International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 3, Issue 3, pp: 269 -274, 2018
https://doi.org/10.5281/zenodo.1315457
http://www.ijzab.com

Research Article

ASSEMBLAGE OF NEST SOIL BACTERIA AND EVALUATE THE ANTIBACTERIAL ACTIVITY OF CARPENTER ANT (*CAMPONOTUS COMPRESSUS*) MANDIBULAR EXTRACTS AGAINST SELECTIVE GRAM STAINING BACTERIA

P. Mohana*[1], C. Gunasekaran[2] and P. Selvarasu[2]

[1]Department of Zoology, Vivekanandha College of Arts and Sciences for Women (Autonomous), Elayampalayam, Tiruchengode – 637 205, Tamil Nadu, India.

[2]Conservation Biology laboratory, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore – 641 046, Tamil Nadu, India.

Article History: Received 2nd April 2018; Accepted 25th April 2018; Published 25th June 2018

ABSTRACT

The present study was under taken to explore the assemblage of ant nest soil microbes and potential of mandibular gland extraction of carpenter ant *Camponotus compressus.* The microbial assemblages in nest soil from different habitats like Grasslands, Shola forest, Wattle plantation, Pine and Tea plantations were investigated. Among the isolated microbes from the nest soil bacteria, *Staphylococcus aeureus* was robust in three sites of the study area. Disc diffusion method was used to evaluate the antimicrobial activity of mandibular gland extraction against two robust microbial strains like *S. aeureus* and *Escherichia coli.* The result revealed that the maximum zone of inhibition was observed against the *S. aeureus* in both 10 µl and 20 µl of the mandibular sample and the minimum in gram negative bacteria *Escherichia coli*. The presences of mandibular gland proved have good capability more than commercially available antimicrobial products to kill or inhibit the growth of microbes inside the nests.

Keywords: Antibacterial, Ant nest, *Camponotus compressus,* Mandibular gland, Mukurthi National Park.

INTRODUCTION

The ant nests are frequently accumulated by the stored food and waste materials, other organic debris by the foragers. This kind of nest alterations leads to determine the changes, size and activity of soil microbial assemblages inside the ant nests (Boulton & Amberman, 2006; Savin, *et al*., 2004). Microbes play an important role in ant communities like mutualists, commensals or pathogens. Ants are able to stimulate a broad range of physiologically different groups of microorganisms (Dauber & Wolters, 2000; Jakubczyk *et al*., 1972). This microbial-rich environment may lead to the risk of infections and disease transmissions in ants. In such pathogenic risk conditions, ants use their unique defense mechanism to cope with the diseases (Hughes, 2005; Poulsen *et al*., 2002). According to (Wilson Rich *et al*., 2008) the social insects have evolved a variety of adaptations to cope with the intense pathogenic pressures in their environments. The secretions of metapleural and mandibular glands of ants contain antimicrobial substances that defend infections. Bot *et al*. (2002) and Poulsen *et al*. (2002) found many ants are secret the metapleural gland, spreading them over the cuticle, while termites secret antibiotics from the salivary glands and sternal gland.

The *Camponotus* ant genus the metapleural gland is clearly absent (Ayre & Blum, 1971; Holldobler & Engel Siegel, 1984) possibly making more susceptible to infection and disease transmission. However, orally associated mandibular glands and salivary glands are also known to produce the antimicrobial factors. The ant mandibular glands are a pair of thin-walled sacs filled with mixture of alcohol, aldehydes and ketones (Blum & Hermann, 1978). These volatile compounds also have sturdy effects on ant behavior. The madibular gland secretions are the main source of nest mate recognition odor and modulators of

*Corresponding Author: Dr. P. Mohana, Assistant Professor, Department of Zoology, Vivekanandha College of Arts and Sciences for Women (Autonomous), Elayampalayam, Tiruchengode-637205, Tamil Nadu, India. Email: drpmohana@gmail.com

alarm signals (Bradshaw *et al.,* 1975;Cammaerts *et al*., 1983; Powell & Clark, 2004). High microbial parasites and pathogen pressure have led to the evolution of immune proteins in social insects including ants (Viljakainen *et al*., 2009). Antibiotic secretions are also associated with the salivary glands of arthropods including ticks, mosquitoes and termites have also been reported by Lu *et al*. (2005).

The available literature claims that the mandibular and metapleural gland are protecting the colony against the invaded micro-organisms of leaf-cutting ants (Hughes *et al.,* 2002; Yanagawa *et al.,* 2008). These compounds were investigated for potential activities against human pathogens particularly resident to conventional antibiotics (de Lima Mendonca *et al.,* 2009). The study reported that the *E.coli* is resistant to common antibiotics Ampicillin and Cefoxitin (Souza *et al.,* 2015) and the *S.aureus* was resistant to Chloramphenicol, Cefalotin, Erythromycin, Sulphonamide and Vancomycin (de Lima Mendonca *et al*., 2009). Junior *et al*. (2001) found that the mandibular gland secretion of leaf-cutter ant *Atta sexdens rubropilosa* inhibits the germination of necrotrophic fungus *Botrytis cinerea* which causes the diseases in economically important crops like Wine grapes and strawberries. Brough,(1983) discovered antimicrobial secretions from the mandibular gland in a Formicine ant (*Calomyrmex* sp.). Very few reports were documented about the bacterial diseases in ants. The *Pseudomonas* genus bacteria killed and fed all *Solenopsis invicta* larvae vegetative cells with-in five days (Lofgren *et al*., 1975). It is estimated that 66% of insect species and 30% ants are infected by *Wolbachia* (Hilgenboecker *et al.,* 2008; Russell *et al.,* 2012). *Wolbachia* infections cause detrimental effects on colony fitness of *Formica truncorum* (Wenseleers, Sundström, & Billen, 2002) and also affects Dorylinae, Formicinae, Myrmicinae, Ponerinae and Pseudomyrmecinae subfamilies (Van Borm *et al*., 2001). There are some beneficial microbes associated with ants, for example *Blochmannia* provides nutritional benefits in carpenter ants and leaf cutter ants use actinomycete bacteria to maintain stable ant-fungus relationship (Feldhaar *et al*., 2007; Mueller *et al.*, 2008). Developing a better understanding of the associations between carpenter ants and microorganisms can reveal the role of *Camponotus* in forest ecosystems (Mankowski & Morrell, 2004). Hence the present study focuses on microbial assemblage in nest soil and the respective defense mechanisms mounted by *C. compress* in various habitats.

MATERIALS AND METHODS

Study site

The study area Mukurthi National Park (MNP: 11 º 26' to 76º 10' to 11º22' N and 76º38' E) is about 78.46 Sq.km and has the elevation of 2400m and it is protected under UNESCO since 1st July 2012. The study area comprises the patches of evergreen forest surrounded by grasslands. The park has a wide variety of flora and fauna. But most of the grasslands have been replaced by exotic species namely wattle (*Acacia* spp.), pine (*Pinus roxburghii*), tea (*Camellia sinensis*) and bluegum (*Eucalyptus globulus*). The climate is highly seasonal, with a dry season extending from December to February and a wet season between June and November. The soil sample was collected from five habitats, namely Grasslands, Shola forest, Wattle plantation, Pine and Tea plantations. The carpenter ant *C. compressus* is one of the most abundant species of ants in South India. They live in enormous communities, travelling long distances from their nests, and are active during both day and night. *C. compressus* is one of the best known robust species in Mukurthi National Park. Three replicates of ant nest soil were sampled to a depth of 5, 10 and 15 cm from ten randomly selected ant nests from five habitats. The collected soil samples with ants were immediately transferred to the laboratory for the further study.

Mandibular gland extraction

The re-sealable plastic bags contains workers of *C. compressus* were placed in a freezer for 1 hr to immobilize the ants. Decapitation and preservation of ant heads in solvents probably 2 ml dichloromethane is a standard method for extraction of mandibular gland secretion of ants when they cannot be analyzed immediately. Decapitation ant heads about 1 gm were homogenized well using 2 ml of 70% alcohol and subsequently the mixture was centrifuged at 5000 rpm at 4˚C for 15 minutes. The pure supernatant was tested against the pathogens in a culture plates. The residuary samples were stored in a defreezer at -20ºC for long-term storage.

Screening of ant nests soil microbes

The pre-treated soil samples were serially diluted using 9 ml of sterilized phosphate buffer saline and 1 gm of the soil sample was added to it in a test tube and thoroughly mixed. The suspension was then serially diluted by transferring 1 ml of the suspension to a series of test tubes containing 9 ml of sterilized phosphate buffer till 10^{-6} dilutions. The swab sticks used for the collection of the samples and the samples were streaked directly on the labeled agar plates and incubated at 37°C for 24 h. After incubation, cultures were examined for significant bacterial colony growth. Subcultures were then made into plates of nutrient agar and incubated for another 24 hours. The primary identification of the bacterial isolates was made based on colonial appearance and pigmentation. Biochemical tests such as standard Catalase test, Citrate utilization, Oxidase, Methyl red, Voges Proskauer, Indole production, Motility, Glucose, Sucrose, Maltose, Lactose were performed to identify microbes, Characterization and identification of the isolates was done using the methods of Bergey's manual of determinative bacteriology (Buchanan & Gibbons, 1974).

Disc diffusion method

Kirby-Bauer disc diffusion method was used to determine the antibacterial activities (Fazeli *et al*., 2007; Molan, 1992; Samy *et al*., 2006) which has been widely used by several researchers. The *Escherichia coli* and *Staphylococcus aeureus* inoculums were inoculated on the surface of the separate nutrient agar plates with a help of sterile cotton swabs. The disc of antibacterial mandibular extraction of the *C.compressus* was placed on the surface of the agar plates. Further, the plates were incubated at 37˚C for 24 hours to determine on the basis of zone of inhibition. Using nutrient agar disk diffusion method, the isolated microbes were tested by the mandibular extraction. The selected isolated microbes were treated with various combinations of 10, 20 µl samples using petri disc. Double distilled water was used as a control. The same test was done with the standard commercial product Ampicillin. After incubation, the antibacterial activity was evaluated by measuring the inhibition zone (mm) ± SD of three replicates.

RESULTS AND DISCUSSION

The carpenter ant, *C. compressus* is a large colonial and social insects consisting of reproductive and sterile castes. The moist soil of the *C. compressus* ant nest is a habitat of high microbial abundance in the field, and the microbial biomass differs among the habitats. A total of 13 bacterial isolates were obtained and the maximum number of microbial isolates with 1X 10^3 (cfu/g) from ant nests in various habitats of Mukurthi National Park. The study identified 13 species of microbes namely *Bacillus alvei, Bacilllus clausii, Bacillus subtilis, Escherichia coli, Enterococcus faecalis, Klebsiella pneumonia, Lactobacillus acidophilus, Micrococcus luteus, Proteus mirabilis.*

Table 1. List of isolated dominant microbes in ant nests of the study area.

Sample plots	Soil code	Isolates (CFU)		Dominative isolate name
Grassland	GL	10^{-3}	644	*Proteus mirabilis*
		10^{-4}	713	*Staphylococcus aeureus*
		10^{-5}	121	*Bacillus clausii*
Shola Forest	SF	10^{-3}	1022	*Bacillus subtilis*
		10^{-4}	747	*Lactobacillus acidophilus*
		10^{-5}	500	*Pseudomonas fluorescens*
Wattle plantation	WP	10^{-3}	1017	*Staphylococcus aeureus*
		10^{-4}	212	*Pseudomonas* sp.
		10^{-5}	321	*Klebsiella pneumonia*
Pine plantation	PP	10^{-3}	742	*Enterococcus faecalis*
		10^{-4}	128	*Bacillus* sp.
		10^{-5}	79	*Pseudomonas aeruginosa*
Tea plantation	TP	10^{-3}	1789	*Escherichia coli*
		10^{-4}	798	*Proteus vulgaris*
		10^{-5}	230	*Staphylococcus aeureus*

Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aeureus and *Streptococcus salivarius* in nest soil of *C.compressus.* Among these microbes *Staphylococcus aeureus* was the most frequently isolated bacteria in three various habitats (Table 1). Ant mounds consist primarily of sand-sized particles, resulting in lower water holding capacity, increased drainage and aeration relative to bulk soil and conditions that can errand particular for microbial populations (Amador & Gorres, 2007).

The present result revealed that the occurrence of microbial biomass is varied greatly according to the condition of soil among the habitats. The composition of the organic materials is not the same throughout the whole nest volume (Coenen stass *et al*., 1980; Horstmann & Schmid, 1986). Nest conditions, such as high humidity and stable temperatures, favor the growth of microbes. The soil moisture affects the microbial activity because it controls both the substrate and oxygen diffusion. If soil is too dry, substrates may not be sufficiently mobile to support the microbial activities. The coarse texture and lower moisture content of ant mound soil could be responsible for the low microbial biomass and activities. In Pine plantation the ant nest soil contains low quantity of microbes and this may be due to resin which inhibits the growth of potentially pathogenic bacteria and fungi in the nest (Christe *et al*., 2003). Froz *et al*. (2005) reported that the microbial communities in European red wood ant *Formica polyctena* ant nest differ from that in the surrounding soil in part because of differences in pH, food availability and soil quality.

In the carpenter ant have the large mandibles and powerful tools for prey catching, fighting, digging, seed crushing, wood-scraping, grooming, brood care and trophallaxis (Muscedere *et al*., 2011), also protecting the colony against pathogenic pressures. In the present work, it has been demonstrated that the main constitutions of the mandibular gland secretions of ants possess strong inhibitory activities against two robust grams positive and gram negative bacteria isolated from ant nests (Figure 1). Antibacterial potency of mandibular extraction of *C.compressus* tested against robust bacterial species *Escherichia coli* (gram negative) and *Staphylococcus aeureus* (gram positive). The maximum zone of inhibition was observed against the *Staphylococcus aeureus* (10 mm, 12 mm) in both 10 µl and 20 µl of the mandibular extracts. The zonal inhibition against the gram negative bacteria *Escherichia coli* was 8 mm and 10 mm.

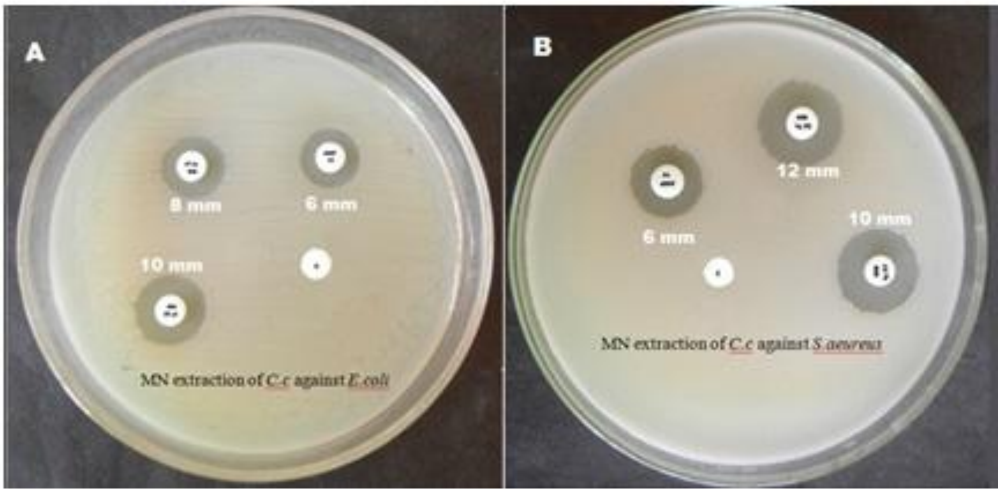

Figure 1. Photographic evidence of an Antibacterial activity of *C.compressus* mandibular extraction against *Escherichia coli and Staphylococcus aeureus*.

Note: (a) AMP - Ampicillin, (b) C- distilled Water, (c) MN - Mandibular gland extraction (10, 20 µl inhibition zone (mm) ± SD of three replicates).

Table 2. Zonal inhibition of *Camponotus compressus* mandibular extraction.

S.No	Bacterial strains	Commercial product	Concentration of *Camponotus compressus* mandibular extraction	
		AMP	MN 10 µl	MP 20 µl
1	*Escherichia coli*	6 mm	8 mm	10 mm
2	*Staphylococcus aureus*	6 mm	10 mm	12 mm

The ants are threatened by numerous predators, parasites and pathogens from various animals, fungi, bacteria and viruses. Most of the ant species possess antimicrobial agent metapleural gland on thorax whose secretions spread over the ants and throughout the nests (Mackintosh *et al*., 1999) also ant venom contains antimicrobial property including alkaloids which inhibit the growth of both Gram-positive and negative bacteria and presumably act as a brood antibiotics as reported elsewhere (Jouvenaz *et al*.,1972; Orivel *et al*., 2001). However, in case carpenter ants the mandibular gland produces compounds that have antibacterial activity that protect the terrestrial ant colonies from soil pathogens (Maschwitz, 1974). Therefore, the present study revealed that the absence of metapleural gland is substituted by the mandibular gland in *Camponatus compressus* species. According to Hermann & Blum, (1981) the glandular secretions are generally acidic in nature, expressed in the form of carboxylic acid or phenol moieties. Consequently, many other ant glandssecrete compounds that are weakly bacterio-static and in the case of formic acid from the poison gland of formicinae ants are significantly bactericidal. Bacterial suppressing effect of a particular acidic glandular secretion therefore may not be its primary function.

CONCLUSION

The present study elucidated the efficiency of *Camponotus compressus* mandibular gland is remarkably active against the gram positive and gram negative bacteria. The strong zone of inhibition found against both gram negative *Escherichia coli* and gram positive *Staphylococcus aeureus*. Further work is needed to determine the chemical constitutions of *C.compressus* mandibular glands. Based on the results, it is concluded that mandibular gland has great potential as antibacterial agent than commercial products against microorganisms and that can also be used in the treatment of infectious pathogen diseases.

ACKNOWLEDGEMENT

We are grateful to Chief Wildlife Warden and District Forest Officer, Tamil Nadu Forest department for issued research permits.

REFERENCES

Amador, J.A., & Gorres, J.H. (2007). Microbiological characterization of the structures built by earthworms and ants in an agricultural field. *Soil Biology and Biochemistry, 39*(8), 2070-2077.

Ayre, G., & Blum, M. (1971). Attraction and alarm of ants (Camponotus spp. Hymenoptera: Formicidae) by pheromones. *Physiological Zoology, 44*(2), 77-83.

Blum, S., & Hermann, H. (1978). *Venoms and venom apparatuses of the Formicidae: Dolichoderinae and Aneuretinae. In: Bettini, S. (ed) Arthropod Venoms, Hand Book of Experimental Pharmacology,* Springer-Verlag, Berlin, 871-894.

Bot, A. N., Ortius-Lechner, D., Finster, K., Maile, R., & Boomsma, J.J. (2002). Variable sensitivity of fungi and bacteria to compounds produced by the metapleural glands of leaf-cutting ants. *Insectes Sociaux, 49*(4), 363-370.

Boulton, A.M., & Amberman, K.D. (2006). How ant nests increase soil biota richness and abundance: a field experiment *Arthropod Diversity and Conservation, 15,* 55-68.

Bradshaw, J., Baker, R., & Howse, P. (1975). Multicomponent alarm pheromones of the weaver ant. *Nature, 258*(5532), 230.

Brough, E.J. (1983). The antimicrobial activity of the mandibular gland secretion of a formicine ant, Calomyrmex sp. (Hymenoptera: Formicidae). *Journal of Invertebrate Pathology, 42*(3), 306-311.

Buchanan, R., & Gibbons, N. (1974). *Bergey's Manual of Determinative Bacteriology*. 8th edn. Baltimore: The Williams and Wilkins Company, Baltimore, 26, 1246.

Cammaerts, M.C., Evershed, R., & Morgan, E.D. (1983). The volatile components of the mandibular gland secretion of workers of the ants Myrmica lobicornis and Myrmica sulcinodis. *Journal of Insect Physiology, 29*(8), 659-664.

Christe, P., Oppliger, A., Bancala, F., Castella, G., & Chapuisat, M. (2003). Evidence for collective medication in ants. *Ecology Letters, 6*(1), 19-22.

Coenen-Stass, D., Schaarschmidt, B., & Lamprecht, I. (1980). Temperature distribution and calorimetric determination of heat production in the nest of the wood ant, Formica polyctena (Hymenoptera, Formicidae). *Ecology, 61*(2), 238-244.

Dauber, J., & Wolters, V. (2000). Microbial activity and functional diversity in the mounds of three different ant species. *Soil Biology and Biochemistry, 32*(1), 93-99.

de Lima Mendonça, A., Da Silva, C. E., de Mesquita, F.L. T., da Silva Campos, R., Do Nascimento, R.R., de Azevedo Ximenes, E.C.P., & Sant'Ana, A.E.G. (2009). Antimicrobial activities of components of the glandular secretions of leaf cutting ants of the genus Atta. *Antonie Van Leeuwenhoek, 95*(4), 295-303.

Fazeli, M.R., Amin, G., Attari, M.M.A., Ashtiani, H., Jamalifar, H., & Samadi, N. (2007). Antimicrobial activities of Iranian sumac and avishan-e shirazi (Zataria multiflora) against some food-borne bacteria. *Food Control, 18*(6), 646-649.

Feldhaar, H., Straka, J., Krischke, M., Berthold, K., Stoll, S., Mueller, M.J., & Gross, R. (2007). Nutritional upgrading for omnivorous carpenter ants by the endosymbiont Blochmannia. *BMC Biology, 5*(1), 48.

Froz, J., Kalcik, J., & Cudlín, P. (2005). Accumulation of phosphorus in nests of red wood ants Formica s. str. Paper presented at the *Annales Zoologici Fennici,* 42(3), 269-275.

Hermann, H.R., & Blum, M.S. (1981). Defensive mechanisms in the social Hymenoptera. *Social Insects, 2*, 77-197.

Hilgenboecker, K., Hammerstein, P., Schlattmann, P., Telschow, A., & Werren, J.H. (2008). How many species are infected with Wolbachia? – a statistical analysis of current data. *FEMS Microbiology Letters, 281*(2), 215-220.

Hölldobler, B., & Engel-Siegel, H. (1984). On the metapleural gland of ants. *Psyche, 91*(3-4), 201-224.

Horstmann, K., & Schmid, H. (1986). Temperature regulation in nests of the wood ant, Formica polyctena (Hymenoptera: Formicidae). *Entomologia Generalis,* 229-236.

Hughes, W. (2005). Life histories and parasite pressure across the major groups of social insects. *Insect Evolutionary Ecology: Proceeding of the Royal Entomological Society, 211*, 139-139.

Hughes, W. O., Eilenberg, J., & Boomsma, J. J. (2002). Trade-offs in group living: transmission and disease resistance in leaf-cutting ants. *Proceedings of the Royal Society of London B: Biological Sciences, 269*(1502), 1811-1819.

Jakubczyk, H., Czerwiński, Z., & Pętal Figielska, J. (1972). *Ants as agents of the soil habitat changes*: Państwowe Wydawnictwo Naukowe. Oddział. *Ekologia Polska.* 20, 153 - 161.

Jouvenaz, D., Blum, M., & Mac Connell, J. (1972). Antibacterial Activity of Venom Alkaloids from the Imported Fire Ant, Solenopsis invicta Buren.

Antimicrobial Agents and Chemotherapy, 2(4), 291-293.

Junior, A.L.M., Della Lucia, T.M., Barbosa, L.C., Maffia, L.A., & Morandi, M.A. (2001). Efeito de secreções da glândula mandibular de Atta sexdens rubropilosa Forel (Hymenoptera: Formicidae) sobre a germinação de conídios de Botrytis cinerea Pers. Fr. *Neotrop. Entomol, 30*, 403.

Lofgren, C., Banks, W., & Glancey, B. (1975). Biology and control of imported fire ants. *Annual Review of Entomology, 20*(1), 1-30.

Lu, L.J., Xia, Y., Paccanaro, A., Yu, H., & Gerstein, M. (2005). Assessing the limits of genomic data integration for predicting protein networks. *Genome Research, 15*(7), 945-953.

Mackintosh, J., Flood, J., Veal, D., & Beattie, A. (1999). Increase in levels of microbiota recoverable from male and larval *Myrmecia gulosa* (Fabricius) (Hymenoptera: Formicidae) following segregation from worker ants. *Austral Entomology, 38*(2), 124-126.

Mankowski, M., & Morrell, J. (2004). Yeasts associated with the infrabuccal pocket and colonies of the carpenter ant *Camponotus vicinus*. *Mycologia, 96*(2), 226-231.

Maschwitz, U. (1974). Vergleichende untersuchungen zur funktion der ameisenmetathorakaldrüse. *Oecologia, 16*(4), 303-310.

Molan, P.C. (1992). The antibacterial activity of honey: 1. The nature of the antibacterial activity. *Bee World, 73*(1), 5-28.

Mueller, U.G., Dash, D., Rabeling, C., & Rodrigues, A. (2008). Coevolution between attine ants and actinomycete bacteria: a reevaluation. *Evolution, 62*(11), 2894-2912.

Muscedere, M.L., Traniello, J.F., & Gronenberg, W. (2011). Coming of age in an ant colony: cephalic muscle maturation accompanies behavioral development in Pheidole dentata. *Naturwissenschaften, 98*(9), 783.

Orivel, J., Redeker, V., Le Caer, J.P., Krier, F., Revol-Junelles, A.M., Longeon, A., Rossier, J. (2001). Ponericins, new antibacterial and insecticidal peptides from the venom of the ant Pachycondyla goeldii. *Journal of Biological Chemistry, 276*(21), 17823-17829.

Poulsen, M., Bot, A.N., Currie, C. R., & Boomsma, J.J. (2002). Mutualistic bacteria and a possible trade-off between alternative defence mechanisms in Acromyrmex leaf-cutting ants. *Insectes Sociaux, 49*(1), 15-19.

Poulsen, M., Bot, A.N., Nielsen, M.G., & Boomsma, J.J. (2002). Experimental evidence for the costs and hygienic significance of the antibiotic metapleural gland secretion in leaf-cutting ants. *Behavioral Ecology and Sociobiology, 52*(2), 151-157.

Powell, S., & Clark, E. (2004). Combat between large derived societies: a subterranean army ant established as a predator of mature leaf-cutting ant colonies. *Insectes Sociaux, 51*(4), 342-351.

Russell, J.A., Funaro, C.F., Giraldo, Y.M., Goldman-Huertas, B., Suh, D., Kronauer, D.J., Pierce, N.E. (2012). A veritable menagerie of heritable bacteria from ants, butterflies, and beyond: broad molecular surveys and a systematic review. *PLoS One, 7*(12), e51027.

Samy, R.P., Pachiappan, A., Gopalakrishnakone, P., Thwin, M.M., Hian, Y.E., Chow, V.T., K Chow,; Ho Bow and; Joseph T., Weng, J. T. (2006). In vitro antimicrobial activity of natural toxins and animal venoms tested against Burkholderia pseudomallei. *BMC Infectious Diseases, 6*(1), 100.

Savin, M.C., Görres, J.H., & Amador, J.A. (2004). Microbial and microfaunal community dynamics in artificial and Lumbricus terrestris (L.) burrows. *Soil Science Society of America Journal, 68*(1), 116-124.

Souza, D., Almeida, A., Andrade, V., Marcelo, N., Azevedo, I., Martins, E., & Figueiredo, L. (2015). Atividade antimicrobiana do óleo essencial de Lippia origanoides e Lippia rotundifolia frente a enterobactérias isoladas de aves. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 67*(3), 940-944.

Van Borm, S., Wenseleers, T., Billen, J., & Boomsma, J.J. (2001). Wolbachia in leafcutter ants: a widespread symbiont that may induce male killing or incompatible matings. *Journal of Evolutionary Biology, 14*(5), 805-814.

Viljakainen, L., Evans, J.D., Hasselmann, M., Rueppell, O., Tingek, S., & Pamilo, P. (2009). Rapid evolution of immune proteins in social insects. *Molecular Biology and Evolution, 26*(8), 1791-1801.

Wenseleers, T., Sundström, L., & Billen, J. (2002). Deleterious Wolbachia in the ant Formica truncorum. *Proceedings of the Royal Society of London B: Biological Sciences, 269*(1491), 623-629.

Wilson Rich, N., Dres, S.T., & Starks, P.T. (2008). The ontogeny of immunity: development of innate immune strength in the honey bee (*Apis mellifera*). *Journal of Insect Physiology, 54*(10-11), 1392-1399.

Yanagawa, A., Yokohari, F., & Shimizu, S. (2008). Defense mechanism of the termite, Coptotermes formosanus Shiraki, to entomopathogenic fungi. *Journal of Invertebrate Pathology, 97*(2), 165-170.

*Corresponding Author: Dr. P. Mohana, Assistant Professor, Department of Zoology, Vivekanandha College of Arts and Sciences for Women (Autonomous), Elayampalayam, Tiruchengode-637205, Tamil Nadu, India. Email: drpmohana@gmail.com