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Research Article

Pimpinella anisum essential oil: A natural approach to amplifying fertility and reproductive well-being in the Saida Region

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Abstract

Infertility poses a significant challenge within the sheep population, hindering herd regeneration. Both rams and ewes are susceptible to infertility, prompting our objective of enhancing reproductive performance through the utilization of essential oils as a means to mitigate this issue. Essential oils have long been acknowledged for their medicinal properties and have been extensively employed in traditional medicine. Some essential oils have been reported to exert effects on male reproductive functions in both animals and humans.Pimpinella anisum, scientifically known as green anise, is a medicinal and aromatic annual plant renowned as one of the oldest medicinal herbs and widely used as a spice. In Algeria, essential oils extracted from Pimpinella anisum are consumed on a wide scale; however, their potential in enhancing ram fertility has remained unexplored.To improve the fertility of the Rembi breed sheep population, rams were randomly selected for this study. These rams underwent various experiments to evaluate their response to different concentrations (0, 0.5, and 1 mm/ram) of essential oil obtained from anise seeds using the hydrodistillation extraction method. Furthermore, the study encompassed an assessment of their key zootechnical characteristics over a one-year period.

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1. Introduction

Algeria stands as a key player in the national livestock sector, primarily due to its extensive sheep population, which serves as the cornerstone of the country's animal resources. This substantial population accounts for an impressive 78% of the total livestock count, signifying its pivotal role in shaping the agricultural framework of the nation (Zemour, 2022 ; Zemour & Sadoud, 2021). Currently, the country boasts over 26 million sheep, contributing to approximately 60% of the nation's red meat production, showcasing the immense potential of this industry (Ministère d'Agriculture et du Développement Rural Algérie , 2013; Zemour et al., 2020). Sheep farming holds substantial economic significance and serves as a crucial source of animal protein (Zemour & Sadoud, 2021).

Among the diverse spectrum of sheep breeds in Algeria, the Rembi breed prominently features as one of the top three breeds, constituting 12% of the nation's sheep population, accounting for a substantial total of two million individuals (Lakhdari et al. 2015; Bousta et al. 2020; Zemour, 2022). Originating primarily from the Saida region, the Rembi breed is found in multiple regions. This extensive sheep farming industry is pivotal to the nation's economy, given that reproduction forms the cornerstone of livestock profitability. Breeders' income is intricately linked to fertility and prolificacy, emphasizing the critical role these factors play in sustaining the industry's success. Moving forward, the research focuses on harnessing the potential of *Pimpinella anisum* essential oil to enhance fertility within these breeds. This transition presents a clear direction for the study, aiming to address the pressing need for improved reproductive outcomes within Algerian sheep farming.

Several factors intricately influence the productivity of a sheep flock. Among these, infertility stands as a significant challenge, notably characterized by prolonged seasonal sexual rest periods. This issue predominantly impacts ewes, presenting a substantial hurdle compared to its effect on rams (Belhouadjeb, 2017). Given these circumstances, it becomes imperative to explore avenues for enhancing the productivity of our sheep population. Reproduction optimization stands as a fundamental component of economic efficiency in any livestock operation. As a result, it is high time to consider transitioning from current farming systems to more efficient approaches similar to those implemented by major sheep-producing nations. The research specifically aims to explore the impact of *Pimpinella anisum* essential oil on sheep fertility. This transition signifies a crucial shift in the discussion towards investigating a potential solution to enhance reproductive efficiency in the Algerian sheep population.

Historically, essential oils have been recognized for their medicinal properties, dating back to ancient times. They continue to be utilized in traditional medicine, pharmacology, and modern medical practices. Among the various medicinal plants commonly used, some have been reported to affect male reproductive functions in animals and humans (Franchomme & Sajaloli, 2015). Additionally, certain essential oils are aromatic products rich in phytoestrogens, although their safety is yet to be fully established. These oils possess properties that may impact the physiological processes of reproduction, potentially influencing aspects such as hormone balance or reproductive cycles (El- Kalamouni, 2010).

2. Materials and methods

2.1 Plant material

The Anise seeds were procured from an herbalist based in the city of Saida, located in the Saida province. These seeds were obtained in a dried form and have their origins in the Tlemcen region, specifically sourced from the Maghnia District. Following their acquisition, the seeds were carefully packaged in a sachet for subsequent extraction.

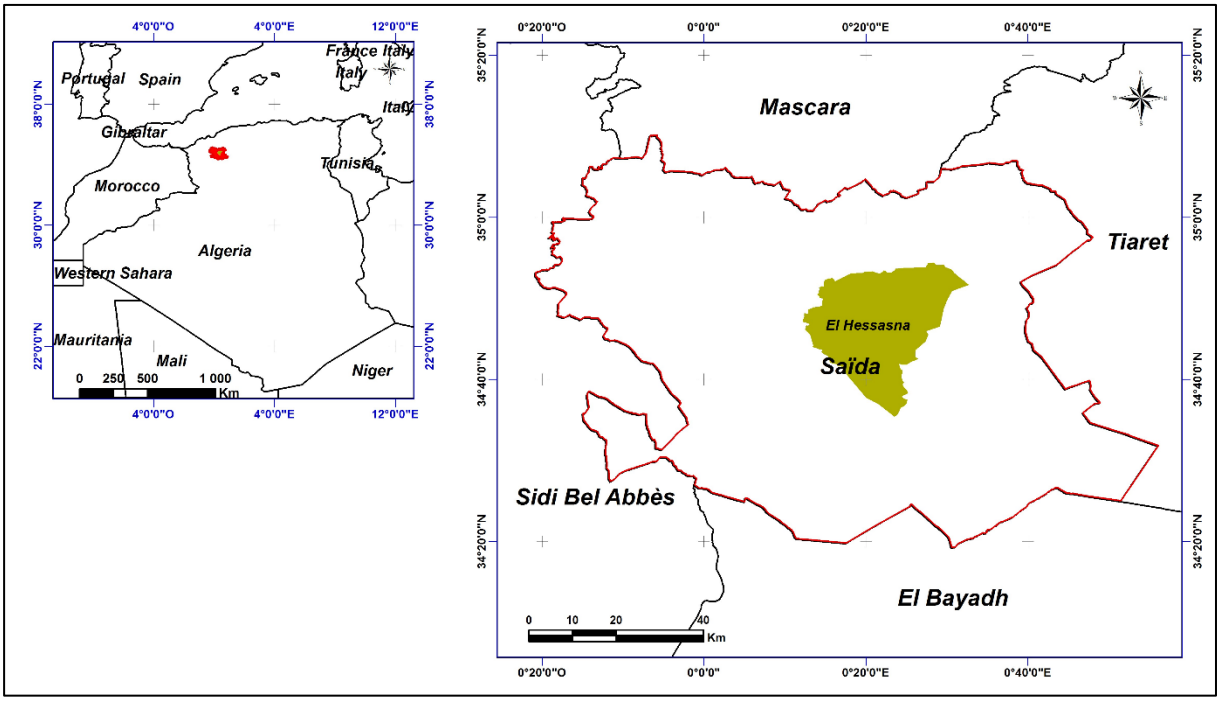

Figure 1. The region where leaves of Anise seeds were collected, Saida, Algeria

Figure 2. Pimpinella Anisum seed

2.2 Methods

The extraction and comprehensive study of various biological activities associated with anise seed essential oil were conducted at the Research Laboratory of Biotoxicology, Pharmacognosy, and Biological Valorization of Plants, situated within the Department of Biology at Dr. Tahar Moulay University in Saida between 2022 and 2023. This state-of-the-art laboratory is equipped with high-resolution gas chromatography-mass spectrometry (GC-MS) equipment for precise analysis of the essential oil constituents. Additionally, specialized bacteriological culture and susceptibility testing facilities were used to assess the antibacterial effects.

The research encompassed a multifaceted investigation:

1 *Antibacterial Effect*: The essential oil's impact on various bacterial strains was assessed using standardized antibacterial assays, including disc diffusion and minimum inhibitory concentration (MIC) determination.

2 *Ram Reproductive Parameters*: The study specifically delved into analyzing reproductive parameters in rams. This involved a comprehensive examination of semen quality, including sperm motility, concentration, and viability. Moreover, hormone levels related to reproductive

function, such as testosterone and luteinizing hormone, were evaluated pre- and post-administration of the essential oil.

3 *Methane Production*: The assessment of methane production involved the utilization of specialized equipment to measure methane emissions from controlled samples. This included in vitro experiments to simulate rumen conditions and quantify the potential reduction in methane output following the administration of the essential oil.

These investigations were carried out using robust scientific methodologies, ensuring precise measurements and reliable results. The combination of these analyses enabled a thorough exploration of the essential oil's diverse impacts, from its antibacterial properties to its effects on reproductive parameters in rams and methane production.

2.3 The extraction of essential oil from Anise seeds.

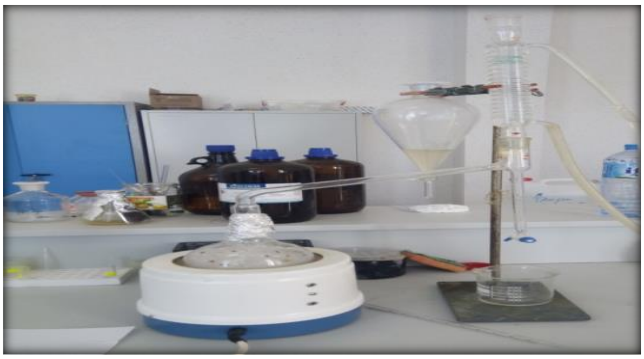

Figure 3. Assembly of the hydrodistillation apparatus

Phytochemical screening of aqueous extracts of Anise seeds

Detection of Tannins

The test for detecting tannins followed the protocol outlined by Aiyegoro & Okoh (2010). In order to ascertain the presence of tannins in *Pimpinella Anisum*, the ethanolic extract of essential oil (70%) was subjected to a reaction with a 1% solution of ferric chloride (FeCl3). If tannins were present, the reaction would manifest as the appearance of a blue color (Cheurfa et al. 2016).

To characterize the tannins, the reaction with ferric chloride (FeCl3) was employed. Two milliliters of each aqueous extract of Anise seed were combined with one or two drops of a 2% alcoholic solution of ferric chloride. The development of a bluish-black or dark green coloration indicated the presence of tannins.

The identification of tannins holds paramount importance in phytochemical analysis due to their substantial biological and pharmacological significance. Tannins are linked to diverse health advantages, encompassing antioxidant and antimicrobial properties (Khenafou, 2017). Analyzing the presence of tannins in Anise seed extract yields crucial information about its potential medicinal qualities, particularly highlighting its robust antioxidant and antimicrobial capabilities (Bekara et al. 2020).

Detection of resins

A volume of 10 mL of the filtrate obtained from the extraction process was combined with 20 mL of hydrochloric acid (HCl). The formation of turbidity or cloudiness in the mixture signifies the presence of resins in our extract, as highlighted by the findings of Khenafou (2017) and Bekara et al. (2020). Detecting resins is crucial in phytochemical analysis, as they often denote specific chemical constituents within plant extracts. Resins, known for their therapeutic and medicinal properties, contribute significantly to the pharmacological activities of plant extracts. Understanding their presence aids in assessing the potential biological and therapeutic applications of the

Anise seed extract. Khenafou's work delves into the pharmacological activities of resins, shedding light on their medicinal relevance, while Al-Balany's research likely provides additional context specific to resin identification in plant extracts. The simplicity and reliability of the turbidity-based test allow for a quick assessment of resin presence in the Anise seed extract, providing valuable insights into its potential medicinal properties.

Detection of coumarins

The presence of coumarins was assessed using the methodology outlined in references by Bekro et al. (2007) and Tipu et al. (2006). To perform the test, 5 mL of the filtrate was transferred into a test tube, and the opening of the tube was sealed with a filter paper soaked in sodium hydroxide (NaOH). After an incubation period of 10 minutes in a water bath, the filter paper was exposed to UV light. The appearance of a green and yellow color on the filter paper confirmed the presence of coumarins.

Detecting coumarins is vital in phytochemical analysis as these compounds possess various pharmacological properties, including anticoagulant and anti-inflammatory effects. The references by Bekro et al. (2007) and Tipu et al. (2006) likely delve into the identification and pharmacological significance of coumarins in plant extracts, offering insights into their potential medicinal applications. Coumarins are known for their diverse pharmacological activities, and their presence in the Anise seed extract could indicate potential health benefits, making their detection essential in assessing the extract's medicinal potential. The simplicity and reliability of the color change test allow for a quick assessment of coumarin presence, providing valuable insights into the potential medicinal properties of the Anise seed extract.

Detection of saponins

The detection of saponins involves vigorously agitating the filtrate. Subsequently, the mixture is allowed to settle undisturbed for a duration of 20 minutes. The presence of saponins is evaluated by observing the formation of foam, and specific criteria are used for interpretation:
- Foam less than 1 cm: Indicates a negative test.
- Foam between 1-2 cm: Indicates a positive test, as per the guidelines outlined by Trease & Evans (1987).

Understanding saponins in plant extracts is crucial due to their diverse pharmacological properties. Trease & Evans (1987) likely detail the significance and identification of saponins, offering guidelines for their detection in plant extracts. Saponins are known for their various biological activities, such as their roles as natural detergents, immune modulators, and potential cholesterol-lowering effects. Identifying their presence in the Anise seed extract is essential in determining its potential therapeutic applications. The simplicity and reliability of the foam test allow for a quick assessment of saponin presence, providing valuable insights into the medicinal potential of the Anise seed extract.

Detection of terpenoids

To detect the presence of terpenoids, a mixture of 1 mL of acetic acid and 2 mL of sulfuric acid was added to 1 mL of the filtrate. If terpenoids are present, the reaction will result in the development of a reddish-brown color, as described by Yu et al. (2013).

Detection of flavonoids

This technique involves preparing two solutions:
Solution A: 5 mL of the ethanolic extract.
Solution B: 5 mL of ethanol + 5 mL of KOH (50%).
The appearance of a yellow color after mixing the two solutions indicates the presence of flavonoids, as detailed by Trease & Evans (1987).

2.4 Animal material

Selection and Preparation of Subjects for the Experiment

For our year-long study, three 18-month-old rams of the Rembi breed were selected. Among them, one ram served as the control, while the remaining two were assigned to the essential oil treatment group.

Statistical analysis

The effect of Anise seed essential oil *(Pimpinella Anisum*) was evaluated using one-way analysis of variance (Anova) with MINITAB 20 software, version 2021. The parametric Tukey test was utilized for post hoc analysis. Significance was determined at $p \leq 0.05$, indicating that means annotated with the same letter were not significantly different.

3. Results

3.1 Animal material

Weight measurement

The Anova test ($p \leq 0.05$) assessing the impact of Anise seed essential oil on the weight development of the rams over the study duration revealed no significant differences among the groups. The means annotated with the letter 'A' ($p = 0.166$) were similar to the control, suggesting that Anise seed essential oil did not affect the weight of the rams. The detailed data showing normality test results are provided in Table 1.

Table 1. Grouping information using Tukey's method with a 95% confidence level

Factor	N	Mean	Grouping
0 ml	12	67	A
0.5 ml	12	65	A
1 ml	12	63	A

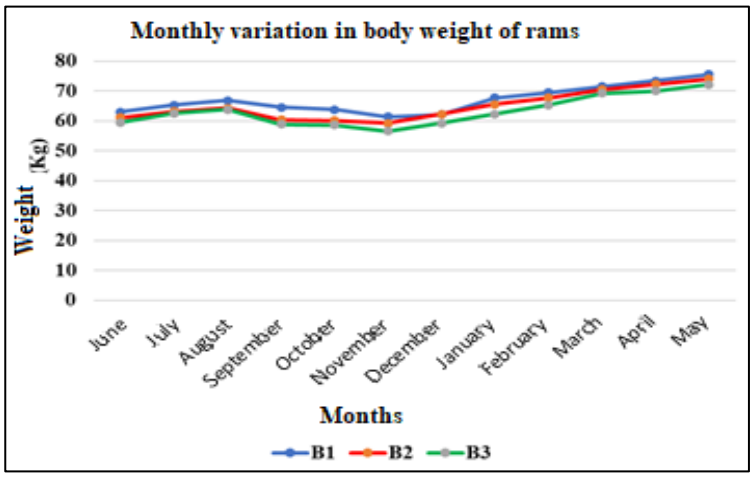

Figure 4. Monthly variations in weight among rams

Based on Figure 4, it's evident that the weight of the rams exhibits seasonal fluctuations. Spring emerges as the most conducive period for weight gain, while weight decreases during winter. This disparity is closely tied to the variance in food resource availability. The spring season typically offers abundant resources compared to the winter, which experienced a critical drought period. These observed variations in weight due to seasonal changes lay a crucial foundation for understanding the animals' overall development. Now, let's delve into the scrotal circumference results to further assess the rams' physiological changes.

Scrotal circumference results

Scrotal circumference in rams is a vital reproductive parameter. It serves as an indicator of testicular development and, by extension, reproductive potential. Generally, an increase in scrotal circumference is associated with sexual maturity and improved fertility in rams. Larger scrotal circumference often correlates with higher sperm production and quality, thereby influencing the ram's ability to impregnate ewes.

In studies, variations in scrotal circumference across seasons or under different conditions might reflect changes in reproductive readiness, potentially influenced by factors like nutrition, environmental conditions, and overall animal health. For instance, ample nutrition and favorable environmental conditions tend to promote better reproductive performance and larger scrotal circumference. Figure 5 shows the technique of measuring scrotal circumference using a measuring tape.

Figure 5. Measurement of scrotal circumference

The Anova analysis on the impact of *Pimpinella Anisum* essential oil revealed no significant difference ($p = 0.555$) in scrotal circumference between the experimental rams and the control group over the one-year study duration. The 'A' annotations in Table 2 further confirm the absence of statistically significant variation.

Table 2 Grouping information using Tukey's method with a 95% confidence level

Factor	N	Mean	Grouping
0 ml	12	30	A
0.5 ml	12	28	A
1 ml	12	30	A

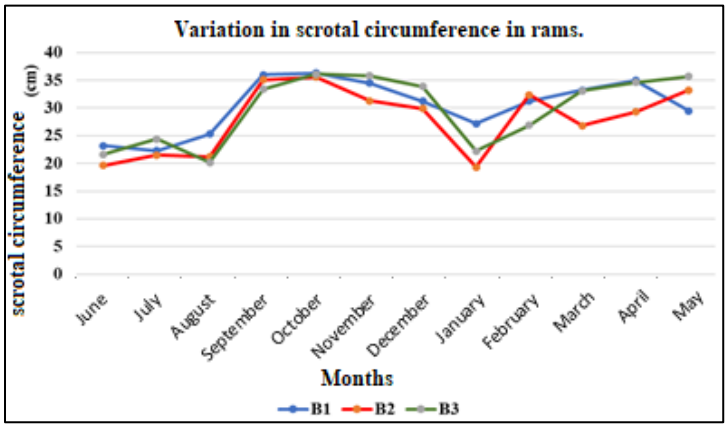

Figure 6. Monthly variations in scrotal circumferences among rams

Figure 6 displays evident fluctuations in scrotal circumference over the study year. The measurements reveal a distinct seasonal pattern, with the highest average values (B1: 35.60 cm; B2: 34 cm; B3: 35.10 cm) observed during autumn. In contrast, the summer season showcases lower average measurements (B1: 23.60 cm; B2: 20.77 cm; B3: 22.08 cm). This indicates that scrotal circumference is notably influenced by seasonal factors, with autumn having a notably favorable impact on the measurements.

Sperm Collection

Sperm collection utilized an artificial vagina, enabling semen retrieval transferred into graduated tubes for ejaculate volume measurement.

Ejaculate Volume

The ejaculate volume for the rams ranged from 1 to 2 ml. Anova analysis found no significant variation between the experimental and control groups ($p = 0.900$). Table 3 denotes 'A' values aligning with the control, confirming no statistical difference in ejaculate volume.

Table 3. Grouping information using Tukey's method with a 95% confidence level

Factor	N	Mean	Grouping
0 ml	12	2	A
0.5 ml	12	1	A
1 ml	12	1	A

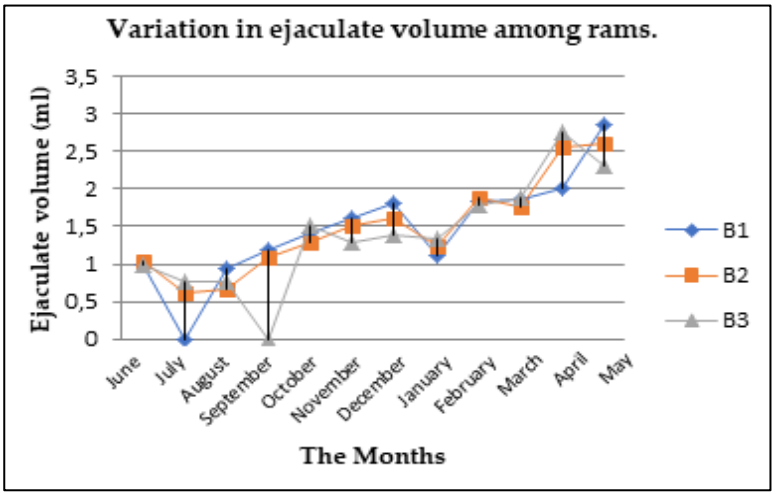

Figure 7. Monthly variations in ejaculate volume among rams

Ejaculate volume and histological study

Figure 7 illustrates significant seasonal variations in ejaculate volume. Higher average values (B1: 2.25 ml; B2: 2.30 ml; B3: 2.31 ml) were observed during autumn and spring, contrasting the lower values in summer (B1: 0.96 ml; B2: 0.77 ml; B3: 0.84 ml). This suggests a seasonal influence on ejaculate volume. In the histological study, both the control ram (B1) and experimental rams (No. 02 and No. 03) exhibited normal testicular morphology without abnormalities. The testicular parenchyma appeared normal in all rams. However, the experimental rams showed seminiferous tubules with lower density, housing germ cell lineages at various stages of maturation, while maintaining normal Sertoli, interstitial tissue, and Leydig cells.

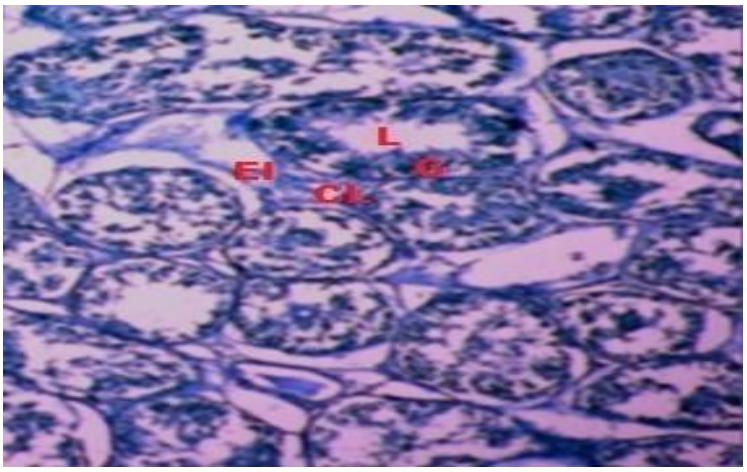

Figure 8. Histological section of the seminiferous tubules in Ram 01 (control)

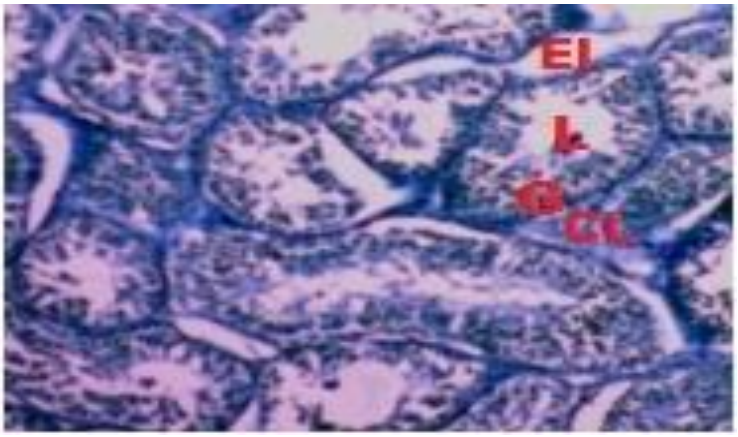

Figure 9. Histological section of the seminiferous tubules in Ram 02

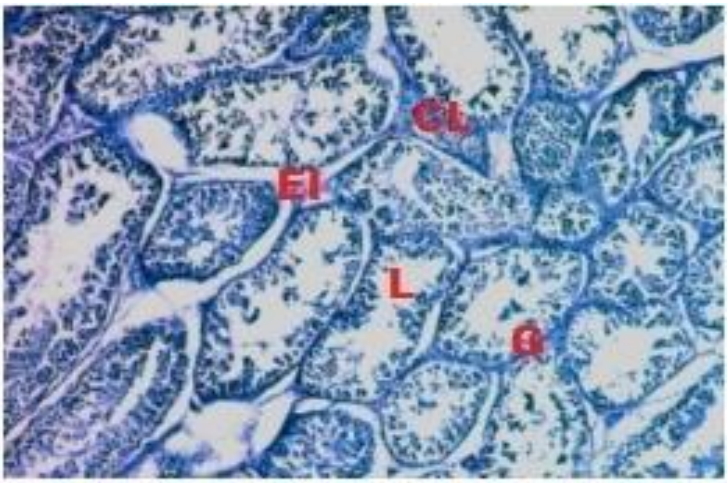

Figure 10 . Histological section of the seminiferous tubules in Ram 03
L: Lumen, EI: Interstitial space, G: Spermatogonia, CL: Leydig cell.

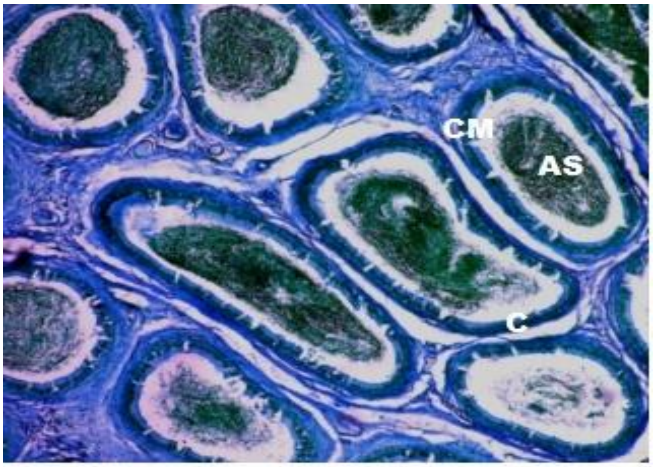

Figure 11. Histological section of the epididymis in Ram 01 (control)

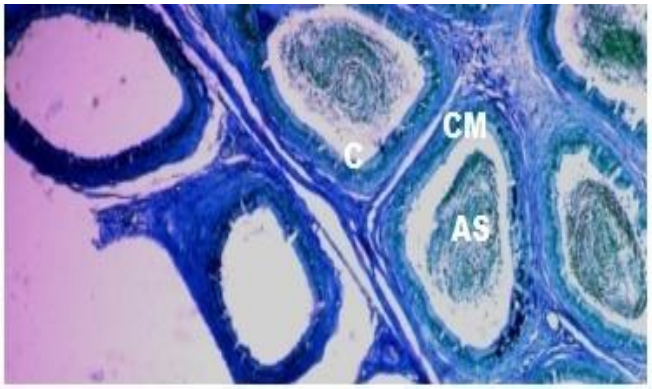

Figure 12. Histological section of the epididymis in Ram 02

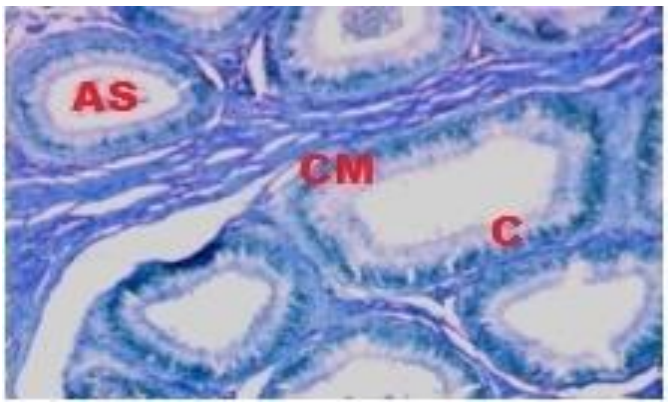

Figure 13. Histological section of the epididymis in Ram 03
AS: Cluster of spermatozoa, CM: Myoepithelial layers, C: Ciliated columnar cells.

The manuscript should present the complete information on the materials and method, so that anyone can duplicate the experiment. When using standard procedure, provide complete reference. In case a modified procedure has been applied, then the modification must be described. Materials and methods should not more than 1500 words with line numbering. The method was appropriate to approach the problem solving. This can be divided into subsections if several methods are described (Chavas & Mitchell, 2018).

3.2 Discussion

Plant Material

Yield

Pimpinella anisum, commonly known as Green Anise, holds a significant place in medicinal and culinary applications due to its long-established history as a medicinal herb and spice plant (Gülçın et al. 2003; Mosavat et al. 2019). Previous studies by Kosalec et al. (2005) and Eisenreich et al. (2021) focused on screening antioxidant and antimicrobial activities from *Pimpinella anisum* seed extracts, affirming its aromatic attributes and its classification within the Apiaceae family. These studies reported essential oil yields ranging between 1% and 4%, consistent with our findings. In our study, we observed an essential oil yield of 1.6%, which aligns with the yield reported by Ghouati et al. (2012), ranging from 1.2% to 1.35%. Notably, our yield is lower than the average of 3.55% reported by Ghouati et al. (2012). The new knowledge generated through our study reveals that the essential oil yield of *Pimpinella anisum* was within the expected range observed in earlier research, solidifying its consistency in oil production. Our results align with the ranges seen in previous studies, confirming the consistent nature of essential oil yield in *Pimpinella anisum*.

Animal Material

Body Weight

In our year-long study, we closely monitored a fundamental zootechnical aspect in Rembi rams—their body weight. Our aim was to assess the potential impact of anise seed essential oil on their weight development. Body weight stands as a crucial indicator of ram reproductive activity and correlates directly with age. However, it's important to note that this parameter can be affected by various factors, including diet, season, breed, and climatic conditions (Taherti et al. 2016).

The results of our study depicted the progression of body weight in rams treated with the anise seed essential oil solution, from 18 months to 30 months of age, compared to the control ram. Through statistical analysis using Anova, we determined that the weight variation among the three rams was not statistically significant, leading us to conclude that the essential oil had no notable influence on the animals' weight. In contrast, other studies focusing on the integration of green anise as a supplement in broiler chicken feed have shown considerable enhancements in various zootechnical parameters by adding 0.5 g/kg to 1.5% of green anise to their diet (El-Deek et al. 2002; Al-Kassie et al. 2008; Mosavat et al. 2019).

The homogeneous body growth observed in our rams can be attributed to several factors, including the consistent feeding practices (uniform ration) and the genetic characteristics specific to the Rembi breed. Most adult rams included in our study displayed weights comparable to those reported for Rembi rams in other studies (Benzidour, 1995; Benyoucef, 1994). Our investigation has unveiled a discernible relationship between body weight and seasonal variations among sheep. The substantial weight gain observed during the spring season is attributed to the ample forage available within the farming environment. Conversely, a modest decline in body weight during the winter season appears linked to heightened reproductive activity and physical exertion among rams during the autumn mating season. Sheep husbandry in Algeria predominantly adheres to a semi-extensive system reliant on year-round grazing, significantly impacted by climatic factors such as rainfall and seasonal fluctuations. Given the drought-like conditions experienced in 2021, the observed decline in animal body weight becomes explicable. Additionally, our study showed an increase in weight gain during the spring season due to forage availability. Several studies have indicated that animals tend to lose weight during periods of high sexual activity, particularly in autumn, compounded by dietary restrictions in winter months (Salomon, 1997).

Scrotal circumference

Scrotal circumference stands as a reliable indicator of rams' reproductive activity directly associated with age and body weight. Other factors such as diet, season, breed, and climate can significantly influence this parameter (Taherti et al. 2016). Presently, essential oils (EO) are being investigated for their potential to improve fertility. The animal model used in this study has previously been utilized by several researchers to assess the potential adverse effects of medicinal plant extracts on male reproductive functions (Ortavant, 1956).

Throughout our year-long study, we measured the scrotal circumference of the testicles in three Rembi rams aged 18 to 30 months. The objective was to ascertain the influence of anise seed-derived essential oil (*Pimpinella Anisum*) on gonadal development. However, our findings indicated that the scrotal circumference of the experimental rams, who received doses of anise essential oil over the study period (from 18 to 30 months of age), did not exhibit significant variation compared to the control ram. Employing statistical analysis with Anova, we concluded that the observed differences in scrotal circumference among the three rams were not statistically significant, implying that essential oil does not affect scrotal circumference development. These findings align with a study by Roberts et al. (1988), demonstrating an insignificant effect on the evolution of rat gonads.

The recorded average scrotal circumferences in our study were as follows: ram 01 (control) = 30.41 cm, ram 02 = 27.94 cm, and ram 03 = 29.83 cm. These values are lower than those reported for Ouled Djellal and HAMRA rams and smaller than those documented for British breed rams (Roberts et al. 1988). In a previous study, scrotal circumferences of 34 to 36 cm for adult rams and 28 to 30 cm for young rams were indicative of excellent fertility (Kafi et al. 2004).

The observed decrease in testicular diameter during winter and summer in our study can be attributed to the decrease in body weight primarily caused by restricted grazing and unfavorable rearing conditions during these seasons. Given that our study year experienced mediocre rainfall and the ram rearing system was semi-extensive (relying on concentrate and grazing), the diet during these periods was both quantitatively and qualitatively reduced. This suggests that food availability is a significant factor influencing scrotal circumference variation.

Previous studies have indicated that severe underfeeding, such as a loss of 400 g of live weight per month for 30 weeks, consistently decreases testicular weight and diameter, as well as the concentration and total number of spermatozoa in ejaculated semen. Conversely, overfeeding Merino rams with protein-rich lupin seed for 15 days increased testicular weight by 66% and live weight by 39%, likely due to increased LH activity in males (Baril et al. 1993).

The seasonal variation observed in our research aligns with reports by Wolfire et al. (2010) for Suffolk and Lincoln rams, where testicular growth remains low from the end of winter until the beginning of spring and starts to recover in April-May, reaching its maximum in autumn (October). Similar patterns were observed in Texel, Suffolk, and Île-de-France rams (Wolfire et al. 2010; Chemineau et al. 1992; Mandiki et al. 1998; Gastel et al. 1995). The reduction in scrotal circumference during the winter months aligns with observations made by Litim (2014), Baril et al. (1993), Milczewski et al. (2015) in Awassi rams, El-Bouyahiaoui et al. (2022) in adult Ouled Djellal rams, Atashi et al. (2012) in Suffolk rams, and Pourseif et al. (2013) in Algerian white breed rams.

Ejaculate volume

Based on the mean ejaculate volumes obtained (ram 01 volume = 2.86 ml, ram 02 volume = 2.60 ml, ram 03 volume = 2.3 ml), there is no significant difference in the annual ejaculate volume between the control ram and the rams that received doses of *Pimpinella Anisum* essential oil (0.5 mg/ram and 1 mg/ram) respectively. This conclusion is supported by the statistical analysis using ANOVA, which indicates that the effect of anise seed essential oil on sperm volume is not significant. The administration of anise seed oil solution did not have a detrimental effect on sperm

production in the rams, as their ejaculate volume remained within the normal range and varied with the season.

Several studies have demonstrated that the season can influence the reproductive characteristics of rams (Baril et al. 1993; Belkadi et al. 2017). In Algerian breeds, although there are seasonal variations in sperm production, it is possible to produce semen throughout the year in Ouled Djallal and Hamra breeds (Milczewski et al. 2015). The recorded values for ejaculate volume were lower during the winter and summer, as reported by Milczewski et al. (2015) and Belkadi et al. (2017). Additionally, an increase in volume was observed with a peak in early autumn, followed by a decrease in early January that persisted until the end of February, and then a progressive increase reaching a new peak in March. Similar findings were reported by Kafi et al. 2004 during the same seasons. In contrast, Walkden et al. (1994) showed that the quantity of ejaculated semen reaches its maximum values in June and appears to vary with the duration of daily illumination.

Testicular histology

Our histological examination of the testicles in all experimental rams revealed robust spermatogenic activity in the seminiferous tubules of the control ram, more pronounced compared to ram 02 and 03, subjected to essential oil from anise seeds at doses of 0.5 mg/ram and 1 mg/ram. In contrast to Martin et al. (2010) findings indicating a negative influence of photoperiod on spermatogenesis, our examination of histological sections from the control ram showed various stages of maturation in germ cell lineages. The Sertoli cells appeared normal, while the interstitial tissue displayed typical Leydig cells and a vascular structure. We did not observe the phenomenon of a negative photoperiodic effect on spermatogenesis in the studied gonads. This could be attributed to a low degree of variation in the duration of illumination, considering the location closer to the tropics rather than a temperate region. Additionally, the reduction in spermatogenic activity during this period is likely influenced by dietary factors.

Eman et al. (2019) observed a 20% rise in spermatogenic activity in Australian rams between February and June, correlating this increase with grazing periods and implicating nutrition in the modulation of testicular parenchyma. They noted a direct impact of nutrition on the diameter of seminiferous tubules and accessory glands, which reportedly tripled in volume during spring in Soay rams. The authors described the diameter of seminiferous tubules as an indicator of spermatogenesis, denoting heightened Sertoli cell activity due to increased circulating gonadotropin hormones, leading to amplified germ cell production. Our results for ram 02 and 03, which received varying doses of anise seed essential oil, displayed normal testicular parenchyma but with less dense seminiferous tubules compared to the control ram. The germ cell lines were at various stages of maturation but less dense than those in the control ram. The Sertoli cells appeared normal, and the interstitial tissue showed normal vascular conjunctival characteristics with Leydig cells. These findings align with those of Belayachi (2016), where histological sections of testicles from prepubertal animals exhibited histopathological changes, including a decrease in Sertoli cell number, necrotic spermatocytes, and a notable reduction in germ cells. Eman et al. (2019) also reported decreased levels of FSH, LH, and testosterone due to the administration of anise seed essential oil.

Conversely, Mansour et al. (2013) found that treatment of rats with Nigella sativa essential oil had no effect on the weight of reproductive organs (testicles and epididymis), sperm parameters, plasma testosterone concentration, FSH, LH, and inhibin β. Several studies have demonstrated the androgenic properties of certain essential oils. For instance, Al-Saaidi et al. (2009) showed stimulation of hormone synthesis responsible for spermatogenesis (LH, FSH, and testosterone) and an increase in spermatogenic activity in male rats treated with extracts from Nigella sativa seeds. Similarly, Nasseem et al. (1998) observed increased hormone synthesis and spermatogenic activity in rats treated with extracts from Momordica charantia seeds. Kdogan et al. (2004) reported that peppermint essential oil (M. piperita) increased gonad weight by enlarging the

diameter of seminiferous tubules, likely due to increased testosterone levels. Haeri et al. (2006) demonstrated a significant weight increase in the testicle, epididymis, and seminal vesicles following the administration of essential oil from Satureja khuzestanica (savory), attributed to increased numbers of spermatogonia, spermatids, Leydig cells, and spermatozoa, as well as an enhancement of spermatogenesis. Therefore, the enlargement of reproductive organs is a result of elevated levels of FSH and testosterone in the serum induced by this oil.

4. Conclusion

Our study examined the impact of Anise seed essential oil on reproductive metrics in Rembi breed rams. In vitro analysis revealed no significant differences in weight, scrotal circumference, or ejaculate volume between treated and control groups. Statistical tests confirmed no significant variations ($p = 0.166$, $p = 0.555$, and $p = 0.900$), indicating the oil's negligible influence on these parameters. While the oil had no effect on Sertoli and Leydig cells, it did lower sperm density in specific tubules and epididymis in rams 02 and 03, suggesting a possible adverse impact on sperm quantity. Areas for Future Investigation would be to conduct a longitudinal study to understand the extended consequences of continued essential oil exposure on reproductive health. Dosage Effects: Investigate the effects of varied essential oil concentrations to determine the threshold for adverse impacts on sperm quality. Underlying causes is to explore the reasons behind the observed decrease in sperm density in the specific anatomical regions to pinpoint the mechanisms affected by the essential oil.

Institutional Review Board Statement (Ethical considerations)

All experiments complied with the Algerian legislation (Law Number 95-322/1995) inherent to protection of animals designed to experimental and other scientific purposes as well with the guidelines of the Algerian Association of Experimental Animal Sciences (AASEA) and were specifically approved by the latter (AASEA authorisation number 45/DGLPAG/DVA/SDA/14).

Author's declaration and contribution

The authors certify that they have no conflicts of interest. Author contributions across all phases of this research article were diverse and crucial, spanning from idea inception and methodological refinement to data collection, analysis, manuscript drafting, critical review, project supervision, and highlighting a comprehensive collaborative effort.

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