, the study also revealed that the trialed medicinal plants had prevented the hatching of eggs, but not their embryonation. As a result, further studies are required to elucidate this mechanism particularly the comparison between eggs recovered from susceptible and resistant nematodes to renowned antinematicidals.

The presence of a synergistic combination between natural phytotherapeutics to control antinematicidal-resistant GI nematodes has not been proven. In fact, studies on such topic are seldom carried out by investigators in the world. On the other hand, most reports concerning the haphazard use of mixed herbs against various human ailments have been emanated from the traditional Indian and Chinese ethno-medicine.

Therefore, ancient practitioners of this type of medicine claim that the medicinal plants have synergic effects (Sabu and Kuttan, 2002; Li, 2009). Moreover, the study of Javed and Akhtar (1990) revealed that methanol extract of combined Vernonia (V.) anthelmintica and Embelia (E.) ribes had reduced FECR% to (93) when administered to goats infested with GI nematodes. It should be explained that they didn’t assess each plant extract individually to determine its sole antinematicidal activity, thus, the impact of this combined extract is back chiefly to E. ribes and not to synergism with V. anthelmintica because the later plant
has a low antinematicidal potency (Iqbal et al., 2006) as confirmed in the present study (Hamad, 2012). It could be mentioned that there is an absence of any type of synergy, additive and antagonism impacts between N. tabacum and A. indica when the pharmacological determinants for interaction of drugs are applied (Katzung, 2007). At the same time, the hypothesis of unavailability of any kind of drug interaction between trialed combined plants, which was reported by the author, may not be 100% accurate because such assumption requires a comprehensive study to be conducted on a vast number of medicinal plants. In this regard, we should remember that the kingdom of plant has a big diversity and each plant contains many various active ingredients, so presence of interaction between them could be expected.

7. Conclusions

AR is almost prevalent in every country particularly where sheep and goats are reared intensively. Resistance has developed by GI nematodes against the renowned broad-spectrum anthelmintics, especially among the benzimidazole/pro-benzimidazole group. Furthermore, AR is less common in remote rural areas where ethno-veterinary medicine is practiced through the use of ethnobotanical dewormers and access to allopathic antinematicidals is limited. It is noteworthy to mention that efforts to prevent rampancy of AR are ineffective because it has been rooted on all continents. It could be said that non-chemical alternatives such as, biological control, vaccination, genetic approaches, nutritional supplementation, and grazing management have not achieved a considerable result in the field. Alternatively, as a result of the futility of the aforementioned substitutes, phytomedicines are the suitable choice to control antinematicidal-resistant nematodes in small ruminants. In light of the studies that have been executed in different regions of the world, particularly in Asia, South Latin America and Africa, further phytopharmacological and phytotoxicological assessments are required when a given medicinal plant extract is trialed for its secondary impacts on the host and constructive influence on the parasite. Moreover, synergism between various active constituents within the same plant extract could be available and this, in turn, restricts the ability of parasitic nematodes to evolve resistance against herbal medicines. On the other hand, presence of synergy among ethnobotanicals remains controversial and, hence, more in vitro and in vivo studies are needed. Undoubtedly, trials for the evaluation of phytotherapeutical agents against antinematicidal-resistant nematodes are very rare; therefore, studies in this field should be elaborated by veterinary nematologists. Ultimately, more studies must be conducted in the field of pharmacognosy for this purpose.

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Conflict of interest statement

The authors of this review article attest no conflicts of interest regarding the information incorporated in this manuscript.
Tables (n=3) in 3 pages

Table 1 Prevalence of antinematicidal resistance against different dewormers among gastrointestinal nematodes in small ruminants

<table>
<thead>
<tr>
<th>1- Benzimidazoles</th>
<th>2- Imidazothiazoles</th>
</tr>
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<tbody>
<tr>
<td>Very common</td>
<td>Common</td>
</tr>
<tr>
<td>Less common</td>
<td></td>
</tr>
<tr>
<td>3- Tetrahydropyrimidines (Most members)</td>
<td>(Levamisole HCL) (Morantel and Pyrantel)</td>
</tr>
<tr>
<td>4- Macroyclic Lactones</td>
<td>5- Salicylanilides</td>
</tr>
<tr>
<td>6- Amino acetonitrile derivatives (Ivermectin &amp; Moxidectin)</td>
<td>(Closantel) (Monepantel)</td>
</tr>
<tr>
<td>Common</td>
<td>Common</td>
</tr>
<tr>
<td>Perhaps not yet in field!!</td>
<td></td>
</tr>
</tbody>
</table>

Brazil          BZ<sub>s</sub>, LEV, ML
Echevarria et al., 1996
Bulgaria         BZ<sub>s</sub>
Iliev et al., 2014
Denmark          BZ<sub>s</sub>
Maingi et al., 1996
France           BZ<sub>s</sub>, LEV
Kerboeuf et al., 1988 ;
Palcy et al., 2010
Germany          BZ<sub>s</sub>, LEV
Bauer, 2001; Duwel et al., 1987
Greece           BZ<sub>s</sub>
Papadopoulos et al., 2003;
Gallidis et al., 2011
India            BZ<sub>s</sub>, Morantel,
Closantel        Uppal et al.,
1992 ;
Yadav et al., 1993
Manikkavasagan et al., 2015
Italy            LEV, ML
Traversa et al., 2007
Kenya            BZ<sub>s</sub>, ML, Closantel
Mwamachi et al., 1995
Malaysia         BZ<sub>s</sub>, LEV, ML,
Closantel        Dorny et al.,
1994 ;
Sivaraj et al., 1994

Table 2 Geographical distribution of antinematicidal resistance among gastrointestinal nematodes of sheep in different countries (selected references)

<table>
<thead>
<tr>
<th>Country</th>
<th>Antinematicidal drug</th>
</tr>
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<tbody>
<tr>
<td>Australia</td>
<td>BZ&lt;sub&gt;s&lt;/sub&gt;, LEV, Morantel, Closantel Waller et al., 1995</td>
</tr>
<tr>
<td>Brazil</td>
<td>BZ&lt;sub&gt;s&lt;/sub&gt;, LEV, ML</td>
</tr>
<tr>
<td>Echevarria et al., 1996</td>
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<tr>
<td>Bulgaria</td>
<td>BZ&lt;sub&gt;s&lt;/sub&gt;</td>
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<tr>
<td>Iliev et al., 2014</td>
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<tr>
<td>Denmark</td>
<td>BZ&lt;sub&gt;s&lt;/sub&gt;</td>
</tr>
<tr>
<td>Maingi et al., 1996</td>
<td></td>
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<tr>
<td>France</td>
<td>BZ&lt;sub&gt;s&lt;/sub&gt;, LEV</td>
</tr>
<tr>
<td>Kerboeuf et al., 1988 ; Palcy et al., 2010</td>
<td></td>
</tr>
<tr>
<td>Germany</td>
<td>BZ&lt;sub&gt;s&lt;/sub&gt;, LEV</td>
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<tr>
<td>Bauer, 2001; Duwel et al., 1987</td>
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<tr>
<td>Greece</td>
<td>BZ&lt;sub&gt;s&lt;/sub&gt;</td>
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<tr>
<td>Papadopoulos et al., 2003; Gallidis et al., 2011</td>
<td></td>
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<tr>
<td>India</td>
<td>BZ&lt;sub&gt;s&lt;/sub&gt;, Morantel, Closantel Uppal et al., 1992 ; Yadav et al., 1993</td>
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<tr>
<td>Manikkavasagan et al., 2015</td>
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<tr>
<td>Italy</td>
<td>LEV, ML</td>
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<td>Traversa et al., 2007</td>
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<td>Kenya</td>
<td>BZ&lt;sub&gt;s&lt;/sub&gt;, ML, Closantel</td>
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<td>Mwamachi et al., 1995</td>
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<td>Malaysia</td>
<td>BZ&lt;sub&gt;s&lt;/sub&gt;, LEV, ML, Closantel Dorny et al., 1994 ; Sivaraj et al., 1994</td>
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</tbody>
</table>
Table 3 Assessment of antinematicidal activity of *Nicotiana tabacum* and *Azadirachta indica* extracts and their combination utilizing fecal egg count reduction ([FECR (%)] test, egg hatch assay (EHA) and adult motility test (AMT))

<table>
<thead>
<tr>
<th>Plant’s name</th>
<th>FECR (%)</th>
<th>EHA AMT (LC_{50} values)</th>
<th>Plant’s rank (LC_{50} values)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>N. tabacum</em></td>
<td>88.6</td>
<td>(0.566)*</td>
<td>2 (0.496-0.642)**</td>
</tr>
<tr>
<td></td>
<td>(8.24)*</td>
<td>(5.09-14.55)**</td>
<td></td>
</tr>
<tr>
<td><em>A. indica</em></td>
<td>85.14</td>
<td>(1.169)*</td>
<td>3 (1.047-1.303)**</td>
</tr>
<tr>
<td></td>
<td>(15.40)*</td>
<td>(9.99-26.05)**</td>
<td></td>
</tr>
<tr>
<td><em>N. tabacum</em> &amp; <em>A. indica</em></td>
<td>94.59</td>
<td>(0.523)*</td>
<td>1 (0.465-0.586)**</td>
</tr>
<tr>
<td>(Combined)</td>
<td>(5.39)*</td>
<td>(3.46-8.81)**</td>
<td></td>
</tr>
</tbody>
</table>

*= denotes LC_{50} value of each plant extract  
**= denotes 95% fiducial confidence interval (Lower-Lower)  
*N.: Nicotiana*  
*A.: Azadirachta*
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