



A Field Study to Evaluate the Efficacy of Medicinal Plants, Artemisia Herba Alba, Coriandrum sativum, Allium sativum, and Their Combination on Internal Parasites in Horses in The Western Region of Libya

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دراسة ميدانية لتقييم فعالية النباتات الطبية الشيح الأبيض والكزبرة والثوم ومزيجها في مكافحة الطفيليات الداخلية في الخيول في المنطقة الغربية من ليبيا

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Abstract

The experimental work was conducted between March and April 2023 at the research laboratory of the Faculty of Veterinary Medicine and Agriculture, University of Zawia. Fifty fecal samples were collected from horses in the western area of Libya and sent to the lab. The medical plants used in this experiment were Coriandrum sativum, Artemisia herba alba, and Allium sativum. The EPG values (Mean \pm SD) pre-treatment of the control group was 585 ± 187.48 on day 0, and the highest EPG value was 1020 ± 140.17 , on day 21, while the EPG values (Mean \pm SD) in the post-treatment groups on day 21 were 21 ± 10.14 , 30 ± 14.25 , 180 ± 56.35 , and 342 ± 142.22 respectively, which was higher than the pre-treatment group 1020 ± 140.17 at day 21. The efficacy of three medical plants was significantly higher ($p < 0.05$) than Coriandrum sativum or Allium sativum alone. The EPG values (Mean \pm SD) in horses of group 2 (T2) on days 7, 14, and 21, were 210 ± 30.85 , 53 ± 23.12 , and 21 ± 10.14 respectively. The highest EPG values (Mean \pm SD) were in group 5 (T5) which was treated with Allium sativum on all day of the experiment, and the lowest EPG values (Mean \pm SD) were in group 2 (T2) which was treated with the mixture of plants powder Coriandrum sativum 20gm, Artemisia herba alba 20gm, and Allium sativum 20gm by 1:1:1, on all day of the experiment.

Keywords: Horses, intestinal parasites, helminths, protozoa, efficacy.

الملخص

تم إجراء العمل التجريبي خلال الفترة من مارس إلى أبريل 2023 في مختبر الأبحاث بكلية الطب البيطري والزراعة بجامعة الزاوية. تم جمع خمسين عينة براز من الخيول في المنطقة الغربية من ليبيا وإرسالها إلى المختبر. النباتات الطبية المستخدمة في هذه التجربة هي الكزبرة والشيح والثوم. بلغت قيم (EPG) المتوسط \pm الانحراف المعياري قبل المعالجة لمجموعة التحكم 187.48 ± 585 في اليوم (0)، وكانت أعلى قيمة 140.17 ± 1020 في اليوم 21، بينما بلغت قيم (EPG) المتوسط \pm الانحراف المعياري في مجموعات ما بعد المعالجة في اليوم (21) 10.14 ± 21 ، 14.25 ± 30 ، 56.35 ± 180 ، و 142.22 ± 342 على التوالي، وهي أعلى من مجموعة ما قبل المعالجة 140.17 ± 1020 في اليوم (21). كانت فعالية ثلاث نباتات طبية أعلى بشكل ملحوظ عند ($p < 0.05$) التي كانت اعلي من الكزبرة والثوم لوحدهما. كانت قيم (EPG) \pm الانحراف المعياري في خيول المجموعة 2 (T2) في الأيام 7 و 14 و 21، 30.85 ± 210 و 23.12 ± 53 و 10.14 ± 21 على التوالي. كانت اقل قيم (EPG) المتوسط \pm الانحراف المعياري في المجموعة 5 (T5) التي عولجت بالثوم طوال أيام التجربة، وكانت أعلى قيم (EPG) المتوسط \pm الانحراف المعياري في المجموعة 2 (T2) التي عولجت بمزيج من مسحوق النباتات (الكزبرة 20 جرام والشيح 20 جرام والثوم 20 جرام) بنسبة 1:1:1 طوال فترة التجربة.

الكلمات المفتاحية: الخيول، طفيليات الامعاء، ديدان، الاوآلى، كفاءة.

Introduction

Horses are classified as members of the equine group. They are herd animals and contentedly coexist in groups with other animals of different types, including sheep and goats. They are primarily found in temperate, semi-arid, or highland regions. Horses are amiable creatures that love being around people. Their significance has not changed in recent years in many areas of Eastern Europe, Africa, South America, and Asia [1]. Over 45,000 horses were in Libya in 2016, according to estimates from the country's Ministry of Agriculture [2]. Horse gastrointestinal parasites have been the subject of several investigations in many countries, including Libya [3], Western Australia [4], and Turkey [5].

Numerous intestinal parasite species attack horses' digestive systems. These parasites cause serious clinical disorders, which can occasionally be deadly, decreased performance, and physical disease [6]. Internal parasites cause emaciation, weakness, loss of activity and vitality, dry skin, and loss of hair luster and shine. Horses are always exposed to various types of intestinal parasites. The most important source of this infection is green fodder, despite its importance because it is impossible to wash or clean it before offering it to the horse to eat. It is often a carrier of Endoparasitic eggs, which explains why horses are infected with intestinal helminths at various times of the year. Temperature and moisture determine the growth and survival of helminthic larval eggs with feces on pasture, creating an ideal habitat for nematode and Trematoda larvae to reach an infected stage [7].

The problem that has a long-term impact on animals' health is internal horse parasites. Veterinarians, horse breeders, and horse owners commonly fail to recognize endoparasitic infections since they are often asymptomatic [8].

The ascarid *Parascaris equorum*, the pinworm *Oxyuris equi*, the lungworm *Dictyocaulus arnfieldi*, tapeworms (*Anoplocephalidae*), and small and big strongyles are the most prevalent helminths that infest horses. The parasites that are most frequently cited as the main contributors to parasitic illnesses in horses are strongyles. Even though there are more antiparasitic medications available, deworming is frequently done poorly, making it useless [9]. The growing number of national and international studies showing anthelmintic resistance as well as the anticipated future resistance of horse parasites to other classes of anthelmintics have recently raised concerns. Frequent use of the same or similar pharmacological treatments, such as ivermectin and praziquantel, which are most commonly used, causes many parasites, particularly gastrointestinal ones, to acquire partial or total resistance that these medications can no longer remove [10,11].

Representatives of the Ascarididae, Strongylinae, Cyathostominae, Anoplocephalids, and botflies from *Gasterophilus* species are among the parasites that have developed or have been progressively developing resistance to the preparations now in use [12,13]. There are contradictory suggestions about the use of anthelmintic regimens in equestrian facilities to either prevent the development of resistance or address an existing resistance issue. In some studies conducted on sheep in Libya, parasites showed progressive immunity to some types of anthelmintics and not others [14]. Also, the difference between the breeds of horses, such as Thoroughbreds and Arabian horses, affects their resistance to parasites. This applies to most animals, and in some studies, the difference in the breeds of animals or birds

affects the hematological and biochemical blood characteristics [15]. There is a long history of using different herbs and therapeutic plants. Since ancient times, they have been in use, particularly in eastern nations. The use of medicinal plants, plant parts, or compounds produced from plants to help fight against infections, and illnesses, or improve general health is known as herbal medicine, or “phytomedicine” [16]. According to published research, the best vegetable raw materials for making the aforementioned additions are garlic, oregano, black cumin, and plants of the genus Basil, as well as combinations of these plants [17,18].

The plant species on the list exhibit distinct anti-nematode and anti-parasitic properties for both humans and animals [19,20]. Adaptogens, immunostimulants, or both can be used to describe herbal supplements that have an impact on the immune system. Immunostimulants trigger the nonspecific or innate defensive systems against bacterial, viral, or cellular infections, whereas adaptogens boost tolerance to physical, chemical, or biological stresses. In addition, some nutrients used as supplements can cause changes in blood levels of protein and cholesterol [21]. This study will focus on evaluating the efficacy of medicinal plants such as *Artemisia herba alba*, *Coriandrum sativum* coriander, and *Allium sativum*, and their combination against internal parasites in horses in the western region of Libya.

Materials and methods

Study area

The study was conducted in the districts, of Zawia, West Zawia, and Surman, State of Libya. The region is located 50 to 60 km west of Tripoli capital of Libya. The district comprises many farms. The horse populations are estimated to be around 5 to 10 heads per farm. The animals' housings are built near the farmers' homes. The horses in these farms are usually fed on both concentrated ration and free pasture during the year. The horses graze on the same pasture and rotational grazing is not followed by the farmers which increases the probability of pasture contamination by Gastrointestinal nematode eggs.

Sources of samples

Ten farms were included in the study after the owner visited the veterinary service centers. The samples were obtained from horses raised on farms in Zawia, West Zawia, and Surman cities. A total of 150 cases of horses are diagnosed as heavily infected with an internal parasite. The horses were examined for the presence of Helminths from Mars to the end of April 2023, as the climatic conditions, such as temperature, rainfall, and humidity, were suitable for the survival of nematode larvae at that time.

Ethical approval

The present experimental work was carried out at the Research Laboratory belonging to the Faculty of Veterinary Medicine and Agriculture Science (Al-Ajeelat), University of Zawia, between March to April 2023.

Collection of fecal samples

The horse stables were visited four times in two weeks for the collection of fecal specimens. The first sampling was just before drug administration while the second fecal collection was 7 days after therapy, the third fecal collection was 14 days after therapy, and the last fecal collection was 21 days after drug administration. A total of 150 fecal samples were collected. About 10 grams of fresh fecal samples were collected directly from the rectum using disposable polythene gloves, kept in plastic sachets. Then, they were cooled down to the temperature of 4 °C for the time of transport and then transported to the laboratory for examination in the Research Laboratory belonging to the Faculty of Veterinary Medicine and Agriculture (Al-Ajeelat), University of Zawia.

Analysis of fecal samples

The collected fecal specimens were put in cooled containers during transportation to the laboratory, which usually took less than two hours. The samples were then kept refrigerated at 5°C until the egg count was performed. The fecal samples were analyzed by the Direct Smear Method and Salt Flotation Technique for the presence of eggs of helminths. The specimens were examined with a Leica DM3000 microscope and imaging system. The (EPG) was conducted in a period that did not exceed five days after sample collection. Eggs per gram (EPG) were counted by using Mac-Master Technique [22]. The examination and identification of helminthic ova were carried out by using a key as described by Soulsby (1982).

Chemotherapy trials

Table 1 presents the medical plant used against GIT helminths in horses in the Libyans field.

Table 1: The medical plant used against GIT helminths in horses in the Libyans field.

Medical plant name	Administration form	Dose(gm)	Route
Coriandrum sativum	Powder in feed	20	Mix with feed
Artemisia herba alba	Powder in feed	20	Mix with feed
Allium sativum	Powder in feed	20	Mix with feed
*Mix	Powder in feed	20	Mix with feed

*Mix (mixture of plants powder Coriandrum sativum 20gm, Artemisia herba alba 20gm, and Allium sativum 20gm by 1:1:1).

50 animals positive for GIT helminths were chosen from the 150 and were randomly divided into five groups T1, T2, T3, T4, and T5 each comprising 10 animals. The animals in each group were marked using suitable identification methods. The first group (T1) served as the untreated control, while the members of group 2 (T2) were given a mixture of plant powder Coriandrum sativum 20gm, Artemisia herba alba 20gm, and Allium sativum 20gm by 1:1:1 then mixed feed with 20gm of this mixture on day 0 after collecting the fecal samples, after 7 days samples were collected for the second time, and animals were given the same mixture (20gm in feed), and in day 14 samples were collected for the third time then, 20gm of the same mixture were administrated in feed for horses, then in day 21 samples were collected for last time, and no drug administrated.

The members of group 3 (T3) were given powder of Coriandrum sativum 20gm in feed, only for all days of the experiment day 0, day 7, and day 14. Animals in group 4 (T4) were treated with powder of Artemisia herba alba 20gm in feed, only for all days same as in group 3. The animals in group 5 (T5) were given powder of Allium sativum 20gm in feed, only for all days same as in group 3 and group 4. Egg per gram (EPG) of the horses in all groups were counted at Day 0 (Pre-treatment) and Days 7, 14, and 21, (post-treatment) using the Mac-Master technique.

$[(\text{Pretreatment EPG} - \text{Post-treatment EPG}) / \text{Pretreatment EPG}] \times 100$ This formula calculated the efficacy of drugs [23].

Side effects

No side effects were observed during the trial in the present study.

Statistical analysis

The collected data were analyzed at different angles by calculating the percentage positivity of helminths. Whereas data on drug efficacy was analyzed by using one-way ANOVA by using SPSS software for Windows (version 27; IBM, USA).

Results and discussion

Infection Rate

The results of the present study revealed an overall infection rate of GIT helminths in horses of 65%.

The Infection rate of different gastrointestinal helminths in horses is shown in Table 2.

The highest infection rate of helminthic species was Moniezia spp. (21.4%), and the lowest spp., was Oxyuris equi (2.6%). Moniezia spp., Trichostongyliode spp., Parascaris equorum spp., Anoplocephala spp., Strongyliodes westri., Paragonimus westermani., Paranoplocephala mamillana., Gongulonema pulcurum Capllaira spp., and Oxyuris equi were the main helminthic species found in horses.

Table 2: Infection rate of different gastrointestinal helminthic in horses.

GIT helminthic species	Tested	+ve (%)
Moniezia spp.	50	21.4%
Trichostongyliode spp.	50	18.9%
Parascaris equorum	50	17.6%
Anoplocephala spp.	50	17.2%
Strongyliodes westri	50	11.4%
Paragonimus westermani	50	6.2%
Paranoplocephala mamillana	50	4.8%
Gongulonema pulcurum	50	3.8%
Capllaira spp	50	2.9%
Oxyuris equi	50	2.6%

The results of the present study revealed an overall infection rate of GIT protozoa in horses of 85%.

The Infection rate of different protozoan species in horses is shown in Table 3. The highest infection rate of protozoan species was Cryptosporidium parvum (34.4%), and the lowest spp., was Eimeria spp.

(14.5%). *Cryptosporidium parvum*, *Blantidium coli*, *Entamoeba coli*, and *Eimeria* spp., were the main protozoan species found in horses.

Table 3: Infection rate of different protozoan species in horses.

Protozoan species	Tested	+ve (%)
<i>Cryptosporidium parvum</i>	50	34.4%
<i>Blantidium coli</i>	50	30.2%
<i>Entamoeba coli</i>	50	15.7
<i>Eimeria</i> Spp.	50	14.5

The EPG values (Mean \pm SD) in horses of various groups:

The EPG values (Mean \pm SD) pre-treatment of the control group was 585 \pm 187.48 at day 0, and the highest EPG value was 1020 \pm 140.17, on day 21. The EPG values (Mean \pm SD) in the post-treatment groups on day 21 were 21 \pm 10.14, 30 \pm 14.25, 180 \pm 56.35, and 342 \pm 142.22 respectively, which was higher than the pre-treatment group 1020 \pm 140.17 on day 21.

The highest EPG values (Mean \pm SD) were in group 5 (T5) which was treated with *Allium sativum* on all days of the experiment and the lowest EPG values (Mean \pm SD) were in group 2 (T2) which was treated with a mixture of plants powder *Coriandrum sativum* 20gm, *Artemisia herba alba* 20gm, and *Allium sativum* 20gm by 1:1:1). The EPG values (Mean \pm SD) in horses of various groups at days 0, 7, 14, and 21 are shown in Table 4.

Table 4: EPG values (Mean \pm SD) in horses of various groups at days 0, 7, 14, and 21.

Group	EPG at day			
	0	7	14	21
T1	585 \pm 187.48	735 \pm 100.21	874 \pm 127.91	1020 \pm 140.17
T2**	623 \pm 189.39	210 \pm 30.85	53 \pm 23.12	21 \pm 10.14
T3	517 \pm 239.17	431 \pm 148.41	290 \pm 102.21	180 \pm 56.35
T4*	520 \pm 150.45	335 \pm 72.54	65 \pm 14.35	30 \pm 14.25
T5	495 \pm 225.22	448 \pm 165.74	390 \pm 177.21	342 \pm 142.22

** Highly significant, * significant, \pm SD P<0.05

The Efficacy of various medical plants.

The efficacy of various medical plants against GIT parasites in horses on different days is shown in Table 5. The efficacy of the mixture of plants powder *Coriandrum sativum*, *Artemisia herba alba*, and *Allium sativum* were 36, 59, and 79% on days 7, 14, and 21 respectively in group 2 (T2) which was recorded as the highest efficacy in this study, while the efficacy of *Coriandrum sativum* in the group 3 (T3) was 35, 53 and 73% at day 7, 14, and 21 respectively. The efficacy of *Artemisia herba alba* was 33, 54 and 75% on days 7, 14, and 21 respectively in group 4 (T4), while the efficacy of *Allium sativum* was 32, 51, and 72% on days 7, 14, and 21 respectively in the group 5 (T5) which recorded as the lowest efficacy in this study.

Table 5: Efficacy of various medical plants against GIT helminthic in horses on different days.

Medical plant	Group	Efficacy (%) at day		
		7	14	21
Mix	T2	36	59	79
<i>Coriandrum sativum</i>	T3	35	53	73
<i>Artemisia herba alba</i>	T4	33	54	75
<i>Allium sativum</i>	T5	32	51	72

*Mix (mixture of plants powder *Coriandrum sativum* 20gm, *Artemisia herba alba* 20gm, and *Allium sativum* 20gm by 1:1:1).

Chemotherapy

The EPG values and percent efficacy are shown in Tables 4 and 5, respectively. The mixture of plant powder *Coriandrum sativum*, *Artemisia herba alba*, and *Allium sativum*, significantly reduced the EPG, followed by *Artemisia herba alba* then *Coriandrum sativum*, and *Allium sativum* compared with a positive control group.

The efficacy of mixture plants powder *Coriandrum sativum*, *Artemisia herba alba*, and *Allium sativum*, in group 2 (T2) was 36, 59 and 79% at day 7, 14 and 21, respectively, while the efficacy of *Artemisia herba alba* in group 4 (T4), was 33, 54 and 75% at day 7, 14 and 21, respectively.

The lowest efficacy of *Allium sativum* in group 5 (T5), 32, 51, and 72% at day 7, 14, and 21, respectively was observed. The efficacy of the mixture of three plants powder were significantly higher ($p < 0.05$) than *Coriandrum sativum* or *Allium sativum* alone. The EPG values (Mean \pm SD) in horses of group 2 (T2) at days 7, 14, and 21, were 210 ± 30.85 , 53 ± 23.12 , and 21 ± 10.14 respectively.

The study showed that using a mixture of medical plants at different stages of the treatment days gives significant results in reducing the number of nematode eggs. Similarly, to study reported that when evaluating the potential use of nine plants for the control of cyathostomy eggs and L3 stage larvae, only *Acacia nilotica*, *Cucumis Prophetarum*, *Rumex abyssinicus*, *Allium sativum*, *Artemisia absinthium*, *Chenopodium album*, and *Zingiber officinale* showed anthelmintic activity [24].

The study showed that using *Allium sativum* inhibited the growth of protozoa, which agrees with the study that showed that allicin, a molecule that belongs to the organosulfur group, is the most abundant in *Allium sativum* and that it is responsible for its characteristic odor when crushed, as well as the inhibition it exerts on the growth of *Babesia caballi* and *Theileria equi* [25], [26].

In contrast, using *Allium sativum* alone did not give high efficiency in eliminating GIT parasites, but it must be used with a mixture of medicinal plants. This is consistent with a previous study that found in the short term (15 days), there is no effect of *Allium sativum* on the elimination of intestinal strongyle eggs in horses, while when evaluating the control of *Parascaris equorum* using five plants with anthelmintic properties, only *Artemisia dracuncululus*, *Mentha pulegium*, and *Zataria multiflora* showed potential for larval control [27].

Eucalyptus camadulensis and *Allium sativum* did not exert effective control over this parasite [28].

Results indicate altitude prevalence of gastrointestinal helminthic and protozoa in horses in western area of Libya. This result might harmonize or diverge from previous studies in the country, and other countries worldwide. The infection rate of *Moniezia* spp., *Trichostongyliode* spp., *Parascaris equorum*, *Anoplocephala* spp., *Strongyliodes westri*, *Paragonimus westermani*, *Paranoplocephala mamillana*, *Gongylonema pulcurum*, and *Capllaira* spp., were 20.4%, 16.9%, 16.9%, 16.9%, 10.2%, 5.1%, 5.1%, 3.4%, and 3.4% respectively.

The infection rate of GIT protozoa in horses *Cryptosporidium parvum*, *Blantidium coli*, *Entamoeba coli*, and *Eimeria* spp., were 33.3%, 34.4%, 16.1%, and 13.9% respectively which are congruent with the findings of helminths from equestrian clubs horses in Misurata, Libya [3]. The other study reported Large strongyles were found in 31.8% and verminous aneurysms in 36.4% of the horses. While *Anoplocephala perfoliata*, *Setaria equine*, *Parascaris equorum*, *Gasterophilus intestinalis*, and *Oxyuris equi* were found in all the horses, 33.1%, 16.2%, 14.4%, 9.3%, and 0.4% respectively [29].

The study from Urmia, North West of Iran showed the highest prevalence and intensity rate belonged to small strongyles which recorded strongyles at 72.9%, *Oxyuris equi* at 22.6%, *Parascaris equorum* at 12.2%, *Anoplocephalidae* 6.3%, *Fasciola* spp. 3.2% and *Eimeria leuckarti* 0.5%. Moreover, small strongyle larvae were most frequent (97.6%) followed by *Strongylus edentatus* (22.6%), *S. equinus* (18.5%), and *S. vulgaris* (6.5%) [30].

Oxyuris equi is a common parasite of equines with worldwide distribution, which follows a direct life cycle with adults and inhabits mainly in the right dorsal colon [31]. The percentage of horses infected with *Oxyuris equi* in this study was 2.6% and it's not reported in previous study from Libya [3]. Often referred to as "the horse killer," the equine bloodworm, *Strongylus vulgaris*, is thought to be the most dangerous gastrointestinal parasite [32].

The region, climate, sample size, laboratory equipment, management systems, animal origin, and the presence of repellent resistance are some of the factors that may have contributed to the disparity in prevalence levels between this study and studies carried out locally and in some other countries [33].

Anthelmintic resistance may be divided into three categories: multiple resistance, lateral resistance, and cross-resistance. Whereas lateral resistance results from selection for an alternative anthelmintic with a different mechanism of action, cross-resistance occurs when parasites can withstand therapeutic dosages of an anthelmintic with a different mechanism of action. Cross and lateral resistance in a parasite population results in similar activity and multiple resistance [34].

Despite the benefits of plants, few studies have been carried out on their use in controlling gastrointestinal parasites in monogastric animals, particularly horses, and in a previous study on the effect of intestinal anthelmintics on sheep in Libya, the gastrointestinal parasites showed high resistance, and to overcome this type of resistance, the drug was changed [15]. Because of the increasing need to find non-synthetic antiparasitic alternatives that are not only effective but also sustainable and do not harm animals, plants are now being considered as a viable option for controlling gastrointestinal parasites in herbivores, including horses [35].

Conclusions

The study concluded that GIT parasites and protozoa are prevalent in horses in the western area of Libya, as in many countries, and it could be observed at various rates. The results of the present study show that using medical plants was a very effective strategy to overcome gastrointestinal nematode resistance in horses, and it will not give the helminths a chance to form a resistance against the drugs. More studies from Libya are recommended to assess the efficacy of various medical plants against GIT parasites in horses and different animal species. These findings can make equine practitioners aware of the possible advantages and disadvantages of herbal dewormers.

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