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Evaluation of Insecticidal and Antimicrobial Effect of *Boerhavia diffusa*

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***Abstract*—** *Boerhavia diffusa*, commonly known as 'Punarnava' in the Indian system of medicine is a perennial creeping herb found throughout the waste lands. The insecticidal activity of aqueous and acetone extracts of *B. diffusa* was tested against the pest insect *Dysdercus cingulatus*, commonly known as Red Cotton Bug. The plant extract exhibited ovicidal effect, nymphicidal effect, varying degree of moulting disruption, reduced fecundity rate along with prolonged mating period. Insecticidal property exhibited by this plant extract can be exploited in the field of Integrated Pest Management, so as to reduce the hazardous effects of chemical pesticides. The toxic principles present in the plant extract are actually secondary metabolites that are evolved as a protective measure against herbivorous animals. Antimicrobial activity of *B. diffusa* was also evaluated using gram negative bacteria, *Pseudomonas aeruginosa* and showed significant level of sensitivity to *B. diffusa* extracts. Thus it is evident that *B. diffusa* possess toxic principles having insecticidal as well as antimicrobial activity which can be employed in the field of agriculture and medicine respectively.

Keywords — Biopesticide, Antimicrobial agent, *Dysdercus cingulatus*, *Pseudomonas aeruginosa*, *Boerhavia diffusa*

I. INTRODUCTION

Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend against attack from predators such as insects, bacteria, fungi and even herbivorous mammals. Among these phytochemicals, secondary metabolites are organic compounds which are found in a smaller range and serving a more specific function. Plants contribute a rich source of bioactive molecules which are useful alternatives to synthetic chemical pesticides in the control of insect pests. Globally greater awareness has been created on plant derived substances as they are ecofriendly and possess more insecticidal properties through multifarious functions [1].

Pathogenic bacteria have always been considered as major cause of morbidity and mortality in human. Due to increase of microbial resistance to antibiotics, there is need for developing new innovative antimicrobial agents. There is a long tradition of using dietary plants in the treatment of many infectious diseases [2].

Dysdercus cingulatus, commonly known as Red Cotton Bug is a serious pest of cotton and okra. Nymphs and adults feed on developing cotton bolls and heavy infestations affect crop mass, oil content and the marketability of the cotton crop. Cotton is the most economically important natural fiber material in the world. India has the largest area under cotton cultivation. In recent years, yield of cotton has become declining due to infestation of pest attack [3, 4]. Insecticidal potential of *Boerhavia diffusa* was tested against this particular pest insect.

Pseudomonas aeruginosa is a common gram-negative bacterium found in soil, water and most man-made environment, and cause disease in animals including humans. The symptoms of infections are generalized inflammation and sepsis. If such colonization occurs in critical body organs such as lungs, urinary tract, kidney, the results may be fatal [5]. *P. aeruginosa* is naturally resistant to a large range of antibiotics which is attributable to a concerted action of multidrug efflux pump with chromosomally encoded antibiotic resistant gene [6].Antimicrobial activity of *B. diffusa* was assessed using this microorganism.

B. diffusa commonly known as 'Punarnava' in the Indian system of medicine is a perennial creeping herb found throughout the waste lands. Plant is known to have medicinal properties and have a long history of use by indigenous and tribal people in India [7]. In Ayurvedic system of medicine, it is widely used in jaundice, oedema, anaemia, inflammation, immunomodulation, antifertility and eye diseases [8]. The important bioactive phytochemicals in this plant are glycosides, flavonoids, tannins, terpenoids, phenolic compounds etc [9].

In the present study, the insecticidal efficacy of acetone and aqueous extracts of *Boerhavia diffusa* were tested against *Dysdercus cingulatus*, the serious cotton pest. *Boerhavia diffusa* aqueous extract were used to test the antimicrobial property against the pathogenic bacterium, *Pseudomonas aeruginosa.* The results obtained in this study clearly demonstrate the insecticidal potential of *B. diffusa* which may find significance as a safe biopesticide against the cotton pest *D. cingulatus*, especially in the present scenario of dangerous chemical pesticides. Antimicrobial effect of this plant was also successfully proved against *P. aeruginosa*, a pathogenic bacterium, which can be exploited in medical field.

II. METHODOLOGY

Pest collection and rearing

Dysdercus cingulatus were collected from Kerala Forest Research Institute (KFRI) Thrissur and Vandithavalam, Palakkad District, Kerala. The collected insects were maintained in the insectory under laboratory conditions in transparent plastic containers covered with cotton cloth. Insects were fed with their natural food, cotton seeds. Before experimentation insects were maintained for 3 generations in the laboratory. 12-24 hr old fifth stadium and newly emerged adult female *D. cingulatus* were used for the experiment.

Collection of plant for extract preparation

The plant, *Boerhavia diffusa* was collected from different locations of Kerala and identified at Department of Botany, University College, Thiruvananthapuram, Kerala. The fresh leaves were washed thoroughly with water and air dried in shade and powdered using domestic grinder. Then Soxhlet extraction was done [10] using solvents acetone and distilled water. The extracts were further evaporated to a dry residue and then tested for various biological activities against the insect, *Dysdercus cingulatus* and the microbial organism, *Pseudomonas aeruginosa*

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Bioassay:

Nymphicidal Activity

Nymphicidal effect was studied by using uniform sized, 12-24hr old 5th stadium nymphs of *D. cingulatus* which were randomly selected from the stock culture. Ten insects were placed in a transparent plastic container and fed with cotton seeds soaked with four different concentrations of plant extracts. Control insects were maintained in another container and fed with distilled water soaked cotton seeds.

1 %, 5%, 10% acetone and aqueous extracts of the plant were dissolved in l0ml distilled water. Then the mixture was stirred well with glass rod. After this procedure, seeds of cotton were soaked and stirred well. The insects were allowed to feed for 96hrs for testing mortality. Four replicas were maintained for each concentration and mortality was recorded after 96hrs. Then the percentage mortality was calculated.

$$\text{Percentage mortality} = \frac{\text{No. of dead larvae}}{\text{Total no. of larvae}} \times 100$$

Fertility Test

1day old adult females were selected from the normal culture. 10% stock solution of acetone and aqueous extracts of *B. diffusa* were prepared. From that 0.1 ml were applied on the second tergite of the abdomen by using a micro syringe. Control insects were treated with distilled water. After 3 days, these females were released into transparent plastic jar and normal healthy males were released for mating. Four replicas were maintained for each concentration and observed the mating behavior and oviposition. Number of eggs laid by experimental insects was compared with control insects. Control experiment was also set up with normal untreated females and males.

Repellent Activity

For testing repellency [11], six 5^{th} stadium nymphs were randomly selected from the culture and kept in a petri dish which contained a Whatman's filter paper on the bottom. These petridishes were closed with transparent plastic sheet and made some small holes for aeration. After some time, different concentration (5%, 10%) of acetone and aqueous extract of the plant (0.5ml) was injected into the filter paper in petridish slowly and observed the behavioral response of the insects. Four replicas were maintained for each concentration.

$$\text{Percentage Repellency} = \frac{\text{No. of larvae repelled}}{\text{Total no. of larvae}} \times 100$$

Ovicidal Effect

30 numbers of newly laid eggs were collected and they were placed over cotton pads sprayed with plant extracts at different concentrations (5%, 10%). Control eggs were maintained in cotton pads soaked with distilled water. Four replicas were maintained for each concentration and observed the hatchability and calculated the percentage mortality.

$$\text{Percentage mortality} = \frac{\text{No. of unhatched eggs}}{\text{Total number of eggs}} \times 100$$

Antimicrobial Test

Pseudomonas aeruginosa was inoculated in Brain Heart Infusion Broth (BHIB) and compared the turbidity with that of the standard 0.5 McFarland solution. Mueller-Hinton Agar (MHA) plates were prepared for each experiment. The microbial lawn was prepared on agar plates by adjusted inoculums using a sterile cotton swab. Allowed to dry for 1-2 hrs and then an 8mm biopsy bore was used to <<punch out>>, 3 equally spaced wells on Mueller Hinton Agar plates. 200µl of sample was then delivered to the wells. A positive control such as commercially available $\text{Chloramphenicol}_{50}$

mcg disc was placed on the agar. Plates were then incubated for 18-24hrs. All tests were performed in triplicate and inhibition zone were measured using a millimeter scale.

III. RESULTS AND DISCUSSION

Nymphicidal activity

Data regarding the effect of *B. diffusa* extracts on 5th instar nymph of *D. cingulatus* are summarized in Figure 5, 6. Significant level of mortality was observed on 5th instar nymphs in a dose dependant manner at 1 %, 5% and 10% of acetone extracts (Figure 5) and aqueous extracts (Figure 6) of the plant. No significant degree of mortality was observed in control insects. After 96hrs of treatment, morphological and anatomical abnormalities were predominantly noticed at higher concentration of acetone and water extracts of the plant. It included incomplete moulting, shrunk abdomen, crumbled wings, feeding deterrence, and black shades on the body (Figure 1 and 2). Treated insects also showed malformed wings and died after moulting. Feeding deterrence was also showed at high doses of the plant extract.

Repellent activity

Repellent activity of randomly selected 5th instar nymphs of *D. cingulatus* was found to be dose dependant. Acetone extracts showed more repellent activity at high dose compared with control insects. Aqueous extracts showed negligible average repellent activity (Table 1).

Effect on reproduction

Copulation period was shown to be prolonged between 2-3 days in the case of treated insects. The number of eggs laid by acetone treated insects were greatly reduced (50 - 60 eggs). Aqueous extracts also affected egg laying (60-65 eggs). Control insects laid 120- 150 eggs (Table 2). Topical application of the plant extract on newly emerged adult females showed low fecundity rate compared with control females.

Ovicidal effect

30 numbers of newly laid eggs were collected and they were placed over cotton pads sprayed with different dilutions of plants extracts. Control eggs were placed on cotton pads soaked in distilled water. Treated eggs turned pale brown and dead. Dried and shrunk eggs were observed in almost all experimental eggs. Few eggs hatched but the nymphs could not survive. In the case of control eggs 100% hatchability was observed (Table 3).

Antimicrobial test

The minimum inhibitory concentration and zone of inhibition values were carried out by using the bacterial strain *P. aeruginosa*. The minimum inhibitory concentration (MIC) and Zone of inhibition (ZOI) values were calculated with standard chloramphenicol$_{50}$. From the results of MIC values, it indicates that *B. diffusa* showed significant antibacterial property against *P. aeruginosa* (Fig 3, 4). Table 4 indicated ZOI.

Discussion

Plants synthesize a number of secondary metabolites. Among them some of the compounds are recognized as insecticidal molecules. In the present study, the insecticidal effect of *B. diffusa* was tested on the insect *D. cingulatus*, the common red cotton bug. Antimicrobial activity was checked on *P. aeruginosa*. Four different concentrations 1%, 5%, 6%, and 10% of acetone extract and aqueous extracts 5%, 10% and 20% concentration of the plant was tried against *D. cingulatus* 5th stadium nymphs. Significant mortality was observed in dose dependent manner which demonstrates nymphicidal potential of the plant extract. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the body [12].

Dose dependent mortality due to extract of stem bark of *Streblus asper* [13] was reported for *D. cingulatus.* Extracts from the stem bark of *Streblus asper* possess insecticidal activity against the fifth instar of *D. cingulatus.* In 2012, Asha *et al*., [14] studied the biocidal activity of two marine green algae, *Ulva fasciata Delile* and *Ulva lactuca Linnaeus* against third instar nymph of *D. cingulatus* at different concentrations. Methanol extracts of both algae showed nymphicidal and anti ovipositional activity, reduced fecundity, hatchability, adult longevity and relative growth rate.

In 2010, Sahayaraj and Mahesh Tomson [15] studied the impact of two pathogenic fungal crude metabolites on mortality, biology and enzymes of *D. cingulatus. Beauvaris bassiana* (Balasmo) vuillimin and *Metarhizium anisopliae* (Metchnikoff) Sorokin crude metabolic extracts and fungal spores were tested against *D. cingulatus* under invitro conditions and both showed insecticidal properties. Antifeeding effect of varied concentration of *Adathoda vasica* and *Vitex negundo* crude methanolic leaf extracts was evaluated against *D. cingulatus*. *V. negundo* caused more nymphal deaths. Both plant extracts produced more than 50% nymphal mortality at two higher concentrations and *V. negundo* showed both antifeedant and nymphicidal activities [16].

Screening of insecticidal activity of brown macroalgal extracts against *Dysdercus cingulatus* were studied by [17]. The biocidal activity of hexane, chloroform, methanol and water extracts of brown algae, *Sargassum wightii* and *Padina pavonica*. The chloroform extract of *S. wightii* caused more nymphicidal effect at 96hrs than *P. pavonica.* In *P. pavonica*, mating period was highly prolonged by the aqueous extract.
In the present study, the repellency and feeding deterrence were observed at different concentration. *D. cingulatus* nymph showed repellence on acetone extract of *B. diffusa*.

Feeding deterrence was showed on high concentration of aqueous and acetone extract treated food. The results showed that the insects preferred untreated control food than any of the treated food. The compounds that prevent, block or otherwise interfere with food selection and consumption are referred to as feeding inhibitors. Antifeedants or feeding deterrents or phagodeterrents constitute a class of preingestive feeding inhibitors that act through gustatory receptors and evoke the rejection of plant material [18].

Repellency in the present study has been noted visually in the treated insects. *D. cingulatus* nymphs showed repellency in acetone extracts of *B. diffusa* treated areas on filter paper. Botanical pesticides generally have ovicidal and repellent activities. Repellents are the chemicals which cause an insect to make an oriented movement away from its source. Due to their odoriferous nature, oils of plants have been used for this purpose. *D. cingulatus* showed 95% repellency in *Ailanthus excelsa* extract of n-Hexane, chloroform and methanol extracts [19].

Prabhu and John [20] studied on ovarian development in juvenilized adult *D. cingulatus* affected by some plant extracts. Topical application of different doses of acetone extracts of the plants *Anthocephalus cadamba, Lantana camara, Tectona grandis, Calophyllum sp*. and *Phyllanthus emblica* to the newly moulted last (5th) instar larvae resulted in 6^{th} instars retaining varying degrees of nymphal characters. However, there was a different extent of ovarian inhibition in some of these individuals. When such individuals were mated, their eggs developed normally. The adultoids in all cases produced normal eggs. The corpus allatum of the 6^{th} instar was only about half the size of that of the normal adult female, the size difference being statistically significant.

In 2010, Tanu Sharma and Ayesh Qamar *et al*., [21] studied on evaluation of neem extracts against the eggs and adults of *Dysdercus cingulatus*. The toxic efficiency of neem leaves, neem green seed coat, neem yellow seed coat and neem seed kernel were tested against *D. cingulatus* eggs and adults at different concentrations. Neem extracts showed highest mortality in low concentration and least mortality in yellow seed coat. Treated with the extracts noticed least survival of eggs.

Kitherian Sahayaraj and Mary Jeeva [22] in 2012 investigated the role of a brown seaweed alga, *Sargassum tenerrimum* against *Dysdercus cingulatus*. The impact of benzene (BN), chloroform (CH) and a mixture of benzene and chloroform (BNCH) extracts, as well as two chromatographic fractions of BNCH (FR1 and FR2) were investigated for their insecticidal and ovipositional properties against *D. cingulatus*. The BN extracts showed the best insecticidal activity, higher than BNCH and CH extracts and all the extracts reduced the total nymphal developmental period of the pest in a dose dependent way. Adult longevity of both males and females was reduced by the CH and BN extracts. The CH extract reduced *D. cingulatus* oviposition, preoviposition and post-oviposition periods as well as fecundity. Females emerging from the BN category could not lay eggs. The BNCH fractions, FR1 and FR2, also exhibited nymphicidal activity against the pest. However, these fractions did not significantly alter the nymphal developmental period.

Evangelin *et al*., [11] demonstrated ovicidal, nymphicidal and juvenomimetic effect of *Adathoda vasica* extract on the red cotton stainer, *D. cingulatus*. The extract of *A. vasica* showed ovicidal, nymphicidal and juvenomimetic activity against this pest. Fertility and fecundity test were carried out by aqueous and acetone extract of *B. diffusa* in this study. The results showed that the extracts of *B. diffusa* affected the fertility and fecundity in *D. cingulatus.* The number of egg laid and percentage of hatching were significantly low. Aqueous extract of *B. diffusa* treated females showed prolonged copulation period and laid low number of eggs.
In the present study antibacterial property of aqueous extract of *Boerhavia diffusa* leaf was conducted by gram negative bacteria *Pseudomonas aeruginosa*. The extracts were tested by agar well diffusion method. A positive control Chloramphenico1_{50} mcg disc and negative control DMSO were used. The bacteria showed sensitivity to the plant extract. Phytochemicals present in the leaves of *B. diffusa* shows potent antibacterial activity against gram positive and gram-negative bacteria [23]. In 2008 Girish and Satish [24] observed that *B. diffusa* showed maximum inhibition on *Staphylococcus aureus*, *Bacillus megaterium* and *Bacillus cereus* at 50µL concentration. Mahesh *et al*, [25] reported that *B. diffusa* leaves have potential antibacterial activity against bacteria *S. aureus, B.subtilis, S. faecalis, M. luteus, K. pneumoniae, P. vulgaris, S. marcescens* and *S. flexneri*.

IV. CONCLUSION AND FUTURE SCOPE

The present study obviously indicates the insecticidal as well as antimicrobial potential of the plant *B. diffusa.* Ovicidal, nymphicidal and repellent activity were exhibited against the serious cotton pest *D. cingulatus*. Antibacterial property of the plant was also demonstrated against the pathogenic strain of bacterium *P. aeruginosa*. Thus it can be concluded that this preliminary results may find significance in future research for developing a bioinsecticide as well as an antibacterial agent, thereby reducing the hazards of toxic chemicals.

Figures and Tables

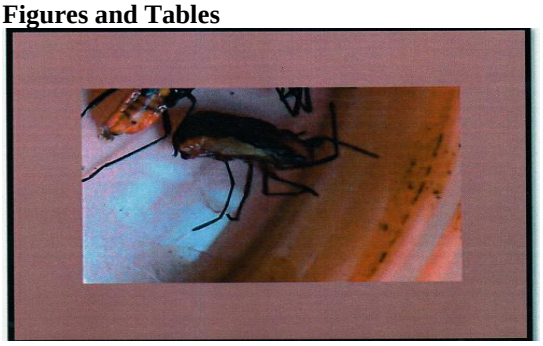


Figure 1: Incomplete Moulting 5th stadium nymphs of *D. cingulatus*

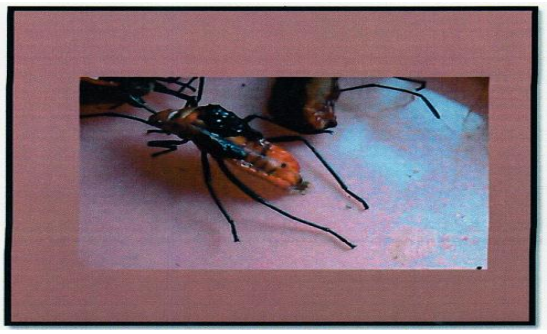


Figure 2: **Shrunken Abdomen**

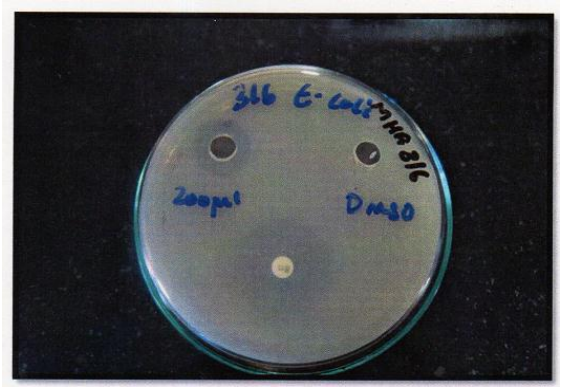


Figure 3: Antimicrobial effect of Boerhavia diffusa

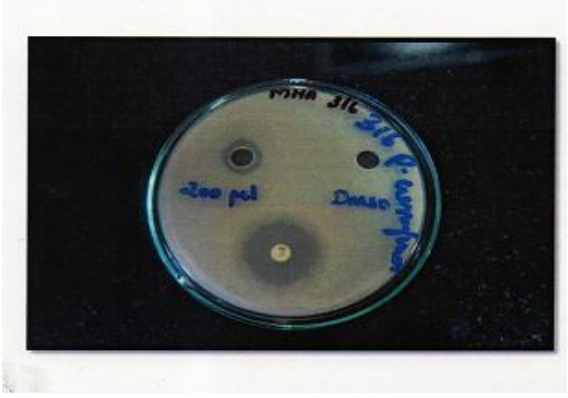
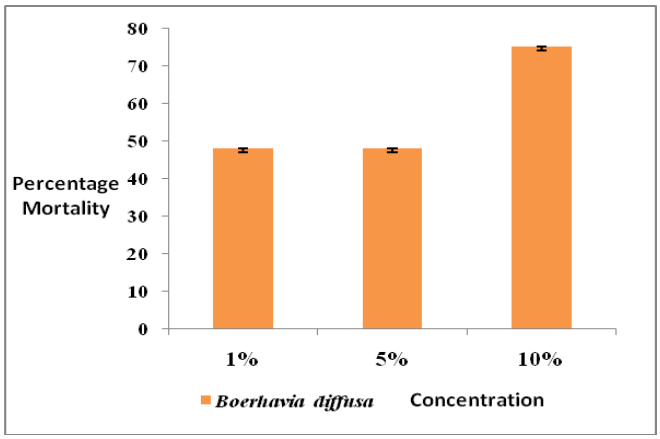


Figure 4: Antimicrobial effect of Boerhavia diffusa

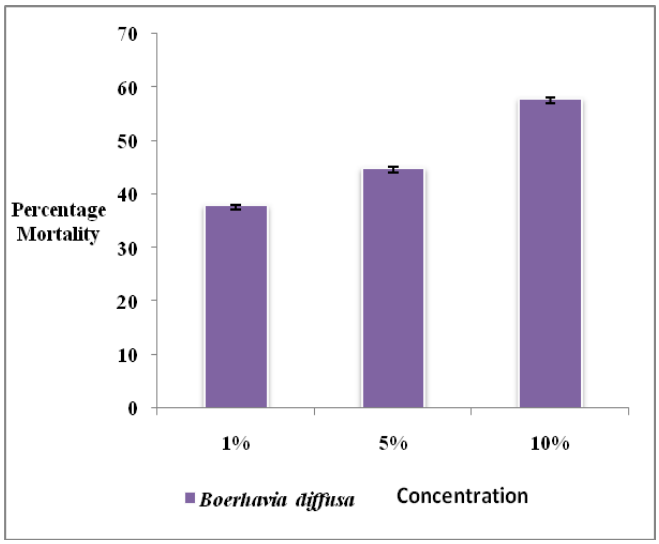
Table 1: Repellent effect of acetone & aqueous extract of *Boerhavia diffusa* on *Dysdercus cingulatus*

Plant	Dose (100 µl)	No. of eggs laid
Boerhavia diffusa	Acetone extract	50 - 60
	Aqueous extract	60 - 65



Each value is Mean ± S.D, n=4.
Each value is significant at $p \leq 0.05$

Figure 5: Effect of *Boerhavia diffusa* acetone extracts on 5th nymph of *Dysdercus cingulatus*



Each value is Mean ± S.D, n=4.
Each value is significant at $p \leq 0.05$

Figure 6: Effect of *Boerhavia diffusa* aqueous extracts on 5th nymph of *Dysdercus cingulatus*

Table 2: Effect of Acetone and Aqueous extract of *Boerhavia diffusa* on Fecundity rate of *Dysdercus cingulatus*

	Concentration (%)	Percentage Repellency	
		Acetone extract	Aqueous extract
Boerhavia diffusa	5	7.33	0.33
	10	9.33	0.66
Control	-	0	0

Table 3: Ovicidal effect of acetone & aqueous extract of *Boerhavia diffusa* on *Dysdercus cingulatus*

Concentration	Percentage Mortality	
	Acetone extract	Aqueous extract
5%	86.6	7.3
10%	93.3	83.3
Control	0	3.3

Table 4: Effect of antimicrobial activity of *Boerhavia diffusa*

Sl. No.	Parameters	Test Method	Sample Volume	Test Result
1	Sample concentration - 500 ppm			
2	*E. coli*	agar well diffusion	200ul	S-17mm
3	*P. aeruginosa*	Agar well diffusion	200ul	S-13mm

*S-Sensitive

V. ACKNOWLEDGMENT

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