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Activity of Garlic (*Allium sativum*) Bulb Against Bacterial Isolates from Body Wash of American Cockroach (*Periplaneta americana*) from Lagos, Nigeria

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ABSTRACT

Cockroaches have huge health importance; they may be involved in the passive mechanical transfer of pathogens from humans to animals and food. The study aimed to isolate the external bacterial flora of *Periplaneta americana* and to determine the antimicrobial activity of crude garlic extract and conventional antibiotics on the bacterial population. A total of 45 Cockroaches (n=45) were collected aseptically from Ojo and Iyana-era all in the Ojo local government area of Lagos State, Nigeria. Bacteria were isolated on nutrient agar, MacConkey agar, and Salmonella-Shigella Agar and were putatively identified based on cultural, morphological, and biochemical characteristics. The identification system was complemented with an Analytical Profile Index (API). A total of 30 bacterial strains were identified, of which 23 were Gram-positive, and 7 were Gram-negative, belonging to the *Pseudomonas*, *Bacillus*, *Streptococcus*, *Escherichia*, *Staphylococcus*, *Actinomycetes*, *Enterococcus*, *Serratia*, and *Listeria* genera. The antimicrobial activities of garlic extract on isolates revealed that strain OJ9, IE4, OJ8, and OJ3 had inhibition zone values of 26.5 ± 2.1 , 26.0 ± 1.4 , 24.5 ± 2.1 , and $24. \pm 1.4$. A high inhibition zone was also obtained with strains OJ5, OJ10, and OJ4, respectively, whereas OJ1, OJ2, and OJ12 did not show inhibition zones. The antibiotic susceptibility and resistance patterns showed that Strain OJ1 was susceptible to ciprofloxacin but resistant to gentamycin and zinnacef, whereas OJ7 was susceptible to erythromycin and perfloracin but resistant to other antibiotics, including. Also, strain IE7 was susceptible to perfloracin, rocephin, and septrin. The study provided clear insight into the antimicrobial potency of the *Allium sativum* crude extract.

Keywords: Cockroach; Crude-garlic extract; Antimicrobial activity; Allicin; *Staphylococcus*; *Bacillus*; *Pseudomonas*.

INTRODUCTION

Cockroaches are among the most widely distributed insects found in homes, food-handling establishments, hospitals, and health care facilities. They are also found in septic tanks where they feed on faecal matter. Aside from this, Cockroaches also feed on garbage and decaying foods [1]. They are vectors capable of transmitting pathogens to food, kitchen utensils, and different parts of homes. Various pathogenic microorganisms have been associated with cockroaches, which they convey on their cuticles or ingest, or excrete on surfaces. Some medically important bacteria have been isolated from the body surfaces of *Periplaneta americana*, some of which include *Staphylococcus* sp., *Streptococcus* sp., *Salmonella* sp., *Shigella* sp., *Escherichia* sp., *Campylobacter* sp., *Pseudomonas*, and *Klebsiella* sp. [2,3,4].

Among cockroach species, *Periplaneta americana* is consumed in oriental countries like China, where it is bred in large populations, sold to farmers and the general public as feed for livestock, or eaten as food [5]. *Periplaneta americana* is also reared by some farmers because they are found to be useful in the pharmaceutical industry. The insects have been used in drug formulations to cure several ailments such as gastroenteritis, duodenal ulcer, and pulmonary tuberculosis. The insect is also effective in relieving sore throat, fluid build-up, and tonsillitis due to its detoxifying properties [6].

Cockroaches are known for their vectorial capacity to distribute microbial pathogens carried on their body surfaces as mechanical carriers. Such a microorganism is dependent on the environment within which the cockroach circumnavigates. This makes it necessary to characterize the bacteria carried in each environment. Since cockroaches are also being promoted as possible sources of feed for poultry and other consumptive uses, it would be imperative to either eliminate the microbial fauna on the insect's body surface or render them ineffective in causing and spreading disease. Also, there is a need to protect the laboratory and other workers who work with cockroaches from potential infection from the microbial load on the insect's body surface. The acclaimed antimicrobial and insecticidal potency of some botanicals may be relied upon to ensure the safe handling and consumption of cockroaches, such as garlic (*Allium sativum*).

The antibacterial properties of garlic have been known for a very long time. Garlic has been shown to exhibit a wide spectrum of antibacterial activity against Gram-negative and Gram-positive bacteria, including species of *Escherichia*, *Salmonella*, *Staphylococcus*, *Klebsiella*, *Proteus*, *Bacillus*, and *Clostridium*, among others [7]. Farmers involved in breeding cockroaches also use garlic to disinfect the body surface, making the insects less harmful for consumption. These farmers use garlic to eliminate and prevent microbes and pathogens

from contaminating the insects intended for processing as a source of food for livestock and humans [8]. However, the effectiveness of the treatment has not been established, just as little is known about the species spectrum of the cockroach biota used. This calls for the present study, aimed at identifying the bacterial flora on the body surface of *Periplaneta americana* collected from Lagos and investigating the antibacterial activities of Garlic (*Allium sativum*) crude aqueous extract on bacterial isolates from *P. americana*.

METHODOLOGY

Sampling Sites and Sample Collection

In order to determine the bacterial community composition of the body wash of *Periplaneta americana* (Cockroach), septic tanks of selected residential buildings of Iyana-Era and Ojo, Ojo Local Government Area of Lagos State were sampled. In total, 45 live Cockroaches were aseptically collected by handpicking between 1 and 3 a.m. from the two sampling sites. Thirty cockroaches were collected from Ojo, and 15 from Iyana-Era septic tank using a direct collection approach and stored in a sterile large bottle. Samples were transported to the laboratory in the morning for microbial enumeration.

Microbial Enumeration

The bacterial community composition of *Periplaneta americana* was estimated and isolated using the standard plate count technique. Serial dilution of 10-fold of samples using physiological saline was carried out, and a 0.1 ml aliquot was transferred onto nutrient agar, McConkey agar, and Salmonella-Shigella agar. The spread plate technique was used. Petri plates were incubated at 35 ± 2 °C for 18-24 hrs. Colonies were purified by sub-culturing on nutrient agar.

Identification and Characterization of Bacterial Isolates

Isolates were characterized and identified based on their colonial and cellular morphology, biochemical characteristics, following the taxonomic scheme of Cowan and Steel [9] to identify isolates to the genus level. The biochemical test was complemented with an analytical profile index (API V4.0 for Staph, 50 CH and 20 E) to identify the predominant members to the species level. Some of the substrates tested on the isolates were O-nitrophenyl- β -D-galactopyranoside, Arginine, Lysine, Ornithine, Sodium thiosulphate, Urea, Tryptophan, Creatine sodium pyruvate, Mannitol, Inositol, Sorbitol, Maltose, and Arabinose, etc.

Antimicrobials

Activity of Crude Garlic Extract on Selected Isolates

One hundred grams of garlic bulb were peeled, washed and transferred into a washed electrical blender after which 125 ml of distilled water was

added and blended into a paste. The paste was passed through a wire mesh to sieve out the shaft. The filtrate was passed through Whatman No 1 filter paper. The extract was further purified by passing it through a membrane filter (0.45 µm, Millipore). The crude garlic extract was stored in a sterile, dark bottle and refrigerated. A standard solution of the bacterial concentration with the equivalent of 0.5 McFarland (1.5×10^8 cfu/ml) was prepared of which 1ml aliquot was poured on sterile solidified Mueller-Hinton agar plate, decanted and air-dried. A sterile cork borer was used to punch out plugs (discs) on the seeded agar plate adjacent to one another. A 50 µL filter-sterilized crude garlic extract was transferred into the wells and incubated at $35 \pm 2^\circ\text{C}$ for 18-24 hrs. The diameter of the clear zones was measured in millimeters. Agar plates were prepared in duplicates.

Antibiotic Susceptibility Patterns of the Isolates

Antibiotic susceptibility and resistance patterns of the isolates were determined by disc agar diffusion methods following guidelines established by Bauer *et al.* [10]. Isolates were tested against Gram-positive and Gram-negative multidisc. The antibiotics tested with their respective concentrations were Pefloxacin (10 µg), gentamycin (10 µg), ampicillin (30 µg), Zinnacef (20 µg), amoxicillin (30 µg), rocephin (25 µg), ciprofloxacin (10 µg), Streptomycin (30 µg), Septrin (30 µg), and erythromycin (10 µg). The antibiotic disc was placed on Mueller-Hinton agar plates already seeded with 0.1 ml inoculum of the bacterial isolates, and the petri-plates were incubated at $35 \pm 2^\circ\text{C}$ for 18-24 hrs and observed for zones of inhibition. The antibiotic resistance and susceptibility patterns were interpreted according to the Clinical and Laboratory Standards Institute Guidelines [11].

RESULTS

The Cultural Morphological and Biochemical Identification of isolates is shown in Table 1. Also, Table 2 depicts the Analytical Profile Index (API) of predominant isolates obtained from the body of a cockroach. Culture-dependent analysis of the bacterial flora of the body of *Periplaneta americana* identified 30 bacterial isolates. Of the 30 bacterial

isolates identified, 23 were Gram-positive and 7 were Gram-negative. A total of 10 genera of isolates were identified with various numbers of strains, viz., *Pseudomonas* sp. (8), *Streptobacillus* (1), *Actinomyces* (4), *Bacillus* (3), *Streptococcus* (2), *Enterococcus* (1), *Staphylococcus* (6), *E. coli* (1), *Serratia* (3), and *Listeria* (1). Bacterial strains from Ojo were designated as OJ1, OJ2, OJ3, OJ4, OJ5, OJ6, OJ7, OJ8, OJ9, OJ10, OJ11, OJ12 and OJ13 whereas bacterial strains from Iyana Era were designated as IE1, IE2, IE3, IE4, IE5, IE6, IE7, IE8, IE9, IE10, IE11, IE12, IE13, IE14, IE15, IE16 and IE17. Strain OJ1 is a Gram-negative rod with irregular edges, flat smooth surfaces, catalase and oxidase positive, and motile. Strain OJ3 has a circular, creamy colour with flat, smooth surfaces and a Gram-positive, motile rod, which tested positive for catalase and oxidase but does not produce indole nor utilize lactose. Bacterial strain OJ10 is a Gram-positive coccus with circular, raised, smooth surfaces, catalase and oxidase positive, which ferments lactose.

Bacterial strain IE1 is a pinkish circular, smooth, motile rod cell bacterium, catalase and oxidase positive, that does not produce hydrogen sulphide gas. Also, the bacteria strain IE13 is a creamy Gram-positive coccus, catalase- and oxidase-positive. The API analysis revealed that strain OJ3 fermented D-glucose, D-fructose, and D-mannose but did not utilize maltose, xylitol, D-melibiose, raffinose, or xylose. Bacterial strains OJ5, OJ11, and IE12 were able to utilize plant-derived glycogen, such as methyl-D-mannoside, methyl-G-glucoside, N-acetyl-glucosamine, amygdalin, arbutin, esculin, salicin, and cellobiose. While OJ9 and OJ11 tested negative for alcohol sugars such as xylitol and sorbitol.

The abundance of various bacterial isolates from *Periplaneta americana* is depicted in Figure 1. *Pseudomonas* sp. is the most abundant in the Ojo sample, with a prevalence of 38%, compared with the 12% recorded for the Iyana Era sample. Also, *Staphylococcus* was high in the Iyana Era sample, at 30%, compared with less than 10% in the Ojo sample. *Escherichia*, *Enterococcus*, and *Streptobacillus* genera were isolated from Ojo, whereas *Serratia* and *Listeria* were identified from Iyana Era samples.

Table 1: Cultural, Morphological, and Biochemical Identification of Isolates

Isolates codes	Colonial morphology				Gram staining	Cellular morphology	Catalase	Oxidase	Indole	Motility	Citrate	Lactose	Gas	H ₂ S	Glucose	Putative identity
	Colour	Shape	Elevation	Edge												
OJ1	Green	Irregular	Flat	Smooth	-	Rod	+	+	-	+	+	-	-	-	+	<i>Pseudomonas</i> sp.
OJ2	Green	Irregular	Convex	Smooth	-	Rod	+	+	-	+	+	-	+	+	+	<i>Pseudomonas</i> sp.
OJ3	Cream	Circular	Flat	Smooth	+	Rod	+	+	-	+	+	-	+	+	+	<i>Streptobacillus</i> sp.
OJ4	Cream	Circular	Raised	Rough	+	Rod	+	+	-	-	+	-	-	-	-	<i>Actinomyces</i> sp.
OJ5	Yellow	Irregular	Convex	Rough	+	Rod	+	+	-	-	+	-	-	+	+	<i>Bacillus</i> sp.
OJ6	Green	Circular	Flat	Smooth	-	Rod	+	+	-	+	+	-	-	+	+	<i>Pseudomonas</i> sp.
OJ7	Green	Irregular	Flat	Smooth	-	Rod	+	+	+	+	+	-	+	+	-	<i>Pseudomonas</i> sp.
OJ8	Yellow	Circular	Raised	Smooth	+	Rod	+	+	+	+	+	-	+	+	+	<i>Bacillus</i> sp.
OJ9	Whitish gray	Circular	Convex	Smooth	+	Cocci	+	+	-	+	+	-	+	+	+	<i>Streptococcus</i> sp.
OJ10	Cream	Circular	Raised	Smooth	+	Cocci	+	+	-	+	+	+	-	+	+	<i>Enterococcus</i> sp.
OJ11	Yellow	Circular	Convex	Smooth	+	Cocci	+	+	-	+	+	-	-	+	+	<i>Staphylococcus</i> sp.
OJ12	Green	Irregular	Flat	Smooth	+	Rod	+	+	+	+	+	-	+	+	+	<i>Pseudomonas</i> sp.
OJ13	Cream	Circular	Flat	Rough	-	Rod	+	+	-	+	+	+	-	+	+	<i>E. coli</i>
IE1	Pink	Circular	convex	Smooth	+	Rod	+	+	-	+	+	+	+	-	-	<i>Serratia</i> sp.
IE2	Pink	Circular	Convex	Smooth	+	Rod	+	+	+	+	+	+	-	-	-	<i>Serratia</i> sp.
IE3	Green	Irregular	Convex	Smooth	-	Rod	+	+	+	+	+	+	-	-	-	<i>Pseudomonas</i> sp.
IE4	Green	Irregular	Convex	Smooth	-	Rod	+	+	+	+	+	+	-	-	-	<i>Pseudomonas</i> sp.
IE5	Yellow	Circular	Convex	Smooth	+	Cocci	+	+	+	+	+	+	+	-	-	<i>Staphylococcus</i> sp.
IE6	Pink	Circular	Convex	Smooth	+	Rod	+	+	-	+	+	+	+	-	-	<i>Serratia</i> sp.
IE7	Cream	Circular	Raised	Serrated	+	Rod	+	+	-	-	+	+	+	+	-	<i>Listeria</i> sp.
IE8	Cream	Circular	Convex	Smooth	+	Rod	+	+	+	-	+	+	+	-	-	<i>Bacillus</i> sp.
IE9	Whitish	Circular	Convex	Smooth	+	Cocci	+	+	-	+	+	+	+	-	-	<i>Streptococcus</i> sp.
IE10	Yellow	Circular	Convex	Smooth	+	Bacilli	+	+	-	+	+	+	-	+	-	<i>Actinomyces</i> sp.
IE11	Cream	Circular	Raised	Rough	+	Cocci	+	+	+	+	+	+	-	-	-	<i>Staphylococcus</i> sp.
IE12	Yellow	Circular	Convex	Smooth	+	Bacilli	+	+	-	+	+	+	-	-	-	<i>Actinomyces</i> sp.
IE13	Cream	Circular	Raised	Smooth	+	Cocci	+	+	-	+	+	+	-	-	-	<i>Streptococcus</i> sp.

IE14	Yellow	Circular	Convex	Smooth	+	Cocci	+	+	+	+	+		+	-		<i>Staphylococcus</i> sp.
IE15	Whitish	Circular	Convex	smooth	+	Bacilli	+	+	+	+	+		-	+		<i>Actinomyces</i> sp.
IE16	Yellow	Circular	Convex	Smooth	+	Cocci	+	+	-	+	+		+	-		<i>Staphylococcus</i> sp.
IE17	Yellow	Circular	Convex	Smooth	+	Cocci	+	+	+	+	+		+	+		<i>Staphylococcus</i> sp.

Table 2: Analytical profile index of the predominant isolate obtained from the body of the cockroach

Sugar fermentation	OJ3	OJ5	OJ9	OJ11	IE12
D-glucose	+	+	+	-	-
D-fructose	+	+	+	+	+
D-mannose	+	++	+	++	++
Maltose	-	+	-		++
Lactose	+	+	+		++
D-trehalose	+	+	+	++	++
D-mannitol	+		+	+	
Xylitol	-		-		
D-melibiose	-	+	-		++
Potassium nitrite	+		+		
Naphthyl acid phosphate	+		+		
Sodium pyruvate	+		+		
Raffinose	-	+	-		++
Xylose	-		-		
Sucrose	+		-		
Methyl-D-glucoside	-		-		+
N-acetyl-glucosamine	+		+		++
Arginine	+	+	+	+	
Urea	+	-	-	-	
Glycerol	-	++		++	++
Erythritol					
D-arabinose		++		++	++
L-arabinose		++		++	++
Ribose		++		++	++
D-xylose		++		++	++
Adonithol					
Methylxyloside				+	
Galactose		++		++	++
Mannitol		+		+	
Sorbitol		+		-	
Methyl-D-Mannoside		+		+	
Methyl-g-glucoside		+		+	
N-acetyl glucosamine		+		+	
Amygdalin		++		++	++
Arbutin		++		++	++
Esculin		++		++	++
Salicin		++		++	++
Celleboise		++		++	++
O-nitrophenyl- β -D galactopyranoside		+		+	+
Lysin		-		-	-
Ornithine		-		-	-
Sodium citrate		-		-	-
Sodium thiosulphite		-		-	-
Tryptophan		-		-	-
Creatine-sodium pyruvate		+		+	-
Kohn's charcoal gelatin		+		-	+
OX		-		+	+
Nitrite reduction		-		-	-
Motility medium		+		+	+
McConkey medium		+		+	+
OF-o		+		+	+

The antimicrobial activity of garlic extract on isolates is shown in Table 3. High zones of inhibition were recorded with strain OJ9, IE4, OJ8, and OJ3 with values 26.5 ± 2.1 , 26.0 ± 1.4 , 24.5 ± 2.1 , and $24. \pm 1.4$. High inhibition zones were also obtained with strains OJ5, OJ10, and OJ4, while OJ1, OJ2, and OJ12 did not show inhibition zones. A high zone of clearing indicates the potency and efficacy of aqueous garlic extract against microorganisms inhabiting the body surfaces of cockroaches.

Furthermore, the antibiotic susceptibility and resistance patterns of the isolates are depicted in Table 4. Strain OJ1 was susceptible to ciprofloxacin but resistant to gentamycin and zinnacef, whereas OJ7 was susceptible to erythromycin and perfloracin but resistant to other antibiotics. Also, strain IE7 was susceptible to perfloracin, rocephin, and septrin.

DISCUSSION

Cockroaches are found in human habitation, such as environments where food is stored, processed, or served [1]. Aside from these environments, they are found in hospitals, including intensive care units, theatres, and laboratories [12, 13]. Also, they visit septic tanks, drains, and bathrooms. Additionally, they are nocturnal and omnivorous. These attributes make them ideal carriers of pathogens and parasites, including bacteria, protozoans, fungi, viruses, and helminths [14,15].

In this study, 30 bacterial species belonging to 10 genera, namely *Pseudomonas*, *Bacillus*, *Streptococcus*, *Escherichia*, *Staphylococcus*, *Actinomycetes*, *Enterococcus*, *Streptobacillus*, *Serratia*, and *Listeria*, were studied. were identified. Some of these bacterial genera have been reported from cockroaches [16]; however, this is the first report of their isolation from septic tanks in Ojo and Iyan-Era.

Bacillus has been reported as the most predominant in various studies, as shown in Adeleke *et al.* [16] and Isaac *et al.* [17]. *Pseudomonas*, however, was the most abundant in this study. *Staphylococcus* was also dominant in the Iyana-era sample. This genus has been implicated in the causation of certain nosocomial infections. Though various bacteria, viruses, and fungi are known to cause nosocomial infections, *Staphylococcus aureus* is known to be among the most common. In this way, it is complemented with *Escherichia coli*, and other microbes not isolated in this study [18,19,17]. It is not surprising that *E. coli* was isolated from the Iyana-Era sample, as it is a hardy organism that can survive harsh environments. Most members of the organisms isolated in the study belong to the family Enterobacteriaceae, which are normal inhabitants of the human intestine. Their abundance in the cockroach body may have resulted from cockroaches interacting with human fecal matter.

Table 3: Antimicrobial activities of crude garlic extract against bacterial isolates

S/N	Isolate codes	Zone of inhibition (Mean \pm SD)
1.	OJ1	0.00
2.	OJ2	0.00
3.	OJ3	24.0 ± 1.4
4.	OJ4	16.0 ± 1.4
5.	OJ5	22.5 ± 2.1
6.	OJ6	4.0 ± 5.7
7.	OJ7	0.00
8.	OJ8	24.5 ± 2.1
9.	OJ9	26.5 ± 2.1
10.	OJ10	19.5 ± 0.71
11.	OJ11	17.0 ± 2.8
12.	OJ12	0.00
13.	OJ13	12.5 ± 2.1
14.	IE1	16.0 ± 0.0
15.	IE2	14.0 ± 1.4
16.	IE4	26.0 ± 1.4
17.	IE6	8.5 ± 1.2
18.	IE7	17.5 ± 2.1
19.	IE11	6.5 ± 4.2
20.	IE12	15.0 ± 1.4
21.	IE16	15.0 ± 0

Spices are used globally to enhance flavors and preserve perishable foods [20]. Spices are simply any dried, fragrant or aromatic vegetable or plant substances that enhance flavors. Different spices used on a daily basis have been documented to possess antimicrobial and medicinal values [21]. It is important to note that garlic belongs to one of the useful aromatic spices. The antimicrobial activities of garlic against Gram-positive and Gram-negative bacterial pathogens have been reported [22, 23, 24, 25, 20].

The results of the antimicrobial activities of garlic, based on the zones of inhibition recorded, are shown in Table 3. A high zone of inhibition was observed with bacterial strains IE1, IE4, OJ3, OJ4, OJ5, OJ8, OJ9, OJ10, and OJ11. The high inhibition zones observed on Mueller-Hinton agar plates seeded with the respective organisms indicate the efficacy and antimicrobial potential of the aqueous garlic extract. Our findings corroborate the earlier report of Safitri *et al.* [26], which shows the efficacy of aqueous garlic extract against *S. typhimurium*, *S. agalactiae*, *S. aureus*, and *E. coli*.

The study also explored the antibiotic resistance and susceptibility patterns of the isolates. Although antibiotics are not always used against cockroaches, highly resistant strains have been reported, with pathogens associated with food [27, 28]. Also, the great association between cockroach and food could be a probable reason for the isolation of resistant strains in food [1]. Bacterial strains were susceptible to pefloxacin, ciprofloxacin, rocephin, and erythromycin. It is important to note that isolates showing clear zones of inhibition to conventional antibiotics were also susceptible to aqueous garlic extract. The results of

this study make it apparent that garlic or aqueous garlic extract can serve as an alternative antimicrobial to conventional antibiotics, thereby curbing the menace of mechanical transfer of pathogens by cockroaches in homes and other locations.

CONCLUSION

The study involves culture-independent analysis of the bacterial flora on the body surface of *Periplaneta americana*. Ten bacterial genera were isolated, including *Pseudomonas*, *Staphylococcus*, and *Serratia*, which were among the dominant genera. The antimicrobial potential of the aqueous garlic extract against the isolates showed that the extract was highly effective against most bacterial isolates, with clear zones of inhibition observed, even compared with conventional antibiotics. Garlic extract has been shown to be highly effective at curtailing the mechanical transfer of pathogens that cockroaches can convey.

AUTHOR CONTRIBUTION

Denloye AA: Initiator and Principal Investigator (PI)

Alafia AO: Initiated Draft Zero of Manuscript

Ashade AO: Preparation of agar, plating, and identification of isolates

Ajelara KO: Technical Support on extract preparation and storage.

Godonu KG; Adetunji BH, Oke S, Babaniyi PI; Oke R; Ezun SJ; and Ajose RA: Took up the collection, maintenance, and preparation of body wash of experimental cockroach.

Oyefolu AOB: Identification of isolates and initial review of the Draft zero of the manuscript.

Table 4: Antibiotic susceptibility and resistant patterns of the bacterial isolates.

No	Isolate code	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E
1.	OJ1	S (22)	R (0)	R (0)	R (0)	R (0)	R (0)	S (20)	R (0)	R (0)	S (20)
2.	OJ2	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)
3.	OJ3	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)
4.	OJ4	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	I (17)	R (0)	R (0)	R (0)
5.	OJ5	R (0)	R (0)	R (0)	R (0)	R (0)	I (17)	S (26)	R (0)	I (17)	S (20)
6.	OJ6	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)
7.	OJ7	S (23)	R (0)	R (0)	R (0)	R (0)	R (0)	I (15)	R (0)	R (0)	S (20)
8.	OJ8	S (25)	I (16)	R (0)	R (0)	R (0)	I (15)	S (25)	S (21)	I (15)	S (25)
9.	OJ9	S (21)	R (0)	R (0)	R (0)	R (0)	R (0)	S (20)	R (0)	R (0)	S (19)
10.	OJ10	S (20)	R (0)	R (0)	R (0)	R (0)	I (17)	S (28)	R (0)	R (0)	S (19)
11.	OJ11	S (24)	R (0)	R (0)	R (0)	R (0)	R (0)	S (24)	R (0)	R (0)	S (20)
12.	OJ12	R (0)	I (15)	R (0)	R (0)	R (0)	R (15)	R (0)	R (0)	R (0)	R (0)
13.	OJ13	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)
14.	IE3	I (14)	R (0)	R (0)	R (0)	I (10)	R (0)	R (0)	R (0)	R (0)	I (15)
15.	IE4	I (17)	R (0)	R (0)	R (0)	I (13)	S (25)	I (19)	I (13)	S (24)	S (20)
16.	IE6	I (16)	R (0)	R (0)	R (0)	I (14)	R (0)	I (16)	S (20)	I (11)	R (0)
17.	IE7	S (23)	R (0)	R (0)	R (0)	I (12)	S (26)	S (20)	I (17)	S (20)	I (15)
18.	IE8	R (0)	R (0)	R (0)	R (0)	I (13)	S (21)	R (0)	I (11)	R (0)	R (0)
19.	IE9	I (15)	R (0)	R (0)	R (0)	I (13)	R (0)	I (19)	S (23)	I (12)	R (0)
20.	IE10	S (23)	R (0)	R (0)	I (15)	I (12)	S (27)	S (21)	I (17)	S (20)	I (16)
21.	IE15	S (25)	R (0)	R (0)	R (0)	I (10)	S (25)	S (20)	I (15)	S (23)	I (14)
22.	IE17	S (21)	R (0)	R (0)	R (0)	I (13)	I (18)	I (18)	I (19)	S (20)	I (15)

Key: Susceptible: 21mm and above, Intermediate: 11 -20 mm, Resistance: 0-10 mm

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