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RESEARCH ARTICLE

COMPARATIVE STUDY OF THE ANTIBACTERIAL ACTIVITY OF CINNAMON AND ORIGAN ESSENTIAL OILS AND THEIR PRIMARYCOMPONENTS ON AVIAN *Escherichia coli* STRAINS.

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Abstract

The emergence of bacterial resistance to the usual antimicrobial agents is nowadays a major challenge in therapeutics for both humans and animals. As a result, the search for new antimicrobial agents has become a necessity. In this context, a great interest was focused on naturally occurring substances with antibacterial activity. The present study consists of evaluating the antibacterial activity of two essential oils (EO) from two aromatic and medicinal plants commonly used in humans in Morocco, namely, oregano (*origanum vulgare*) and cinnamon (*Cinnamomum cassia*), and their primarycomponents, carvacrol and cinnamaldehyde respectively, against antibiotic-resistant*Escherichia coli* strains of avian origin. Extraction of the EO was carried out by hydrodistillation, and their chemical analysis was carried out by gas chromatography (GC).The cinnamon EO is mainly composed of cinnamaldehyde (nearly 90%), followed by beta-caryophyllen (6.1%), cinnamyl acetate and eugenol with (2.75%) and (0.46%); carvacrol is the primary component of oregano EO (65.51%), followed by gamma-terpinene (11.49%), p-cymene (6.27%), thymol (4%) and borneol (3.58%). The aromatogram showed interesting antibacterial activity of EO and their active ingredients against the used bacterial strains. The diffusion method on agar medium showedthat EO of cinnamon was more active than oregano EO against *E. coli*strains, with mean inhibition zones of 25.8+4.5mm and 17+3.7 mm, respectively. The obtained MICs showed also that cinnamaldehyde was more potent than carvacrol, with average MICs of 0.039% and 0.106%, respectively.

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Introduction:-

During le few last decades, the use of antibiotic agents (AB) encountered two major constraints namely the emergence of antimicrobial resistance andthe strong consumer awareness on the health impact of drug residues in food of animal origin. In front of this situation, the discovery of new antibacterial agents has become more than indispensable. To be innovative and bypass aforementioned problems, the new generation of antibiotics should be safe for the consumer and not or less subjected to bacterial resistance. One of the most promising alternatives toreach such an objective would be the exploration of natural resources as they constitute, by their biodiversity, the largest reserve of bioactive substances. Among these alternatives, plant extracts including essential oils (EO) have a high potential to replace antibiotics.

Essential oils (EO) are aromatic and volatile oily liquids obtained from plant material. They are naturally formed in special cells or groups of cells found in leaves and stems (Oussalah and *al.,* 2006). Although the antibacterial properties of EO have long been recognized, the recent interest in natural derived antimicrobials has led to renewed scientific interest in these substances. Indeed, EOs have many biological properties including antibacterialeffects, without development of resistance phenomenon, antioxidant activity and the stimulation of the immune and digestive processes (Bouhdid and *al.,* 2009). Their antimicrobial activity has been shown *in vitro* by numerous studies (Smith-Palmer and*al.,* 1998; Hammer and*al.,* 1999; Dorman and Deans, 2000; Elgayyar and*al.,*2001), primarily against pathogenic bacteria such as *Clostridium perfringens, Escherichia coli, Staphylococcus aureus, Salmonella typhimurium, Listeria monocytogenes and Yersinia enterocolotica* (Dorman and Deans, 2000; Fabio and*al.,* 2003). Antimicrobial activity of EO is particularly assigned phenols (such as carvacrol, thymol and eugenol), alcohols (such as linalool) and aldehydes (such as cinnamaldehyde). Thus, the antibacterial activity of an EO is determined byits level of these constituents (Bouhdid and *al.,* 2009).
The objective of the present study was to evaluate the*in vitro* antibacterial activity of two essential oils, namely, *Origanum vulgare* EO and *Cinnamomum cassia* OE and their major components, carvacrol and cinnamaldehyde, on *Escherichia coli* strains of avian origin resistant to Enrofloxacin and Florfenicol.

Material And Methods:-

Materiel

Active materials

The essential oils of cinnamon (*Cinnamomum cassia*) and oregano (*Origanum vulgare*), obtained by hydrodistillation, were provided by the agro-food laboratory of IAV Hassan II Institute.
Cinnamaldehyde (>95%) and carvacrol (>98%) were purchased from Sigma-Aldrich.

Bacterial strains

The antibacterial activity of essential oils and their active ingredients was evaluated on *Escherichia coli* reference strain (*ATCC25922*) and on 40 *Escherichia coli* strains of avian origin isolated and identified in the laboratory of Avian Pathology at the IAV Hassan II Institute. The isolation of *E. coli* was made from the lung, liver, heart, bone marrow of the broiler.

Methods:-

Essential oil extraction

The apparatus used for the hydrodistillation of essential oils is of the Clevenger type (Clevenger, 1928). It consists of a balloon heater, a Pyrex glass flask where the dried plant is placed and distilled water, a vapor condensing column (refrigerant) and a Pyrex glass collector which also receives the distillation extracts. The essential oils obtained are stored in a refrigerator in a brown glass bottle sealed at 4 ° C and in the shade.

Essential oil chemical analysis

The obtained EO were analyzed by gas chromatography (GC) according to the following conditions: Chromatograph: PEKRIN ELMER Autosystem XL; Detector: FID; Column: ELITE PE-5; Injector temperature: 250 ° C; Detector temperature: 340 ° C; Temperature program: 50 ° C (4min), 5 ° / min, 230 ° (20min); Injection volume: 0.02ml using a syringe; Carrier gas: N2 (nitrogen); Flow rate of the carrier gas: 1 ml / min.

Determination of Antimicrobial Activity

The antibacterial activity of EO was evaluated using two different methods.

The Aromatogram

The aromatogram technique (diffusion technique in solid medium) is used to evaluate the sensitivity of bacteria to EO (Yashphe and*al.*, 1979). Pure EOs are dispersed in 0.2% agar solution, to promote contact between germs and compounds. According to the method described by Remmal and*al.*,(1993a),a 10%initial emulsion is prepared by adding 100 µl of EO to 900 µl of a sterile aqueous solution containing 0.2% (w/v) of agar. Then sterile petri dishes (9 cm) containing 9 ml of Muller-Hinton medium were inoculated with the bacterial suspension of a density of 10^6 CFU/ml of medium; after solidification of the medium, 6 mm diameter sterile disks, made from the Whatmanpaper, were deposited on the surface of the medium and impregnated with 5 µl of the solution of EO, Carvacrol or Cinnamaldehyde. In the case of the combinationof two products, each solution was used at 2.5 µl. The petri dishes are then placed at a temperature of + 4 ° C. for about one hour, and then incubated at 37 ° C. for 18 to 24 hours. The sensitivity of the organisms tested with EO is characterized by the formation of a clear circle (zone of inhibition) around the disks containing these oils. The inhibitory effect of EO was evaluated by determining the diameter of the inhibition zone formed in mm (Matasyoh and *al.*, 2007).

Macrodilution technique in a liquid medium

The minimum inhibitory concentration (MIC) of the EO was determined by the macrodilution method (Remmal and*al.*, 1993). Briefly, a stock solution is obtained by dissolving 20µl of EO solution with 0.2% of agar in 2 ml of TSB, then serial dilutions of the obtained concentration(between 1% and 0.0039% (v/v))were carried out in tubes containing 1 ml of TSB broth. A seeded tube without EO was used as a positive control and, sometimes, a tube containing EO alone serves as a negative control. The different tubes are inoculated with 10 µl of the bacterial suspension with a density of 10^6CFU/ml. The tubes containing different concentrations of EO and the positive control are incubated at the same time at 37 ° C for 18-24 hours.The minimum inhibitory concentration (MIC) is the lowest concentration of oil to which no bacterial growth is visible to the eye after incubation for 24 hours at 37 ° C (NCCLS, 1999).

Results And Discussion:-

Essential oil chemical analysis

Chemical composition of EO of cinnamon

The composition of cinnamon EO used in our study is given in Figure 1. This figure indicates that Cinnamaldehyde is the main component accounting for almost 90%, followed by beta-caryophyllen (6.1%), cinnamyl acetate (2.75%) and eugenol (0.46%). These results are somewhat different from those specified by the European Pharmacopoeia. Indeed, according to the European Pharmacopoeia (2011), levels of the cinnamaldehyde isup to 75%, and those of eugenoland β-caryophyllene are up to 7.5% and1 to 4% respectively.Theses differences might be ascribed to multiple factors such as extraction mode and analysis technique.

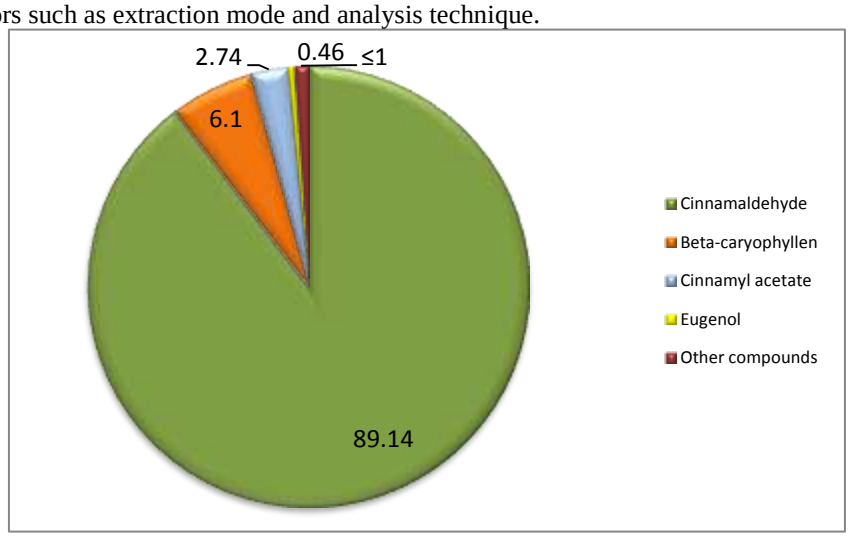

Figure 1:-Chemical composition of cinnamon EO

Chemical composition of EO of oregano:-

The figure 2 shows chemical composition of the EO of oregano (*origanum vulgare*). Overall, 25 constituents, dominated by carvacrol (65.51%) were identified. The others components were gamma-terpinene (11.49%), p-

cymene (6.27%), thymol (4%) and borneol (3.58%); linalool, alpha-terpinene were detected with values less than 1%. Our results are qualitatively, but not quantitively, in agreement with the results reported by Bouhdid and *al*., (2008) and Hammouand *al.*, (2011), who showed that the most represented compound in the EO of oregano is carvacrol (30.53% and 36.31%), followed by the thymol (27.50% and 16.88%) according both studies respectively.

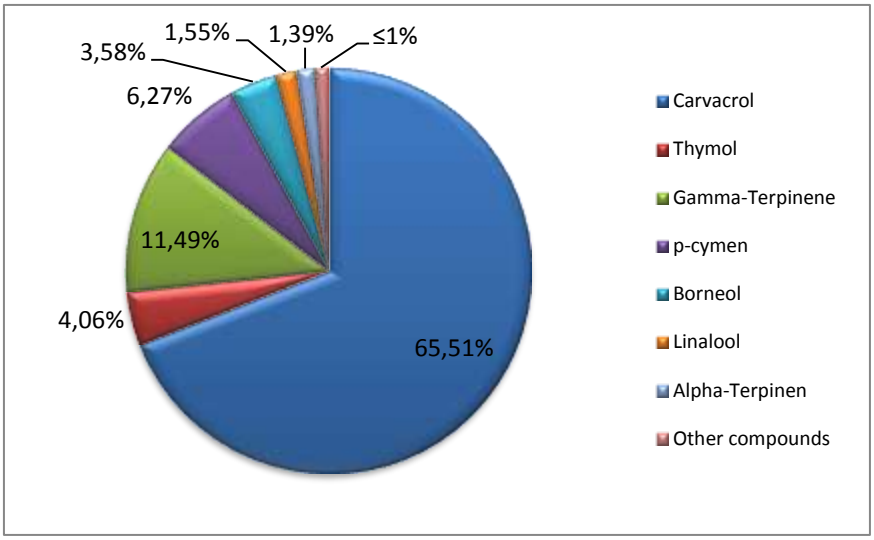

Figure 2:-Chemical composition of oregano EO

Antibacterial activity of essential oils

Out of a total of 40 strains, cinnamon's EO was found to be the most effective against bacteria with an average of 25.8 + 4.5mm inhibition zones compared to 17 + 3.7 mm with oregano EO (figure 3).

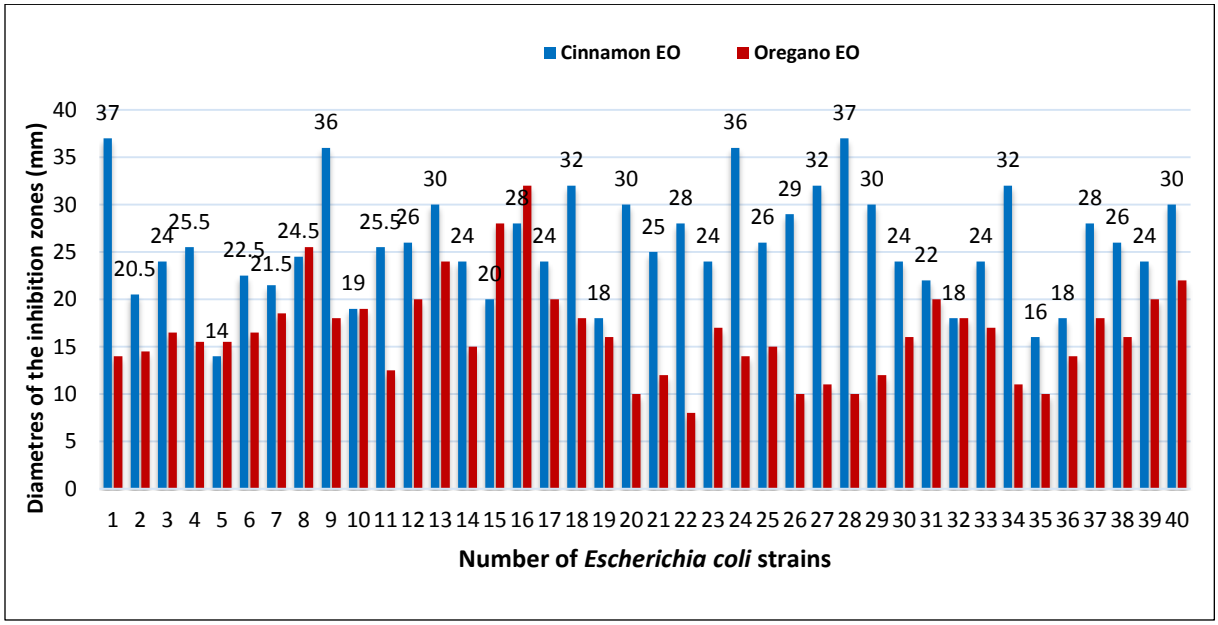

Figure 3:-Inhibition zone diameters (mm) generated by the EO of cinnamon and oregano on *E.coli* strains of avian origin

The EO of cinnamon and oregano are among the EO with the strongest antibacterial power (with thyme, savory and clove). The bactericidal activity of cinnamon EO has been described on various strains of *Escherichia coli*, with a power exceeding streptomycin (Senhaji and *al.*, 2007). The antibacterial effect of oregano EO against *E. coli* has been reported by many studies (Ultee and *al.*, 2002).

The superiority of cinnamon EO compared to that of Oregano observed in our study agrees with the result observed by Bouhdid and *al.,* (2012) has been confirmed in several studies, including the one conducted, while oregano has shown interesting antibacterial activity, particularly against strains of *S. aureus*. However, strains of *Ps. Aeruginosa* expressed some resistance to this oil. These strains were more sensitive to cinnamon EO which is predominantly composed of cinnamaldehyde (Inouye and *al.,* 2001; Friedman and *al.,* 2002). Our results also agree with those of Mithand*al.,* (2014), commercial cinnamon (*Cinnamomum cassia*) and oregano (*Origanum compactum*) essential oils were tested by diffusion technique, against some pathogenic bacteria, cinnamon EO expressed an effect significant inhibitor and superior to that of Oregano, *Listeria monocytogenes*, *Salmonella Typhimurium*and *Escherichia coli* (Mith and *al.,* 2014). These promising antimicrobial effects can be attributed to the presence of major bioactive constituents, including cinnamaldehyde and carvacrol. Our results differ from those of Valero and Salmeron (2003), because among 11 essential oils tested against *Bacillus cereus*, Oregano EO was more active than Cinnamon EO (Valero and Salmeron, 2003).The essential oils of oregano and cinnamon are potential candidates for an application in the treatment of infections. These two essential oils act by increasing membrane permeability, inducing collapse of membrane potential and inhibiting respiratory activity, leading to a loss of cell viability (Bouhdid and *al.,* 2012).

Antibacterial activity of the purecomponents (Cinnamaldehyde and Carvacrol)

Activity on reference bacterial strains

The EO of cinnamon and its main active ingredient cinnamaldehyde showed a higherantibacterial activity than the EO of Oregano and carvacrol. Similarly, the activity of cinnamon EO was, on average, more potent than that of cinnamaldehyde (Table 1).

Table1:-Inhibition zone diameters (mm) generated by tested products on the reference *E. coli* strain.

Test	Cinnamon EO	Cinnamaldehyde	Oregano EO	Carvacrol	Cinnamaldehyde + Carvacrol
1	26	29	16	21	32
2	32	24	18	25	34
Average ± SD	29 ± 3	26,5 ± 2,5	17 ± 1	23 ± 2	33±1

The *E. coli* strain showed variable sensitivity to EO and pure components. It is more sensitive to cinnamon EO than it is to cinnamaldehyde, but more sensitive to carvacrol than oregano EO. However, the cinnamaldehyde and carvacrol combination showed greater inhibitorypower than cinnamaldehyde and carvacrol alone. The antibacterial activity of cinnamaldehyde has been mentioned in the work of Mith and *al.*,(2014) who have shown that this active principle has high antimicrobial activity against *E.coli ATCC 35150*; *E. coli O157: H7 S0575; Salmonella typhimurium ATCC14028* and *Pseudomonas fluorescens ATCC1352*, whereas carvacrol, with the exception of *P. florescence*, had a lower activity. This result is consistent with our observation in the present study. However, according to another study, carvacrol had the strongest effect against *Listeria monocytogenes*, followed by thymol, eugenol, cinnamaldehyde and iso-eugenol (Yamazaki and *al.,* 2004).

Avian strains

Cinnamaldehyde + Carvacrol and their association

Ten avian strains *E. coli* resistant to enrofloxacinbut sensitive to colistin were tested to evaluate the inhibitory effect of cinnamaldehyde and carvacrol and their association. The results of the aromatogram are shown in Table 2.

Table 2:-Inhibition zone diameters (mm) generated by the active products and their association **for *E. coli* avian**

Test	Cinnamaldehyde	Carvacrol	Cinnamaldehyde+ Carvacrol
1	20	16	30
2	22	20	40
3	30	20	40
4	28	30	28
5	32	20	24
6	32	20	24
7	28	20	26
8	32	16	36
9	36	22	30
10	38	14	32

Average ± SD	**30±4**	**20±3**	**31±5**

The pure components showed antimicrobial activity which resulted in varying zones of inhibition depending on the product. They ranged from 14mm to 30mm for carvacrol and from 20mm to 38mm for cinnamaldehyde. Corresponding respectively to means of 20±3mm and 30±4mm. For the cinnamaldehyde and carvacrol combination, the mean value was 31±5mm with variations of 24 to 40 mm depending on the strain. The results confirm the high antibacterial activity of cinnamaldehyde compared to carvacrol on *E. coli* strains.

Essential oils and theirmajor active ingredients

The results show that the highest inhibitory effect against avian *E. coli* strains was obtained with cinnamon products, with mean values of 25.1±4.3mm and 23.35±5.4mm for EO and cinnamaldehyde respectively. While these values averaged only 16.7±2.2mm and 19.35±1.6mm for oregano EO and carvacrol respectively. The figures 4 and 5 illustrate this aspect.

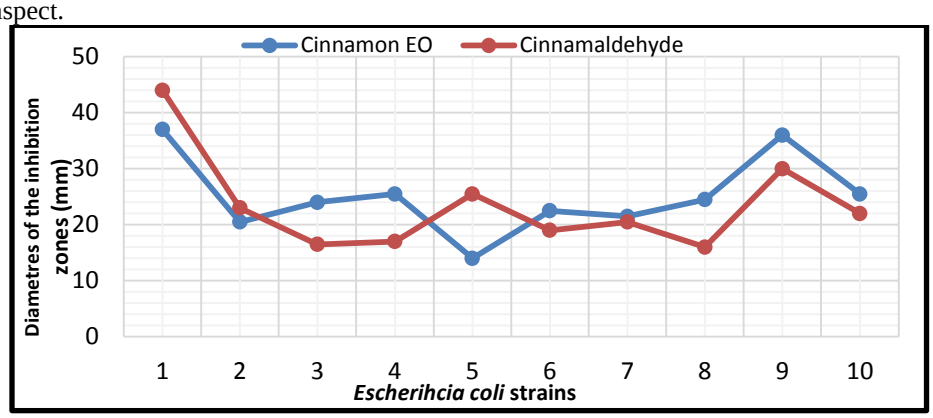

Figure 4:-Comparison of the activity of the EO of Cinnamon and cinnamaldehyde

The antibacterial activity of cinnamon EO was, on average, more potent than that of cinnamaldehyde (Figure 4). This suggests that the minority compounds present in the EO have a significant effect. On the other hand, the figure 5, shows that carvacrol is more powerful than oregano EO. This could suggest that antimicrobial activity of the oregano EO is mainly due to carvacrol which represents only 65% in this EO in our study. This result might be also explained by the presence of antagonistic molecules in the EO.

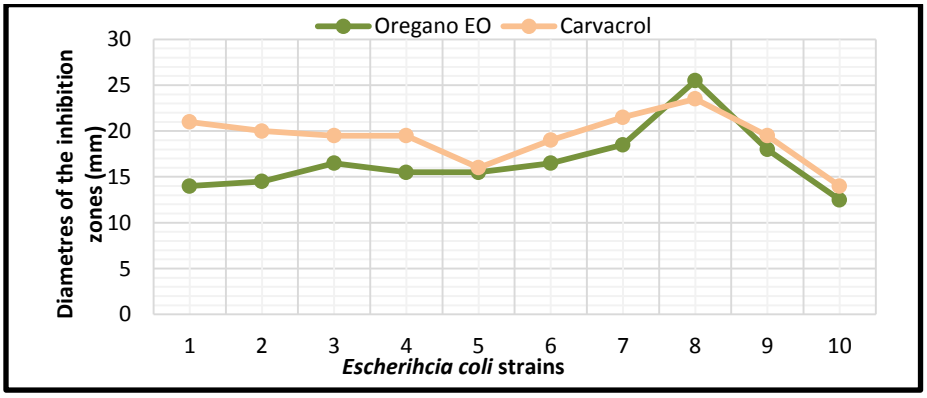

Figure 5:-Comparison of the activity of the EO of oregano and carvacrol

Referring to the results obtained in the present study, it is legitimate to conclude that the antibacterial activity of EO is directly influenced by the nature and the proportion of their constituents. The most represented compounds are often responsible for the observed antibacterial activity (Dormans and Deans 2000; Kalemba and Kunicka 2003). But according to several authors (Chorianopoulos and *al.*, 2004; Sokmen and *al.*, 2004; Peñalver and*al.*, 2005; Bounatirou and *al.*, 2007; Cao and *al.*, 2009), in addition to major compounds, secondary components interact with each other to give an antimicrobial effect to EO. Some studies have concluded that minor components play a role in activity and may have an effect or influence the EO overall activity (Gill and *al.*, 2002; Rota and *al.*, 2008).

According to Kalemba and Kunicka (2003), the sensitivity of a microorganism to EO depends on the properties of the latter and the microorganism itself. In general, Gram- bacteria are more resistant than Gram + due to the structure of their outer membrane. Indeed, the outer membrane of Gram- is richer in lipopolysaccharides and proteins that make it more hydrophilic, and thus prevents hydrophobic terpenes from adhering to it. Nevertheless, some low molecular weight phenolic compounds such as thymol and carvacrol may adhere to these bacteria by attachment to membrane proteins and lipopolysaccharides through their functional groups and thereby reach the more vulnerable inner membrane (Dorman and Deans, 2000).

Minimal Inhibitory Concentration of active ingredients:-

MICs of cinnamaldehyde and carvacrol were calculated using ten *E. coli* strains of avian origin (Figure 6). The average MIC values for each of the active ingredients are 0.039% and 0.106% for cinnamaldehyde and carvacrol respectively, which confirms the high inhibitory activity of cinnamaldehyde in comparison with carvacrol.

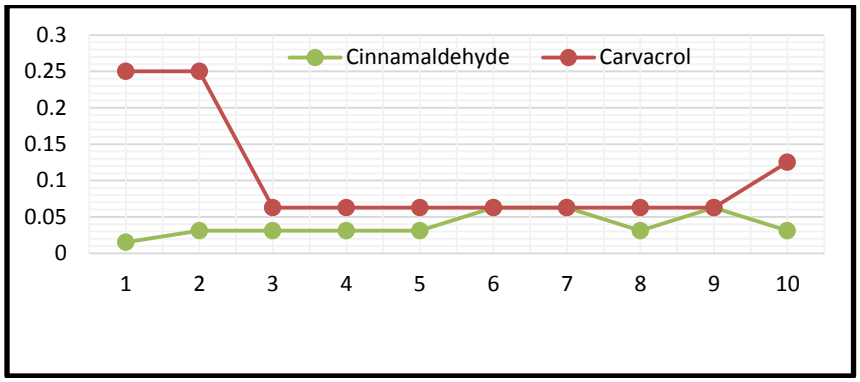

Figure 6:-MICs distribution of cinnamaldéhyde and carvacrol according to strains avian *E.coli*

The comparison of the MIC means d the diameters of the inhibition zones of the two products confirms the higher inhibitory activity of cinnamaldehyde in comparison with carvacrol. The antibacterial effect of cinnamaldehyde was demonstrated in liquid medium against *Listeria monocytogenes* and *Escherichia coli* with MICs equivalent to 0.25% for both bacterial strains (Hawkins and Savannah, 2014). A similar result was obtained in another work, where the cinnamaldehyde MIC against *E. coli* was 0.25% (Kim and *al.*, 2008).

Conclusion:-

The cinnamon and oregano EOs and their major constituents have shown an important inhibitory effect on *Escherichia coli* strains of reference and of avian origin, with a remarkable antibacterial activity of cinnamon EO and its active ingredient, cinnamaldehyde, compared with oregano EO and its major constituent, carvacrol. The effect of the combination cinnamaldehyde and carvacrol was stronger than the activity of the two active ingredients tested separately on *E. coli* strains.

Conflict of interest:

We would like to confirm that there is no conflict of interest associated with this publication.

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