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EFFICACY OF MENTHA PIPERITA L. EXTRACT AGAINST E. COLI IN BROILER CHICKEN

WALEED AHMAD
Department of Zoology, Hazara University, Mansehra, Pakistan.
Corresponding author Email: waleed5844728@gmail.com,

SHUMAILA NOREEN
Associate Professor, Department of Zoology, Hazara University, Mansehra, Pakistan.
E-mail: shumailanoreen@uop.edu.pk

SUMAIRA SALAHUDDIN LODHI
Lecturer, Department of Biochemistry, Hazara University, Mansehra, Pakistan.
Email id. sumaira.bch@gmail.com

NADIA JABEEN
Lecturer, Department of Agriculture, Hazara University, Mansehra, Pakistan.
Email: nadia_khan909090@yahoo.com

NOSHEEN SHAFQAT
Assistant Professor, Department of Agriculture, Hazara University, Mansehra, Pakistan.
Email: noshinshafqat@gmail.com

ALIA GUL
Lecturer, Department of Botany, Hazara University, Mansehra, Pakistan.
E-mail: aliagulbotanist@gmail.com

ASMA
TA, Department of Botany, Hazara University, Mansehra, Pakistan.

NAQASH KHALID
Research Officer, Poultry Research Institute Jaba Mansehra, Pakistan.
Corresponding author Email drnaqash092@gmail.com

MUHAMMAD FIAZ KHAN
Assistant Professor, Department of Zoology, Hazara University, Mansehra, Pakistan.
E-mail: fiazkhanhu333@gmail.com

ZAHEER AHMED
Department of Zoology, Hazara University, Mansehra, Pakistan. E-mail: zaheerkashmeri101@gmail.com

MUHAMMAD MUJTABA
Principal Research Institute/Director, Poultry Research Institute Jaba Mansehra, Pakistan.
Email: mm5769966@gmail.com

ABSTRACT

Antibiotics were commonly employed as food supplements and growth enhancers in broilers, but they were also linked to harmful side effects. As a result, an alternate source to substitute antibiotic growth enhancers was necessary; such a product is Phyto-biotics, specifically Mentha piperita leaves extract. The goal of the

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current study was to determine how different doses of M. piperita leaves extract would affect broiler production and E. coli resistance. 100 milliliters of distilled water were used to dissolve 3.8 grams of Mueller hinton agar powder to prepare the culture medium for the invitro experiment. The disc diffusion technique was used to assess the extract's efficacy. For the in-vivo experiment, six major groups made up of 108 one-day-old broiler chicks were assigned at random. The same management and environmental standards applied to all groups. The results showed significant antibacterial activity of the M. piperita extract against E. Coli. An inhibitory zone of 18.67 mm is created by the extract. The results showed that the No Treatment, Only Inoculation group had the lowest total body weight (515.66g/chicken) while the pre-inoculation 2000ppm group had the highest total body weight (930.22g/chicken). The culture of liver and spleen blood on Mueller-Hinton agar revealed no infection in the broilers who were getting treatment. In conclusion, M. Piperita significantly inhibits the growth of E. Coli.

1 INTRODUCTION

Worldwide Poultry eggs and meat are largely produced and consumed. To supply the ever-growing human population's demand for animal protein, its demand is assumed to rise further. So, there is a challenge for poultry scientists and industries to create enough chicken meat and eggs in the more effective way (Yadav & Jha, 2019). Broilers, a type of poultry birds, is utilized mainly for production of meat around the world. Production, Health and immunity of broilers are all affected by a variety of factors related to the future development of poultry industry. The Key Obstacles to the strategic development of poultry will continue to be Customer confidence, Product types, disease spread, product safety and quality (Hafez & Attia, 2020). Antibiotic usage to promote broiler growth and manage gut flora was once common. Moreover, because of worries about potentially fatal consequences on food animals and indirectly on people, their usage as feed additives is forbidden or controlled in many jurisdictions (Hafez & Attia, 2020).

The bacteria *Escherichia coli* (*E. coli*) belonging to the family Enterobacteriaceae, which is a member of the gamma subgroup of the phylum Proteobacteria. *E. coli* is most commonly recognized as a universal member of the typical gut bacterial microflora in humans, reptiles and other warm-blooded animals (Leimbach et al., 2013). Broilers carry *E. Coli* on skin, in Plumages, Upper Respiratory tract and Intestine. While most E. coli strains do not pose a threat to the health of broilers, some of them have the potential to spread disease outside of the intestines. Colibacillosis, an avian pathogenic *Escherichia coli* (APEC)-induced infection; that can be systemic or localized, is a prominent cause of illness and mortality, in particular, in the worldwide chicken industry (Sargeant et al., 2019).

Mentha piperita L. is usually used in herbal remedies and is thought to be especially beneficial in the development of the immunity, due to its antimicrobic properties, powerful antioxidants, and ability to increase hungriness, due to its active components (Mahmood et al., 2020). It is a fragrant annual herb in the Lamiaceae family. Since ancient Greek, Egyptian, and Roman times, it has been linked to numerous therapeutic principles as a therapeutic cure (Alexa et al., 2018). The major biological activities of volatile oil include anti-infectious (anti-malarial, anti-viral, anti-bacterial and anti-fungal,), digestive stimulant, analgesic, general anesthetic, anti-spasmodic, and astringent (Erdoğan et al., 2016).

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2 MATERIALS AND METHODS

2.1 Preparation of Extract

Ethanolic extract was made by putting 100g of properly cleaned, dried and smashed leaves of *M. Piperita* in 300-400ml of 10% ethanol. The dark glass bottles were used which were well agitated and reserved for 3 days at room temperature.

2.2 Test Organism

The bacteria E. Coli was used to check the antimicrobial action of *M. Piperita*.

2.3 Invitro Test

Agar well diffusion method was used to in this experiment. For making culture media, 3.8g of Muller Hinton agar was dissolved in 100ml of Distilled Water. Dishes were filled with prepared media to a depth of 4mm. The wells of 10mm width were made using sterile cork borer after the drying of media. In wells 100µl of *M. Piperita* extract was putted using sterile micropipette. Broth culture of E. Coli was spread, using cotton swab, on the dishes containing the media and M. Piperita Extract. the results were obtained by measuring the zone of inhibition using measuring scale after incubation. Amoxycillin was used as a standard antibiotic for comparison.

2.4 In-vivo Test

The In-vivo test was conducted on Broilers. In this prospect 108 one-day old chicks were reared up for 4 weeks (28 days) in proper settings having all necessary requirements i.e., bedding of wood shavings, heat lamp for birds' warmth, feeders and waterers.

All the chicks were randomly divided into 12 groups (9 chicks /group) i.e., i) No treatment, Only inoculation; ii) No treatment, No inoculation; iii) Pre-inoculation 2000ppm; iv) Pre-inoculation 4000ppm; v) Pre-inoculation 6000ppm; vi) Post inoculation 2000ppm (7 days); vii) Post inoculation 4000ppm (7days); viii) Post inoculation 6000ppm (7 days); ix) Post inoculation 2000ppm (14 days); x) Post inoculation 4000ppm (14 days); xi) Post inoculation 6000ppm (14 days); xii) Comparative Group (taking Amoxycillin).

The groups were made based on dose and time duration in which *M. Piperita* extract is provided of each group. All the groups were receiving same protocols of management and environmental conditions.

2.5 Administration of E. Coli

Inoculum of E. Coli was given orally to chicks, with the help of Dropper, by dissolving some cultured E. Coli in Distilled water.

2.6 Administration of M. Piperita extract

The extract was administered to chicks in drinking water in different doses according to the groups made.

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2.7 Measurements of Parameters

Weight gain, Feed intake and Food Conversion Ratios were noted on 1st, 7th, 14th, 21st and 28th day and for each group fatality was noted and recorded separately.

2.8 Diagnosis of Infection

History, clinical signs and symptoms, post-mortem inspection, and cultural characterization were the criteria to diagnose infection of *E. Coli* in Chicks.

Enlargement of liver and Fibrin deposits on liver were observed in Post-mortem. A Bacterial culture was performed to confirm the presence of infection on observing the presence of whitish layer on liver and heart.

2.9 Statistical analysis

Statistical analysis was done by using Statistix 8.1 Application and completely Randomized Design ANOVA was applied to check the variance. At 5% ($p<0.05$), the variance between the groups was deemed significant.

3 RESULTS

3.1 Invitro Test

Disc diffusion method revealed the zones of inhibition made by *M. Piperita* and Amoxycillin. The *M. Piperita* extract showed good anti-*E. Coli* activity and made an inhibitory zone of 18.67mm as compare to Amoxycillin which made an inhibitory zone of 15.33mm, as show in fig.3.1.

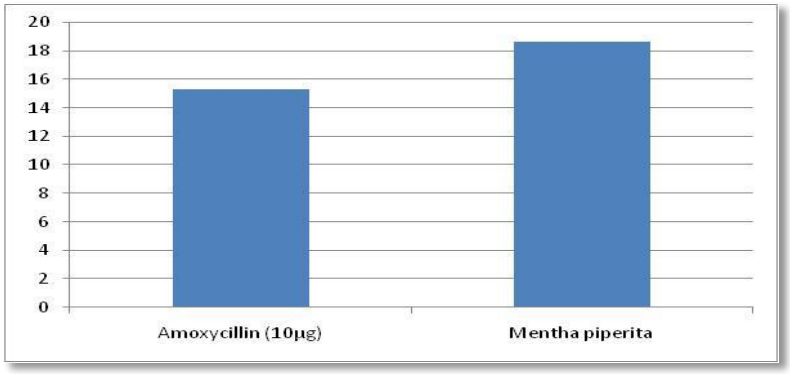

Figure 3.1: Graphical presentation of activity of M. piperita and Amoxycillin against E coli

3.2 In-Vivo Test

The results of adding *M. piperita* extract to broiler chicks' drinking water for 7 days, 14 days, and 28 days, as well as their impacts on performance in regards of feed intake, increase in weight, and FCR, are shown in tables 3.2.1, 3.2.2, and 3.3.3, in that order.

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Table 3.2.1: Feed Intake (Gram/Chick)

Week		1st	2nd	3rd	4th	Total
Group						
Pre-Inoculation	2000ppm	175.02c	367.04de	609.30bc	807.65cd	1958.98f
	4000ppm	177.65b	369.55bc	611.44ab	811.98b	1970.33b
	6000ppm	180.04a	371.98a	611.76ab	813.76a	1977.32a
Post Inoculation (7-Days)	2000ppm	172.06e	368.55cd	607.87c	806.33cd	1954.55i
	4000ppm	172.43de	368.22cd	610.98ab	804.65	1955.96h
	6000ppm	171.98ef	371.22ab	612.33a	805.76de	1960.96e
Post Inoculation (14-Days)	2000ppm	172.54de	367.98cd	607.65c	808.87bc	1956.76g
	4000ppm	173.43d	371.55ab	611.76ab	811.44ab	1967.80d
	6000ppm	170.76f	371.33ab	613.04a	813.22a	1968.00c
Comparative		171.98ef	365.33e	602.98d	798.87f	1938.87k
No Treatment, only inoculation		172.22de	351.76f	593.98e	792.76g	1910.43l
No Treatment, No inoculation		172.98de	365.33e	602.33d	799.22f	1939.33j

To determine whether there was a difference in feed consumption among all broiler groups, the weekly feed intake for each group was computed. Boilers' feed intake is increased by 1977g/chick when 6000ppm of *M. piperita* extract is added to the drinking water, compared to 1958g/chick and 1970g/chick at 2000ppm and 4000ppm, respectively.

Table 3.2.2: Body weight of Broilers (Gram/Chick) During Experiment

Day		1st	7th	14th	21st	28th
Group						
Pre-Inoculation	2000ppm	43.56a	160.12a	446.76a	597.87a	930.22a
	4000ppm	43.45a	135.10c	261.22f	391.22h	565.14j
	6000ppm	44.02a	112.87d	250.22g	329.54j	525.44k
Post Inoculation (7-Days)	2000ppm	44.23a	151.22b	353.98b	472.87c	721.76c
	4000ppm	43.86a	152.87b	327.34c	451.76d	688.44e
	6000ppm	44.45a	150.65b	316.76d	426.76f	633.44g
Post Inoculation (14-Days)	2000ppm	43.56a	150.65b	352.34b	500.68b	793.44b
	4000ppm	44.02a	152.32b	326.76c	417.44g	616.76h
	6000ppm	44.02a	149.30b	316.76d	388.40h	587.44i
Comparative		44.66a	150.65b	309.54e	423.44fg	643.98f
No Treatment, only inoculation		43.77a	150.22b	265.22f	341.76i	515.66l
No Treatment, No inoculation		44.22a	150.65b	322.87cd	443.98e	710.58d

To examine the variation in total body weight among all broiler groups, total body weight was monitored throughout the experiment. Maximum weight gain of 930g was seen in chicks given 2000ppm of *M. Piperita* extract from 1st day, and minimal weight gain of 515g was seen in chicks that were not given *M. Piperita* extract.

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Table 3.2.3: Chicks FCR (g feed/g body weight)

Week		1st	2nd	3rd	4th	Total
Group						
Pre-Inoculation	2000ppm	1.78c	0.68g	1.62f	0.56g	2.12j
	4000ppm	1.94b	1.52ab	1.88ef	1.16b	3.50c
	6000ppm	2.63a	1.54a	3.04bc	1.04c	3.78a
Post Inoculation	2000ppm	1.58e	0.96fg	2.04cd	0.78ef	2.68i
(7-Days)	4000ppm	1.56f	1.14c	1.82ab	0.82e	2.86g
	6000ppm	1.60d	1.16bc	2.24cd	0.94d	3.08f
Post Inoculation	2000ppm	1.60d	0.98fg	1.60f	0.70f	2.66ij
(14-Days)	4000ppm	1.58e	1.14c	2.66c	1.02c	3.17e
	6000ppm	1.60d	1.19bc	3.38a	0.98d	3.37d
Comparative		1.61cd	1.22e	2.06cd	0.88e	3.03fg
No Treatment, only inoculation		1.61cd	1.18f	3.16b	1.16b	3.72b
No Treatment, No inoculation		1.61cd	1.22e	1.95e	1.36a	2.74h

To evaluate the variation in feed conversion ratio (FCR) among different broiler groups, the FCR was determined on a weekly basis. Statistics show that the pre-inoculation 2000ppm group among the experimental broiler groups had the best FCR, but the post-inoculation 14-days 2000ppm group's FCR was only marginally better.

3.3 Clinical Records

The broilers were checked daily for signs of sickness and mortality. The chickens that had received the *E. coli* inoculum became upset and acquired rough feathers. They were significantly more reluctant to move than the group that had not received the vaccination, which showed no symptoms of illness. Compared to the post-inoculation treatment groups and other groups, the pre-inoculation treatment groups experienced fewer severe clinical symptoms. At the end of the trial, the comparison group of broilers showed only mild clinical signs.

In contrast to the other groups, only 4 chicks in the "No Treatment, only inoculation" group died.

3.4 Confirmation of E. Coli infection

The presence of the colibacillosis infection was determined by cultivating the blood from the liver and spleen of deceased broilers on Mueller hinton agar. In the groups that received no therapy, the presence of E. Coli colonies after 24 hours of culture indicated the infection with collibacillosis.

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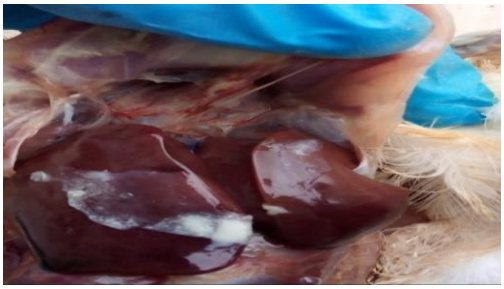

Figure 2: Fibrin layer on Liver

4. DISCUSSION

A test was done in vitro to see how well *M. piperita* extract worked against E. coli. The outcomes demonstrated that the extract of *M. piperita* is antibacterial against E. Coli. A zone of inhibition of 18.66mm is created by the extract. These findings differ slightly from those of (Zamin et al., 2013), which showed that the *M. piperita* leaf extract created an inhibitory zone of 29.31 mm against *E. coli*. This is for the reason that we utilised ethanolic extract, whereas He used cold water extract. Additionally, the phytobiotic concentration varies significantly based on biological factors (such as plant species, growth environment, and harvesting conditions), industrial factors (such as purification, maintenance and extraction), and storing conditions (temperature, oxygen tension, time and light). Additionally, the results of (Pramila et al., 2012) are consistent with our findings; they found that the mint leaves' extract had significant antifungal and antibacterial activity against a range of bacteria and fungi. As a result, we also draw the conclusion that the *M. Piperita* extract has antibacterial action. Our findings are consistent with those of (Zaidi & Dahiya, 2015), who found that *M. piperita* produces an inhibitory zone between 17 and 19 mm against several bacteria. And based on our findings, the peppermint extract causes an inhibitory zone to measure 18.66mm. Our findings were corroborated by (Jeyakumar et al., 2011), which found that the test organisms, including *Bacillus subtilis*, *Klebsiella pneumoniae*, *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were suppressed by *M. Piperita* oil.

Invivo test shows that Different experimental diets provided to broilers resulted in variations in body weight gain, feed intake, and FCR. Despite the fact that it was thought that adding dietary herbs would enhance broiler development performance (Cross et al., 2007). We observed that adding *M. Piperita* to broiler feed can improve feed uptake. Additionally, moderate supplementation—not too much—can improve the broilers' performance in terms of weight gain and FCR. According to our research, feeding broilers with 6000ppm *M. piperita* can boost feed intake and limit weight gain. Our findings are consistent with (Asadi et al., 2017), which found that adding M. Piperita leaf powder to chicks' diets slightly enhances weight gain. Our findings showed that broilers given the E. Coli inoculum gained less weight and consumed less feed. Our findings were comparable to those of (Hussein, 2021) who gave M. Piperita to chicks whose weight gain was impaired as a result of the Eimeria tenella. According to oour results the highest weight

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gain was observed in the group which was taking M. Piperita extract 6000ppm from the day of arrival. This finding is in line with that of (Al-Kassie, 2010), which found that M. piperita significantly affected feed consumption. Another study done by (Petričević et al., 2021) found that broiler diets containing 0.6% M. piperita significantly effect daily feed gain.

According to our research, broilers treated with M. Piperita's extract had an increase in body weight, but as the dose's concentration and duration increased, a weight loss was also seen in the treated broilers. This finding is consistent with (Asadi et al., 2017), which found that adding peppermint leaf powder to chicks' diets somewhat boosts their weight gain. Our findings were comparable to those of (Hussein, 2021) who gave peppermint to chicks whose weight gain was impaired as a result of the E. tenella. The weight gain was accelerated when the chicks were given peppermint. Our findings are consistent with those of (Hasan & M'Sadeq, 2020), who found that M. Piperita supplementation enhances performance of broiler in terms of increase in weight and morphology of gut. Additionally, the research of (Ocak et al., 2008) confirms our finding that peppermint has a beneficial impact on weight intake.

In current we observed best FCR in the chicks that were given *M. Piperita* extract (2000ppm) from day of Arrival. Our findings support those of (Al-Kassie, 2010), according to which adding a small amount of *M. Piperita* to the broiler increases the average feed conversion ratio. According to another study (Asadi et al., 2017), giving *M. Piprita* in a moderate amount had a substantial impact on FCR.

The broilers were routinely checked throughout the experiment for clinical signs of illness and mortality. Chicks given E. Coli inoculum showed signs of the disease. The signs were less severe in the groups that got medication, although the chicks still had rough feathers, had trouble moving, and less weight. Only those chicks in the group who received no care perished. Additionally, the remaining chicks from other groups only showed indications for a short while before they fully recovered. The necropsy of dead chicks revealed that they had swollen livers, minor fibrinous effusion, and pericardium lesions with fibrin deposits. The afflicted organs in the chicks were their liver, heart, and air sacs. The culture of liver and spleen blood on Mueller Hinton agar demonstrated the presence of infection. The results from cultured blood demonstrated that Broilers that had no therapy had E. Coli in their systems. At the end of the experiment, the chicks who received M. piperita leaf extract showed no signs of illness. The findings of (Hussein, 2021) indicating M. Piperita has a strong effectiveness in preventing coccidiosis-related decreases in intestinal health and broilers' performance are consistent with our findings.

When the Broiler strain of *E. coli* was tested for antibiotic resistance, we discovered that there was no amoxycillin resistance present. Because not all strains are resistant to antibiotics, some strains, such as those containing "Extended Spectrum Beta-Lactamases," are resistant to antibiotics, which is why our results did not agree with those of (Olorunmola et al., 2013), in which 137 isolates were tested and found to be almost resistant to commonly used antibiotics (ESBLs).

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The antimicrobial activity of Amoxycillin and *M. piperita* extract against E. coli in broiler chickens is identical when compared, but the weight gain of the chicks treated with Amoxycillin was reduced as compared to the weight gain of the chicks treated with *M. Piperita* extract. Our findings concur with those of (Izat et al., 1989), who investigated the impact of antibiotics on the production of commercial broiler chicks. They claimed that the antibiotic treatment only slightly decreased the proportion of weight loss.

5. CONCLUSION

According to the study's findings, *M. Piperita*'s ethanolic extract shows antibacterial action against *E. coli* both in vivo and in vitro. It was determined that providing the ethanolic extract to the broiler chicks could shield them from contracting colibacillosis. However, it was also discovered that administering *M. Piperita* ethanolic extract at higher concentrations (4000ppm and 6000ppm) for longer periods of time can decrease the weight gain of broilers, whereas administering the extract at a lower concentration, 2000ppm, can increase weight gain while also preventing infection. Additionally, we concluded that *E. coli* did not exhibit any type of resistance to the antibiotic amoxycillin. Both in-vitro and in-vivo tests using the antibiotic Amoxycillin and the *M. Piperita* extract both shown an inhibition of *E. coli*. However, compared to chicks treated with *M. Piperita*, those receiving Amoxycillin had less weight gain. It was determined that a dose of 2000 ppm is safe from death and aids in weight gain. 6000ppm and 4000ppm also work, although due to the large dose, weight loss was seen.

RECOMMENDATIONS

The results of the current investigation suggest that:

1. *M. Piperita* extract should be administered at a lower concentration—less than 2000 ppm—in order to assess its effectiveness against E. coli and its impact on weight.

2. The Extract needs to be evaluated in the broiler against other pathogenic bacteria.

3. For enhanced productive performance, ethanolic extract of *M. Piperita* at a 2000 ppm concentration should be added to water.

Author's contribution:

Waleed Ahmad conducted this research study for partial fulfilment of requirements for reward of M. Phil degree, contributed in data collection, laboratory analysis, interpretation of data and writeup of research publication. Naqash Khalid supervise the experiment and contributed in study method, gathering of data, laboratory analysis and interpretation of data. Shumaila Noreen supervised the experiment and contributed in study method, data interpretation and writeup of the research publication. Mazhar ul Islam aided in provision of extract and help in data collection. Muhammad Mujtaba contributed in research design and laboratory analysis. Muhammad Ayaz contributed in research design, laboratory analysis and writeup of article. Zaheer Ahmed contributed in data collection, laboratory analysis, data analysis and writeup of research publication.

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