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# Comparative structural variations in formicarium required for *In-vitro* studies in two species of ants (Hymenoptera: Formicidae)

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**Abstract**
The development of in-vitro formicariums for the fire ant *Solenopsis invicta* and carpenter ant *Camponotus compressus* was done using methods suggested by Janet (Janet. 1893). Different approaches are required for the two formicaria. For a fire ant formicarium, *Solenopsis invicta*, four different layers are necessary: a drainage layer to prevent a wet condition, a barrier or compartment layer made of coconut husk or shade cloth to stop soil from entering the drainage layer, a layer of charcoal to clean the air or stop microbial or fungal activity and finally a layer of soil. The formicarium of Carpenter ants, *Camponotus compressus*, on the other hand, require a drainage layer made of tiny rocks or clay balls as well as the sole soil layer that causes the carpenter ants to build their colonies deeper. Both formicaria have demonstrated 100% success in raising their offspring.

**Keywords:** ants, formicarium, fire ant, *Solenopsis invicta*, carpenter ant, *Camponotus compressus*, *In-vitro*

## Introduction

Socio-biological works provide important information about various evolutionary theories and that has been summed up in Ant Bible (Holldobler and Wilson 1990). Ants have attracted attention in this regard from ancient times and the study gives useful data for behavioral observations or bio-geographical subgroup studies (Kohout 1990; Leifke et al. 1998). Several authors (Hickling and Brown 2000; Holldobler and Wilson 2009 [5]; Jones et al. 2004 [7]; Quinet et al. 2005 [13]; Roces 1996 [32]) have researched various species of ants for their fascinating behaviour. It has been reported that Camponotus sp. workers exhibit just one of the two circadian activity patterns (diurnal or nocturnal), implying that each worker is 'hardwired' for one or the other (Sharma et al. 2004) [33], which was later acknowledged as an ethological identity. Bingham (1903) [1] discovered two marked worker ants of *Phedole yensis* returning to the nest after finding a food source and leading advance groups to obtain food from that source. *Solenopsis invicta Buren* (Hymenoptera: Formicidae) is a red imported fire ant that is frequently used as a test subject for scientific projects by students and researchers. In Southern regions, Fire ants are easily obtainable and can be easily collected and cultured. However, there are guidelines that forbid the use of live animals. Fire ants are potentially dangerous organisms that can repeatedly sting and in rare situations, result in life-threatening medical issues (Ferguson et. al. 2021) [36]. When using F.
fire ants as test subjects, always use extreme caution to prevent any potential issues. The Ant Formicarium model may infrequently be found normally as fire ants can quickly escape from these gadgets, making them inappropriate for use with them.

A formicarium is a sort of vivarium that houses ant colonies and is also frequently referred to as an ant farm (Janzen et. al. 1974) [26]. The Latin term Formica, which means ant, is where the formic portion of the world originates. It literal means a site of ants. Formicaria is the plural of formicarium. Charles Janet, a French engineer, built the first ant formicarium in the early 1900s (Janet. 1893) [18]. He sought to increase the visibility of ant colony so that it could be observed. His innovative 2D ant nest design was the forerunner of several later, now-famous patentable ideas. Charles was reluctant to have the vivarium displayed at the Exposition Universelle 1900 in Paris since he had no plans to promote it. Frank Austin, an inventor, was the first to commercially offer ant farms and was awarded numerous patents for his reimagined design beginning in 1929. For added aesthetics, several of Frank's formicaria included small above-ground settings like farms, castlesand forests. The creator of Uncle Milton Industries, Milton Levine, registered the name Ant Farm (Lewallen et. al. 2004) [22]. A terrarium-style ant farm is constructed in a vivarium with more conventional meaning. The substrate would be put in a container, along with any additional greenery or ornaments. Any enclosure that is typically used to house animals will work. These kinds of formicaria are incredibly attractive to the eye and provide ants with food. The dark exposed regions will offer security and cause less stress on your ant colony, even if the nesting layer won't be as visible for observational purposes. They will value the solitude and prosper as a group. Formicarium is perfect for keeping the queen ant inside. These patterns were created to make it simple to observe and study the behavioral pattern of ant colony.

## Materials and methods

### Field Collection Fire Ant and Carpenter ant Colonies

Applying a typical bait and pitfall trap techniques one can collect red imported fire ant colonies from the scrubland field using plastic bucket procedure, (Banks et al. 1981). Apply talcum powder to the interior of the bucket using a cotton pad. As long as the surface is dry, ants cannot climb vertical surfaces that have been dusted. Additionally, using talcum powder to more safely prevent ants from creeping up the person collecting them by dusting gloves, rubber boots and shovel handles. It need to dig swiftly and place the dirt in the bucket. Once the ants have been removed, make careful to chop any grass or twigs that might serve as a bridge to the bucket's top and recoat with talcum powder.

**The formicarium of Fire Ant**, ***Solenopsis invicta***

The formicarium is a clear glass or plastic container having length of about 120 cm, width of 30cm and 45cm height with a top lid having minute holes that allow ant to respire easily. The upper surface of formicarium can also be covered by a nylon mesh, preventing ants from exiting the setup but allowing air to pass freely. For constructing the natural ant environment in-vitro requires certain step to be followed that is step. 1. The first bottom layer in formicarium is the drainage layer by adding clay balls or small rocks that prevent the water-logged condition which can cause the substrate to rot (fig.1), thus providing ideal condition for harm full bacteria and fungi to thrive. Any access water in this setup will step down through the substrate above and settle within the small gaps between all the rocks or clay below. Allowing the soil to sufficiently drain and dry out. Step.2. Cover the drainage layer to prevent the soil above from entering and thus inhibiting its effectiveness, for this a shade cloth which has been cut to shape other than this for more natural approach some coconut husk act as excellent alternative (Fig. 2). Step.3. When water passes down through this layer and into the drainage layer below the charcoal helps purify it (Fig. 3). Lessening the chances of harmful microbes building up. Making sure that the charcoal doesn't have any added chemical in it. Step.4. Added soil from the farm or backyard (Fig. 3).

Choosing substrate should depend on what the particular species of ant prefer. Some species might prefer rather dense and sandy soil, whereas other might prefer more of a loose, bark like substrate. Here in the fire ant case, they prefer loose, bark like substrate to live like the way they live in their natural habitat. But carpenter ants prefer a solid one to construct a smooth colony below it. Step.5. Added some plants that best suits the particular setup where they will inhabit it (Fig. 5). It wouldn't be a good idea to pick water loving plants in case of housing fire ants which prefer relatively dry conditions. Making sure that the plant gets enough light naturally from a nearby window. After that add some small rocks, sticks or bark of tree from where the ants were taken.

**Fig 1:** First layer of small rocks

**Fig 2:** Coconut husk layer

**Fig 3:** Charcoal layer

**Fig 4:** Soil layer

**Fig 5:** Gravels

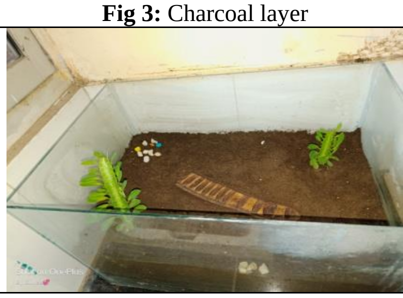

**Fig 6:** layer of succulent shrubs

**The formicarium of Carpenter Ant**, ***Camponotus compressus***

For constructing formicarium for Carpenter ant in-vitro is to make a drainage layer first by adding some small sized rocks or clay balls that drain out all excess water from it. The soil found in the surrounding of natural carpenter ant colony will step down through the substrate above and settle within the small gaps between all the rocks or clay below. Next and the only layer after this for carpenter ant colony construction requires soil in a larger amount so that they can built their nest colony deep inside. One can add plants in that formicarium for making it more natural and for more oxygen content for particular ant in that closed box.

**Fig 7:** formicarium for carpenter ants


**Fig 8:** final view of formicarium

## Results

Based on the behavioral biology of both Fire ants and Carpenter ants, different formicaria have been constructed successfully in-vitro in the laboratory depending on their requirements regarding food, shelter, habitat and ecology as fire ants requires more nutritional care and self sustain non-pathogenic environment with less soil and more substrate content under which they can burrow or hide their eggs or young once whereas Carpenter ants require more soil content so that they can construct more tunnels and chambers for their young once to settle. The soil requirements for both ants are also different as Fire ant requires loose soil whereas Carpenter ants need more compact soil for constructing tunnels.

## Conclusion

The result indicate that the selected species needs two different kind of formicaria for various kind of in-vitro study on the life of these ants. There is wide scope to prepare such kind of formicaria for other species of ants.

## Discussion

Results from manufactured nests in captivity demonstrated that fire ants can attack and kill the hatchlings if a clutch enters the final stage of development and piping occurs, decreasing *C. latirostris* hatchling survival by 10%. On the other hand, ant colonies in the wild resulted in a 43% decline in nest success (taking into account a 28.5% decline in hatching rate and a 14.5% decline in hatchling survival). In the confined study, in-vitro formicarium of red fire ants had a 100% hatching rate and 100% hatchling survival rate, while wild nests had a 100% hatching rate and 100% hatchling survival rate. Red fire ant infestations in *A. mississippiensis* nests resulted in decreased hatchling survival as well (Allen et al., 1997) [14]. Red Fire Ants negatively impacted every nest, whether it was in the wild or in captivity. This is possibly because various Red Fire Ant colonies have variable amounts of aggressiveness (Vander Meer and Alonso, 2002) [15].

Despite the name "carpenter ants" being given to the genus Camponotus, several species did not build their nests in wood. For instance, C. sericeus can construct a nest on the ground (Mody and Linsenmair 2003) [16]; C. senex can build a silk nest in trees with dense copes (Santos 2002) [17]; *C. mirabilis* can construct a nest inside the stem of a bamboo plant (Davidson et al. 2006); other ants can nest either in the ground or inside the wood, such as *C. pennsylvanicus* (Cannon 1998) [20].

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