

# The inhibitory and stimulatory behavior for some of chemical compounds and watery extract of black tea and Arabian coffee on the swarming phenomenon of clinical isolates of *Proteus mirabilis*.



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## ARTICLE INFO

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## ABSTRACT

The effect of different 16 chemical compounds belonging to various categories, Organic and inorganic salts, detergents, surfactants, nutrients, carbohydrates and antiseptics, also, watery extractions of black tea and Arabian coffee were tested against swarming phenomenon of clinical isolates of *Proteus mirabilis* by central spot inoculation method .Triton X100, tris base, watery extract of black tea ,yeast extract and Glucose were appeared stimulatory effect on swarming diameter of *P. mirabilis* since it increased at 25, 49, 72, 77, 90 mm respectively with concentration increase, On contrary sodium acetate, sodium chloride, sucrose, EDTA, Tween 80 and phenol showed inhibitory effect on swarming diameter at 14, 12, 11, 10, 8, 1 mm respectively in comparison to swarming diameter of the control colony 17 mm. However starch, watery extract of Arabian coffee, SDS and peptone revealed various behavior against swarming. The extent of swarming differed according to tested chemical compounds, their concentration and the bacterial strains. Most of the compounds that inhibited swarming were those acted on flagellar mechanism and motility.

## Introduction

Among the most common human infections are those of the urinary tract, after *Escherichia coli*, *Proteus mirabilis*, a motile gram-negative bacterium, is an important pathogen of the urinary tract and is the primary infectious agent in patients with indwelling urinary catheters(1). The urinary tract infections (UTIs) which caused by *P. mirabilis* can be subdivided into two categories: hematogenous infections and ascending infections, in which bacteria colonize and step by step reach urethra, bladder, ureter, and at the end, the kidneys (2). Individuals suffering from urinary tract infections caused by *P. mirabilis* often develop bacteriuria, cystitis, kidney and bladder stones, catheter obstruction due to stone encrustation, acute pyelonephritis, and fever (3) *Proteus* rods, when present in the kidney, can cause severe histological damage, characterized as acute pyelonephritis (4).

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This bacterium has ability to express many virulence factors, some of these including urease(5), metalloprotease(6), hemolysin(7), Lipopolysaccharide(8), Outer-membrane proteins (OMPs) (9), and lecithinase that effective on human organs (10) in order to invade human urothelial cells it is need coordinately regulated these virulence factors with swarming motility (11). The swarming phenomenon helps to distinguishes *Proteus* rods from other members of the Enterobacteriaceae family (12). The presence of flagella on the surface of pathogenic and opportunistic bacteria has been thought to facilitate the colonization and dissemination from the initial site; *Proteus* rods when grown on a solid medium, these cells display swimming behavior and have a distinct morphology; i.e., they are motile, peritrichously flagellated (6 to 10 flagella per cell) rods, 1.0 to 2.0 mm in length, these bacilli, referred to as swimmer cells (7), Characterization of swarming-defective *Proteus* transposon mutants has indicated that a substantial number of proteins are involved in regulation of swarming , including FlhD2C2, FlhA, Umo, Lrp, RsbA, RsmA, SpeB and others, these are involved in regulation of swarming and virulence factor expression (13,14). Among these regulatory proteins, RsbA, which has been suggested to be a His-containing phosphotransmitter of

the bacterial two-component signalling system (15,7), it was act as a negative regulator of swarming differentiation and virulence factor expression in bacterium *P. mirabilis* (16), while protein *rsmA* is a global regulatory widely distributed among many bacteria (7). The integrated of the *RsbA*- and *RsmA*-dependent pathways with other signal pathways to regulate swarming and virulence factors expression is currently not known. *P. mirabilis* swarming requires the sensing and integration of a variety of environmental, cell-to-cell, and intracellular signals. These signals may include those transmitted by high population density, surface contact, peptides and amino acids, and intracellular cations; Although the mechanisms of signal sensing and transduction are still poorly understood, it is generally believed that signals may be sensed and transmitted by two-component regulatory systems and then cytosolic regulators, leading to a complex regulatory network in which the *flhDC* master operon may be the primary site for integration of signals (13,17). Stimulation of the *flhDC* operon initiates swarms cell differentiation, which involves the development of characteristic traits such as cell elongation, multinucleation, and hyper flagellation (18).

The major surface molecule of Gram-negative bacteria interacts with the host and, depending on the dose, induces an inflammatory response (19). The ability to invade cultured human urinary epithelial cells is associated with differentiated swarmer cells and not with undifferentiated swimmer cells (20).

The present paper aimed to detect the inhibitory and stimulatory effect of some of chemicals compounds on an important virulence factor named swarming phenomenon that helps clinical pathogenic isolate of *P. mirabilis* to invade human urinary tract and cause infection.

## Material and methods

### 1- Bacterial strains and culture conditions.

Eight clinical *Proteus* strains used in this study were obtained from Central Public Health Laboratory-quality control unit and from post graduates students Department of Biology, College of Science, University of Baghdad previously collected were collected previously from Central Child hospital and AL-Yarmouk hospital, these isolates were cultured on brain heart infusion broth at 37°C and on MacConkey agar plate (Oxoid). Pale non lactose ferment or with fishy odor colonies were selected and streaked on blood agar plates (blood agar base – Oxoid-supplemented with 5% of human blood) to observe swarming motility. These isolates were identified

by biochemical tests (21). API 20 E test (bio-Merieux) was employed for the confirmation of identification.

### 2- Maintenance of strains

Bacterial strains were maintained on deep Nutrient agar slant (Oxoid) for 5-6 weeks with periodic subculture and in sterilized filter paper for 10 weeks and nutrient broth (Oxoid) with 35 % glycerol at -20°C (6).

### 3- Chemicals

A total of 16 chemical compounds comprising organic and inorganic salts, detergents, surfactants, nutrients, carbohydrates, and other miscellaneous compounds in this study were obtained from BDH, Merck, Oxoid Ltd, or Sigma.

### 4-Effects of chemical compounds on swarming motility

The aqueous solutions (10 ml) of 16 different chemical compounds tested were distributed in test tubes at 10 ml and sterilized by autoclave at 121°C, 15 pound/in<sup>2</sup> for 30 minutes. Sodium acetate, Sodium chloride, Tris, SDS, Tween 80, Triton X100, EDTA, Urea, Glucose, Sucrose, Starch, Peptone and Yeast extract were prepared in distilled water at different concentration (0.5, 1, and 2 %). However, watery extraction of Black tea and Arabian coffee were prepared at 2.5, 5 and 10 % by mixed the required quantity with distilled water and heated at boiling degree 100 °C, Phenol was prepared at 0.25, 0.5 and 1 M). A tube contains 10 ml distilled water only was considered as control. All above tubes were inoculated with 50µl of overnight culture of *Proteus* and incubated at 37°C for 24 hours. Meanwhile, taken 5 µl of culture were taken to inoculate blood agar plates at the center and incubated at 37°C for 24 hours. The outer diameter of swarming zone from the point of inoculation was measured in millimeter and compared with control. The chemical compounds that produced a colony diameter greater than the control was considered stimulatory effect and if the value was less than the control, it was categorized as inhibitory effect.

## Results

Eight clinical isolates of *Proteus* were diagnosed by biochemical tests and API 20 E, five isolates belong to *Proteus mirabilis* and another three isolates belong to *Proteus vulgaris*. All eight isolates differ in their behavior of swarming phenomenon, one of them *P. mirabilis* was selected for further experiments due to their higher ability to express swarming (37 mm from initiate site). The inhibitory and stimulatory effects of various compounds on swarming of *Proteus mirabilis* are presented in figures 1-7. Among carbohydrates, the maximum swarming occurred in the presence of glucose

that possessed a striking stimulation. While sucrose showed an anti-swarming property on test cultures, starch differ in its behavior, , since that it developed partially efficacy and an inhibitory effect at 0.5 and 1 %, respectively, whereas it stimulated the swarming at 2% concentration (Figure1). Increasing the concentration of surfactant Triton X100 from 0.5-2% had much activator effect, whilst Tween 80 revealed an inhibitory effect. Regarding SDS a variable behavior was observed, it acts as an inhibitory agent at 0.5%-1% while no effect appeared

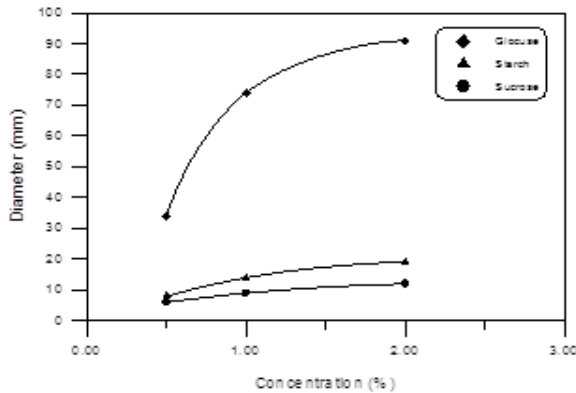


Figure (1): Effect of carbohydrates on the swarming of *P. mirabilis*

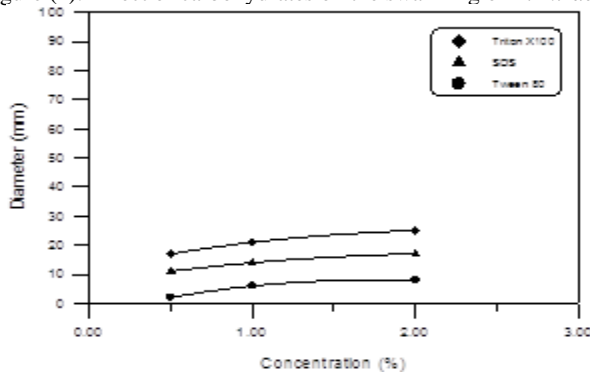


Figure (2): Effect of surfactants on the swarming of *P. mirabilis*

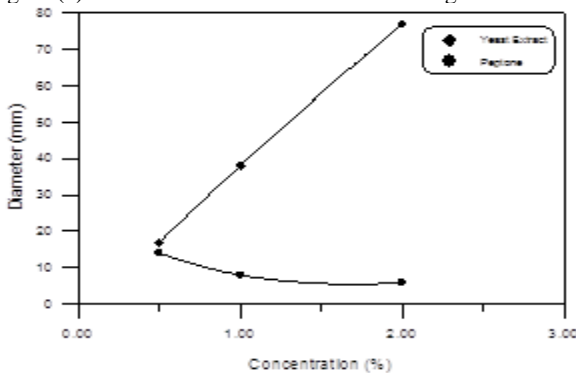


Figure (3): Effect of nutrients on the swarming of *P. mirabilis*

at 2% (Figure 2). The impact of nutrients on growth and swarming of *Proteus mirabilis* is depicted in Figure 3 and the effect of human traditional nutrients are

illustrated in figure 4. Peptone and Arabian coffee had inhibitory behavior on swarming, while yeast extract and tea were activators for swarming with increased concentration. Figure 5 shows the organic compounds, such as Tris base, being a good activator agent for swarming of *P. mirabilis* with increased concentration; the bacterial motility was stimulated only by 1% sodium acetate and inhibited when concentration was increased. Both inorganic compounds; sodium chloride and EDTA were arrested swarming phenomenon with increased concentration (Figure 6). The anti-swarming effect of phenol that recognized as antiseptics was shown in (Figure 7), It was considered as a good antibacterial agent because of its remarkable capacity to arrest swarming with increasing concentration.

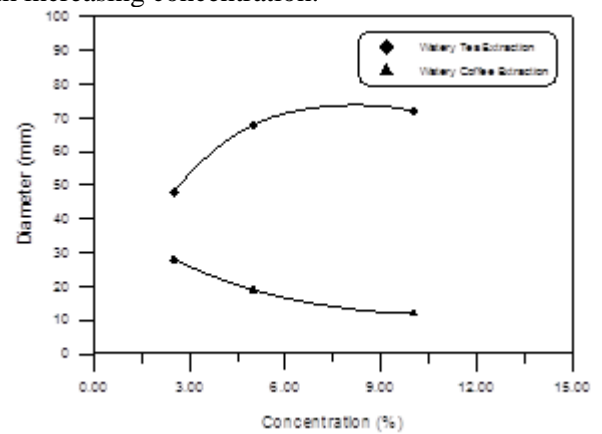


Figure (4): Effect of watery extracts of Tea and Arabian coffee on the swarming of *P. mirabilis*

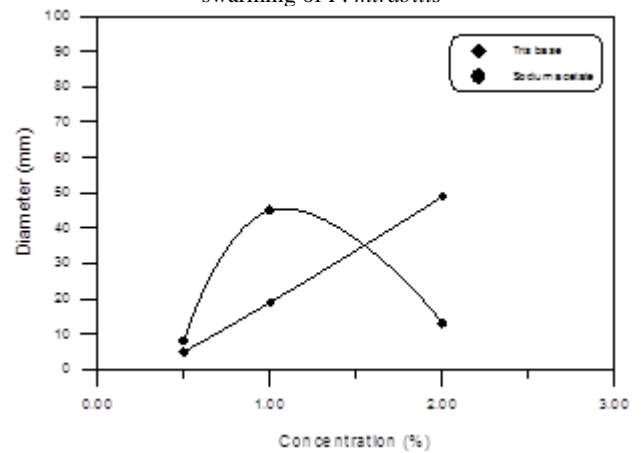


Figure (5): Effect of organic salts on the swarming of *P. mirabilis*

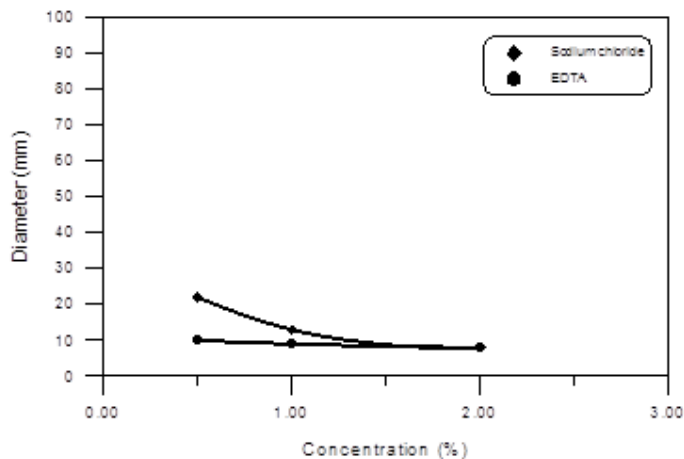


Figure (6): Effect of inorganic salts on the swarming of *P. mirabilis*

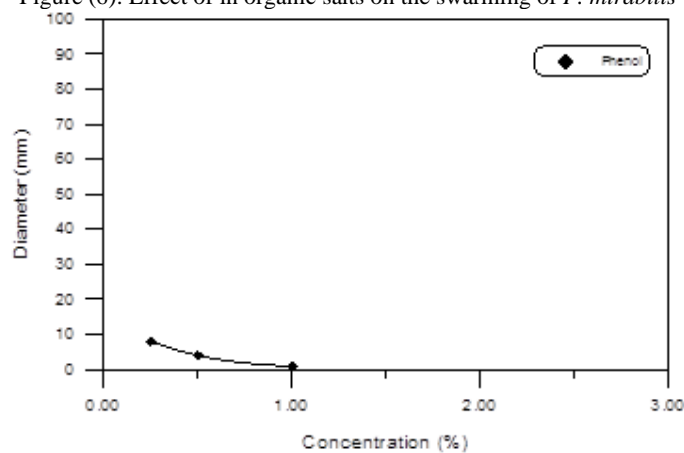


Figure (7): Effect of phenol on the swarming of *P. mirabilis*

## Discussion

The isolates varied in the ability to swarm phenomena, five isolates of *P. mirabilis* showed very clear swarming on semi-solid media. What's more one of them was recognized with diameter of swarming about 37 at 37°C for 24 hr. Furthermore, isolates of *P. vulgaris* developed smaller of swarming under same conditions. In this study, the variation in swarming expression for *Proteus spp* may attribute to different reasons related to isolates themselves (e.g. strain variation, their origin) or growth conditions such as: incubation temperature, media or expression of certain swarming genes. Such results are in agreement with finding of Liaw and hisco-workers (7). The present work results may proved that the pathogenicity of *P. mirabilis* more than *P. vulgaris* due to their ability to produce hemolysin, invade human urothelial cells and cause infections were needed to regulate their virulence factor coordinately with swarming motility (11). This explanation agreed with Senior's opinion when he observed that strong correlation between the ability of *P. mirabilis* to form swarming

growth and ability to produce protease, he also referred to non-swarming isolates of *P. vulgaris* invariably appeared to be non-proteolytic (22). *Proteus mirabilis* has the ability to promote infection of host during swarming and highly motile because the swarming cells could migrate through urinary tract and cause many infections. The swarming phenomena has been studied in different genera *Serratia spp.*, *Salmonella spp.*, *Aeromonas spp.*, *Bacillus spp.*, *Yersinia spp.*, *Pseudomonas spp.*, *Vibrio spp.*, *E. coli* and *P. mirabilis* (23). Swarm cell differentiation and swarming behavior are the results of complex sensory transduction and global control mechanisms. *Proteus mirabilis* swarming requires the sensing and integration of a variety of environmental, cell-to-cell, and intracellular signals and involves regulated expression of gene networks leading to morphological and physiological changes (13). The signals regulating swarming and the pathways for signal transduction are still poorly understood. In this paper, we present evidence that chemical compounds serve as environmental cues to affect *P. mirabilis* swarming. Specifically, carbohydrate such as glucose supports swarming motility with increased concentration, the diameter of swarming reached at maximum value 91mm at 2% concentration (figure1). This role may attribute to enhanced growth rate of swarm cells and utilized glucose as carbon source. The inhibitory effect of sucrose on swarming may assigned to that *P. mirabilis* is incapable to ferment sucrose. Moreover, the *P. mirabilis* lack the ability to express or produce amylase that used to degrade the complex carbohydrate such as starch. To our knowledge, there is no report refers to the effect of any carbohydrates on *Proteus spp.* swarming.

The swarmed cells have been found to be sensitive to sodium dodecylsulphate (SDS) and the diameter of swarming motility was reduced (figure 2), this agent could inhibit translocation of bacteria on solid media. The ability of SDS to inhibit swarming motility may not be unconnected with the demonstrative evidence of its biocidal effect on signal molecules involved in biofilm formation (24), This result was in agreement with Iwalokun and his co-workers results as pointed out to the ability of SDS to inhibit swarming motility at 0.25-1.25 % concentration (25). Yeast extract is among other nutrients that form basic ingredients in culture media also has support action lead to enhanced swarming with increased concentration. The maximum swarming diameter was 77 mm with 2% of yeast extract (figure 3) given that yeast extract is a good source of vitamins that has stimulatory effect on bacterial growth rate. The stimulatory and inhibitory action of coffee and black tea is still unknown. The diameter of swarming was



enhanced with increased concentration of black tea extract; it reached 72 mm from the initiate site of motile (figure 4). Trihydroxymethylglycine (Tris) is widely used in buffer systems involving microbial culture and enzymology, biotechnology and genetic engineering but there have not been clues to show whether this substance could inhibit translocation of bacteria on solid media in vitro. In this study, tris stimulatory effect on swarming was noticed with increased concentration. The diameter of swarming motility was recorded as 5, 19 and 49 mm at 0.5, 1 and 2% concentration, respectively (figure 5). These results were incompatible with Iwalokun and his co-workers who observed effect of tris base as anti-swarming at 0.25- 1.25 % concentration (25). Figure 6 showed that EDTA inhibited swarming of *P. mirabilis*. The anti-swarming effect of EDTA is attributed to its ability to chelate and remove ions like  $Ca^{+2}$  which impair motility (26). The capacity of phenol to arrest swarming with increasing concentration may attribute to their role as antiseptics (figure 7). Noteworthy, in this study, role of other tested compound on swarming of *P. mirabilis* were shown to have variable effect. There are several chemical compounds used for the inhibition of *P. mirabilis* swarming including ferrous ions (27), charcoal (28), ethanol, bile salts, sodium azide and p-Nitrophenylglycerol (PNPG) (29).

There are few information about effect of organic and inorganic salts, detergents, surfactants, nutrients, carbohydrates and antiseptics on the swarming motility and other virulence factors of *P. mirabilis*; while other studies reported that the anti-swarming agents such as sodium dodecylsulphate (SDS), trihydroxymethylglycine (Tris) and urea have inhibitory action on extracellular protease activity of clinical isolates of *P. mirabilis* at 0.2-1.25 % concentration (30, 25). Iwalokun and his co-workers found some of amino acids such as glutamine, serine and methionine enhanced swarming motility; while other 17 amino acids have inhibition effect on swarming (31). Fatty acids act as signals to regulate swarming in *P. mirabilis*, oleic acid enhanced swarming while lauric acid, myristic acids, palmitic acid and stearic acid inhibited swarming (16). p- Nitrophenylglycerol (PNPG) also can inhibit not only swarming but also inhibit the ability of *P. mirabilis* to express other virulence factors such as urease and haemolysin which coupled to swarming and prevent its invasion to human urothelial cells (32,29). Some articles reported that the PNPG may has an inhibitory effect on *rsb A* gene that regulated *P. mirabilis* swarming and lead to a reduction in production of other virulence factors or inhibit genes expression of other virulence factors (7). There are several other theories have been suggested to explain the

mechanism of swarming of bacteria. The negative chemotaxis (33), accumulation of secondary metabolites in the colony vicinity (34), impairment of flagellation (26), enhanced growth rate (35) were some of the reasons suggested to explain the swarming of *Proteus* and *Vibrio* species. But irrespective of nature of flagellation, formation of lateral flagella is requisite for swarming and is aided by signals that trigger the change (36). The chemical compounds that inhibit the swarming may attributed to complex with flagellar proteins of swarming cells and cause its disintegration (37) or impairs formation of flagella and motility (38). The enhanced or inhibited swarming of *P. mirabilis* by all chemical compounds tested in this study may attributed to many reasons include; these compounds may acts as extracellular signals or intracellular signals, may serve as cell-cell communication signals that interact with some of membrane sensor proteins or may affect membrane fluidity, these compounds may interact with activity of RcsC-RsbA proteins or through either an RsbA-dependent or RsbA-independent pathway to regulate swarming and virulence factor expression in *P. mirabilis* or these compounds may have an inhibitory effect on *rsb A* gene that regulated *P. mirabilis* swarming (7,16). In conclusion, this study has been demonstrated the warty extraction of coffee induced swarming inhibition of uropathogenic *P. mirabilis* in vitro; the drinking of coffee could be employed in treatment of patients that suffering from UTI caused by *P. mirabilis* and avoid drinking of black tea due to their stimulatory action for swarming because this action may give chance for *P. mirabilis* to colonize and reach to other sites of urinary tract caused infection. We suggest using these inhibitors in culture media to help of diagnosis of *P. mirabilis* or may using some of these inhibitors (that after tested non-toxic for human) with antimicrobial agents (Antibiotics) to get more bacteristatic or bactericidal effect on dangerous opportunistic *Proteus spp*.

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## السلوك المثبط والمحفز لبعض المركبات الكيميائية والمستخلص المائي للشاي والقهوة العربية على ظاهرة العج (swarming) للعدلات السريية لبكتريا *Proteus mirabilis*

بهاء عبدا لله لفتة

الخلاصة:

اختبرت تأثيرات ستة عشر مادة كيميائية مختلفة تعود لأصناف متنوعة كألاح عضوية ولا عضوية، منظفات، مستحلبات، مغذيات، كاربوهيدرات و مطهرات وكذلك المستخلص المائي للشاي الأسود والقهوة العربية على ظاهرة العج (swarming) للعدلات السريية لبكتريا *Proteus mirabilis* بطريقة التلقيح بالبقعة المركزية. اظهر كلا من Triton X100 و tris base والمستخلص المائي للشاي ومستخلص الخميرة فضلا عن الكلوز تأثيرا محفزا على قطر حركة العج لبكتريا *Proteus mirabilis* لارتفاعه إلى 25، 49، 72، 77، 90 ملمتر على التوالي بزيادة التركيز، في حين اظهر كلا من sodium acetate و sodium chloride والسكرور و EDTA و Tween 80 و phenol تأثيرا مثبطا على قطر حركة العج الى 14، 12، 11، 10، 8، 1 ملمتر على التوالي مقارنة مع قطر حركة العج للمستعمرة البكتيرية السيطرة إذ كان 17 ملمتر . لكن النشا والمستخلص المائي للقهوة العربية و SDS و peptone اظهر تأثيرا متباينا بسلوكها ضد حركة العج. ان مدى حركة العج يختلف باختلاف المركبات الكيميائية وتركيزها والسلالة البكتيرية. معظم المركبات المثبطة لحركة العج تؤثر على ميكانيكية الاسواط والحركة.