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Application of Annona squamosa crude leaf extracts against Lasius niger, a black garden ant

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ABSTRACT
Garden ants in black Lasius niger are a significant agricultural and urban pest that invade both indoor and outdoor living spaces and decimate crops. Workers are 3-5 mm length and lustrous dark black. It has been observed that workers' sizes increase over successive generations as the colony ages. Lasius niger workers were sprayed with aqueous extracts of fresh Annona squamosa leaves at concentrations of 100%, 75%, 50%, 25%, 10%, 5%, 1%, and 0.1%. the dilution determined using the weight per volume formula with RO Water as the solvent. The definition of mMortality is dead / number of subjects x 100%. Both a positive control group that was exposed to the insecticide Chlorpyriphos 2% and a negative control group that was exposed to RO Water were employed as controls in our study. The Annona squamosa leaves extract successfully killed Lasius niger at concentrations of Extract leaves 25%, 50%, 75%, and 100% (w/v), but was less effective when compared to the positive control insecticide Chlorpyriphos 0.2%, and ineffective at concentrations of Extract leaves 0.1%, 5%, and 10%. While the extract's LD50 was extremely high (61.30%).

I. INTRODUCTION

The black garden ant (Lasius niger), commonly known as the common black ant, is a formicine ant that can be found across Asia as well as in some regions of North America, South America, Australia, Europe, and Australasia. It is the type species of the subgenus Lasius. The Asian species were divided into two species; L. platythorax inhabits forests, whilst L. niger lives in open spaces. Since there is just one queen per colony, it is monogynous.

Lasius niger colonies typically have between 4,000 and 70,000 workers, while they occasionally grow to be as large as 40,000. A Lasius Niger queen can live for up to 15 years, however some have reportedly lasted for 30. When the Lasius niger nest is first being established, two to three other queens may be present. They will put up with each other until the first workers arrive, at which point they will probably start fighting until only one queen is left. If two colonies are starting out reasonably close to one another and eventually their two tunnels link, it is conceivable in rare cases for there to be more than one queen in a single colony.

THE Queen phenotype is 9 mm long, glossy black colour but appears to have slight brown stripes on her abdomen. The queen can reach 6-9mm in length and is smaller as a new queen. When a queen is fertilised she removes her wings and digests her wing muscles as food over the winter.

The male phenotype is 3.5–4.5 mm long, slim, colour black. Only produced by queens when the nuptial flights are approaching. They appear with a dark glossy body with a different shape from the workers, almost resembling a wasp appearance. They have wing muscles which stand out from the rest of the body. They are 5-7mm long and have delicate wings.

The worker phenotype is 3–5 mm long, workers are dark glossy black. As the colony gets older it has been known for workers to increase in size over generations.

`This type of ant is a problem for some gardeners. They will farm aphids and scale for the honeydew they excrete, bringing them from host plant to host plant spreading these other garden pests to new healthy plants. The ants will also eat ripe fruits, especially fruits like Tomatoes that lack a thick protective skin. *Lasius niger* also feed on insects and spiders, and other small invertebrates. Black garden ants often explore their surroundings quite extensively during early summer months in an effort to increase the food supply to their queen and her young, and also as a way of testing new ground in preparation for the nests' summer flight. In some cases, these explorations lead to a burrowing through mortar and brick.

Eradication of black garden ants recently conducted using chemical, an insecticide e.g. powder chlordane 5%, solution chlordane 2% or malathion 3% and Chlorpyriphos 2.0 %. Permethrin is used either in the agriculture or for eradication of other household pests, like a spider, bug, other ants, bee, Black garden ants and bedbugs. Though a synthetic insecticide effectively kills insects, but frequently use of it will induce environmental damage and health problems, because of inhaled, ingested insecticides and also because of or its residue in food. In addition, the use of insecticides to kill black garden ants population might finally may can

also kill insects that are playing a key role in environment.

Air Pollution in Higher Groups Plants

Sugar-Apple (*Annona squamosa*) is widespread throughout the tropical regions of the world. *Annona squamosa* leaf contain borneol which had the effect of insecticides. Borneol is a bicyclic organic compound, and is easily oxidized to camphor yealding ketones. Borneol can be synthesized by the reduction of camphor with Meerwein-Ponndorf-Verley Reduction method. Its chemical structure is the molecule $C_{10}H_{18}O$. Until now there is no research on efficacy of the extract *Annona squamosa* against The **black garden ant** (*Lasius niger*). This research aims to unravel the efficacy of leaf extract of *Annona squamosa* against *Lasius niger*. The results of this research are expected to be able to provide information on biological insecticide development in the field of Environmental health. Furthermore, it Next could be developed as a bioinsecticide to prevent infectious diseases through contaminated food, insect bite and reduce the allergies due to insects.

Material and Methods-

Experimental Subjects-

Subjects are adult worker ants (*Lasius niger)*, derived from the houses. Homogenization of subjects could not be done (obtained by rearing in the laboratory) because the life cycle of *Lasius niger* very long , so that experiments conducted by taking *Lasius niger* workers randomly almost the same size (3-5 mm). Sampling was done randomly.

Prepration and use of Annona Insecticede-

Variables in research are a series of concentrations of *Annona squamosa* leaf extract as a free independent variable and mortality ant test (%) as a dependent variable. Concentrations of the extract would be tested are 100%, 75%, 50%, 25%, 10%, 5%, the 0.1% (b/v or v/v), respectively. The dilution calculated by the formula weight per volume, with the solvent RO Water. While mMortality is defined as dead /number of subjects x 100%. As a comparison we used positive control group that was exposed with the insecticide Chlorpyriphos 2.0%) and a negative control group which is exposed by RO Water. Instruments used are tubes(20 cm in length and diameter 2.5 cm), with a gauze to cover the tubes, so that Black garden ants can still respire, tweezers, filter paper and timer were used in this research. While, the material used is the ethanolic extract of leaf of *Annona squamosa leaves*, Chlorpyriphos 2.0 %) and RO Water.

Insecticidal properties: Leaves of *Annona squamosa* were collected from plants and air-dried and powdered. 12.5 g of the powdered material was soaked for 24 h in a glass jar in a solution of 12.5 ml water and 50 ml solvent (methanol). Subsequently, the solutions were filtered through a filter and then the extracts were stored in the refrigerator (4^0C) prior to use. Treatment on all research groups were done by placing Black garden ants into the tube with the filter paper inside. The filter paper was smeared with as much as 2 ml extract. Each research groups consisting of 5 Black garden ants and was done in duplicate with 2 replication. The observation is done by counting the number of died subjects who died after 24 hours of exposure (death rate in %) was observed. Analysis of variance was done to evaluate for knowing the significance of the difference between the mortality research groups. In addition, analysis was done for knowing lethal concentration (LD50). If the negative control mortality were 5-10%, then the treatment group mortality corrected with Abbott's formula (1925). The Abbott's formula is as follows:

$$\% \text{ Corrected mortality} = \frac{\% \text{ Test Group Mortality} - \% \text{ Neg Control Mortaliy}}{100 - \% \text{Neg Control Mortality}}$$

Result and discussion-

The results of study shown on table 1. The table 1 shows that the mortality rate is 100% in positive control group. On the treatment groups, it seems the higher the concentration of the extract of *Annona squamosa* leaves, the higher death rate Black garden ants, that the death rate of Black garden ants related to the dose of the extract. Leaf extract 0.1% seem ineffective to kill Black garden ants after 24 hours of exposure, shown by 0% mortality in the group, together with same as in the negative control group.

The table shows that the lower the concentration of the test substance, the lower the death Black garden ants, even at concentrations of 0.1% there are 100% Black garden ants still alive. Results of statistical tests with *one way Anova* shows that there are significant differences between the research groups (F calculate 41.25 > F table 3.32). The continued test of *Post Hoc Duncan multiple range test* showed that in research groups on *Annona squamosa* leaf leaves extract concentration 0.1%, 5%, and 10% was not effectively kill *Lasius niger* .(p= This is demonstrated by the results of insignificance of that groups with the negative control. Treatment group on extract 25% (p=0.037), 50% (p=0.00), 75% (p=0.00) and 100% (p=0.00) proven effective killing *Lasius niger*, but less effective when compared to the positive control chemical insecticide use.

Annona squamosa leaves have not been much researched as insecticide. These are still in discussion about the active compoud of *Annona squamosa*. According to Duke (2008), one of the *Annona squamosa* leaf contents in leaves that possibily effective as insecticides or repellent is borneol. Borneol is a essential oil with bicyclic

organic compound that belong to essential oil, and has a natural insect repellent effect. It is not yet known, about the mechanism of working of borneol as insect repellent. According to Belaqziz *et al*. (2010), borneol contained within *Thymus broussonetti* has antibacterial effect and ability to kill of *Culex pipiens* larvae.
LD50 of ethanolic extract of *Annona squamosa* leaves in this research against Black garden ants was quite high (61.30%). This is likely due to special body organization of the ants, so it required a large dose to kill the insect. There has been no research report concerning the LD50 of Annonaceae leaves extract against large-sized Insects

Table 1. The number of deaths of Black garden ants (*Lasius niger*) after exposure to the test substance *Annona squamosa* leaf leaves extract in various concentrations (100%, 75%, 50%, 25%, 10%, 1%, the 0.1%), (positive control) and RO Water (negative control)

Number of Black garden ants death					
Study Groups	**R**	**N total**	**N death**	**Average of death**	**% of death**
POSITIVE CONTROL	1 2	5 5	5 5	5.00 ± 0.00	100
EXTRACT 100%	1 2	5 5	4 4	4.00 ± 0.00	80
EXTRACT 75%	1 2	5 5	3 3	3.00 ± 0.00	60
EXTRACT 50%	1 2	5 5	2 3	2.5 ± 0.71	50
EXTRACT 25%	1 2	5 5	1 1	1.00 ± 0.00	20
EXTRACT 10%	1 2	5 5	1 0	0.5 ± 0.701	10
EXTRACT 5%	1 2	5 5	1 0	0.5 ± 0.701	10
EXTRACT 0.1%	1 2	5 5	0 0	0.00 ± 0.00	00
NEGATIVE CONTROL	1 2	5 5	0 0	0.00 ± 0.00	00

According to Trindade *et al*. (2011), there is the influence of the period to collect the leaves. For best results collection should be carried out during the dry season. In this research, a collection of leaves made in October (the wet season), so it is likely to be one of the causes of the low toxicity of extract of *Annona squamosa* leaves against the Black garden ants. The storage of extract also affect the effectiveness as an insecticide. According to Dharmasena *et al.* (2001), storage for six months can decrease the activity of *Annona squamosa* insecticide leaf extract against *Callosobruchus maculatus* significantly. In this research, the fresh leaves has been used. so there is no reason to lack effectiveness due to storage

II. Conclusion

The leaves extract from *Annona squamosa* with Extract leaves 25%, 50%, 75% and 100% (w/v) concentration *Annona squamosa* showed effectively kill *Lasius niger*, but less effective if compared to the positive control (Chlorpyriphos 2.0%)., while extract leaves 0.1%, 5%, and 10% ineffective.While, LD50 of the extract were was very high(61.30%).

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