The International Journal of Indian Psychology
ISSN 2348-5396 (Online) | ISSN: 2349-3429 (Print)
Volume 12, Issue 1, January- March, 2024
DIP: 18.01.163.20241201, DOI: 10.25215/1201.163
https://www.ijip.in

**Research Paper**

# Associative Learning in Plants: An Investigation of *Mimosa Pudica*

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## ABSTRACT

In 1873 Pfeffer published results of one of the earliest experiments in plant learning, demonstrating that the repeated mechanical stimulation of the leaflets of *Mimosa pudica* led to a habituation response. In the following century-and-half span, limited research has been done in the area and we still know comparatively little today about the possibilities and intricacies of learning behaviour in plants. The present experimental research was an investigation in said direction, studying the possibility of acquisition of associative learning in plants. The aim was to establish acquisition or non-acquisition of associative learning in *Mimosa pudica*. A sample of N=40 *Mimosa pudica* saplings were included in the research design, with 20 specimens each in one control, and one experimental group ($n_c$=20, $n_p$=20). A standardised tactile stimulus was administered to the experimental group specimens directly following set duration of exposure to a standardised light source in a delay conditioning arrangement, to induce hiding response in a target leaf for each *Mimosa pudica* specimen in the experimental group. Intervention trials were carried out for 6 days on the experimental group. On day 7, test administration of the standardised light stimulus was found to induce hiding response in many of the experimental group specimens. No control group specimen showed hiding behaviour in response to administration of the light stimulus.

**Keywords:** *Learning, Associative Learning, Mimosa Pudica, Plant Learning, Neutral Stimulus, Conditioned Response*

In 1873 Pfeffer published results of one of the earliest experiments in plant learning, demonstrating that the repeated mechanical stimulation of the leaflets of *Mimosa pudica* led to an apparent decrease in sensitivity to such stimulation – or habituation. A similar tendency in response was demonstrated again by Bose (1906), and extended to electrical as well as mechanical stimulation. He demonstrated also that once habituated, a rest period without the stimulation to which the plant was habituated would be necessary before normal petiole falling response could resume. Holmes and Gruenberg (1965) demonstrated the capacity for stimulus discrimination in *Mimosa pudica* in addition to providing evidence that in the preceding researches, it was indeed habituation, and not simply fatigue that was observed.

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**Received: June 06, 2023; Revision Received: March 07, 2024; Accepted: March 11, 2024**

Sensory faculty, exhibition of behaviour, and eventually the formation of contingencies between these that lead to the most rudimentary forms of ‘learning’, form the foundations on which the vast majority of the behavioural school of psychology and – to a somewhat lesser extent – experimental traditions in psychology stand.

Experimental inquiry into behaviour has often been unorthodox, but resulted in the birth of theoretical frameworks that are central to modern psychology, such as theories of attachment and caregiver deprivation (Harlow, 1958), pioneering theories of learning (Thorndike, 1898; Tolman & Honzik, 1930), the experimental discovery of learned helplessness (Overmier & Seligman, 1967) and many more. The very establishment of the behavioural school of thought in psychology introduced a marked movement away from not only traditions of mentalism and depth psychology, but also from a focus on exclusively human subjects in mainstream psychology. Our acceptance of the history and development of modern psychology makes this familiar to us beyond questioning. Theoretical frameworks and models of behaviour have translated well from their conceptualisation based on animal subjects, to their applicability in the understanding of human psychology today. What one often fails to consider is that at the time of their conceptualisation, there was never any guarantee that any of these studies would be generalisable to human behaviour. Theories of classical and operant conditioning have found application across education, therapeutic intervention, marketing strategy, digital content creation and much more – but at the time of Pavlov’s first experiments, for example, there was no guarantee that behaviour observed in a canine subject would reliably parallel and predict human behaviour. That confirmation has come ex post facto. The expansion of behavioural study into non-animal subjects, thus, can be reasonably pursued.

The demonstration of the applicability of basic behavioural paradigms is a rudimentary step in confirming the suitability of plants to be included in the greater body of knowledge in the field of psychology. For the extremely limited research that has been done in the area, a surprising amount of progress has been made. Not only has evidence of habituation been found in plants, but it has been demonstrated that habituation takes longer to occur when the stimulus is more intense, and is also in such cases longer lasting (Bose, 1906; Holmes et al., 1965, 1966; Applewhite, 1972). A large percentage of research into plant behaviour has been carried out on *Mimosa pudica*, also known as the sensitive plant – no doubt this is thanks to its observably rapid leaf closure (also called hiding response). The most recent of these studies – has been a more sophisticated inquiry into habituation, dishabituation, and retention of habituated response of *Mimosa pudica* carried out by Gagliano et al. (2014), demonstrating greater retention of habituation in plants whose environment was less than optimal to survival. This raises the possibility of complex, adaptive behavioural mechanisms in plants. Learning paradigms, however, have not been explored much further, presenting a research gap.

The more groundbreaking of Gagliano’s researches has been her team’s pioneering demonstration of the exhibition of associative learning in a sample of pea plants (*Pisum sativum*), where the plants showed a conditioned preference for growing towards a blowing fan, even when it meant going against the biological default of phototropic growth (Gagliano et al., 2016). The authors also explored the possible influence of metabolic demands of the plant on the acquisition of associative learning. The experimental findings – at the time of writing of this paper – have not been further confirmed by any other researchers. Markel (2020) however, attempted to replicate the same experiment independently. While Markel’s results do not falsify Gagliano et al.’s (2016) findings entirely, they were not as strongly indicative of associative learning as in the original experiment. Markel reported the results were not significant enough to be conclusive evidence of the possibility of associative learning being observed in plants. No further evidence has been presented to verify or challenge these experimental results.

The present paper attempts to further the research into the possibility of associative learning being demonstrable by plant specimens. Instead of replicating the setup already employed by both Markel and Gagliano et al., this study returns to the tradition of using *Mimosa pudica* for behavioural research.

### *Rationale*

As discussed in the preceding section of the paper, there has been extremely limited inquiry into the applicability of behavioural theory to plant specimens, thus presenting a significant research gap – one that points towards the possibility of an entirely new avenue of advancement for the study of psychology.

While the general assumption of many may be to outright reject the idea of 'learning in plants' due to their dissimilarity to the typical subjects of behavioural research, it would be unscientific and in bad faith to claim it an impossibility without investigative research into the area. Whether plant behaviour can or cannot be included under the purview of psychology is a question that may only be answered definitively after significant evidence has been presented on either side of the argument. The present research aims to take a step towards providing such evidence.

## METHODOLOGY

***Aim:*** To investigate the acquisition of associative learning in *Mimosa pudica*.

### *Objectives*

- To establish acquisition or non-acquisition of associative learning in specimens of *Mimosa pudica*.
- To establish ability of *Mimosa pudica* plant to acquire associative learning.

### *Hypotheses*

- ***Null hypothesis:*** No associations between a neutral light stimulus and a leaf-closure inducing stimulus will be acquired by *Mimosa pudica* specimens.
- ***Alternative hypothesis:*** *Mimosa pudica* specimens will show the acquisition of an association between a neutral light stimulus and a leaf-closure inducing stimulus.

### *Description of the sample*

A total of N=40 specimens of healthy *Mimosa pudica* saplings were selected to be part of the experiment. The saplings were all approximately the same height and sourced from two different nurseries based on availability. The only exclusion criteria were any visible signs of damage or disease. The saplings were divided equally into two groups such that the control group (C) consisted of 20 specimens ($n_c$=20), and the experimental group (P) also consisted of 20 specimens ($n_p$=20).

### *Research Design*

A sample of N=40 *Mimosa pudica* saplings were included in the research design, with 20 specimens each in one control, and one experimental group.

The research was designed to be a quasi-experimental study, following a pre-test/post-test design suitable for studying the effect of an administered intervention. This would make it possible to test for any effect that the intervention (or its absence) could have on the specimens. The specimen sample would be divided into two equal groups – one control group (C), and one experimental group (P). The aim of the experiment was to investigate the acquisition or non-acquisition of associative learning in *Mimosa pudica*. A neutral standardised light stimulus was selected to expose both groups P and C to. A standardised tactile stimulus capable of triggering hiding response in the *Mimosa pudica* leaves was selected to form part of the intervention.

A period of trials was to be carried out to administer the chosen intervention to group P, after which the Test would be carried out. The Test was to consist of exposing specimens of both groups to the chosen light stimulus. Any significant difference in the subsequent responses of groups P and C would establish whether the administration of the intervention had any significant effect on the experimental group P.

### *Variables*
The independent variable would be the intervention – the attempted association of a standardised tactile stimulus with a standardised light stimulus. The dependent variable would be the hiding response of the *Mimosa pudica* leaf.

### *Description of the Tools Used*
A standardised light source was chosen as the ‘neutral stimulus’. This light source was an LED flashlight with a narrow beam, chosen so the light could be focused on a single leaf at a time. The battery was changed before each set of trials (including the Test trial) to maintain constant brightness.

The chosen tactile stimulus was a metal bead. The same metal bead was used for each specimen to maintain uniformity. The weight of the bead was not measured, but it was ascertained over multiple pre-experiment tests that when dropped from the chosen height, the bead was able to induce complete leaf closure in hiding response of a *Mimosa pudica* leaf.

A single leaf was selected on each specimen and marked near the leaf stalk to ensure that the same leaf would be exposed to the experiment stimuli each trial.

### *Procedure*
The two groups P and C consisting of 20 specimens each were kept separately, but in the same area to ensure all specimens would be exposed to similar conditions over the course of the experiment. Pre-experiment observations were made to determine optimum available conditions for the experiment. The specimens were kept in an isolated outdoor area to make exposure to sunlight possible for all specimens, since it was found before the trials were started, that the *Mimosa pudica* leaves would not reopen unless exposed to ambient sunlight. Trials were conducted during early evening after direct sunlight had receded to ensure:

A. The leaves would remain open long enough for the trials to be conducted.
B. Ambient light would be dim enough for the light of the narrow-beamed flashlight to be reasonably perceptible by the specimens’ leaves.

Whether *Mimosa pudica* leaves would show hiding response to mere flashlight beam exposure was also tested in the pre-experimental phase: some leaves did indeed close upon

180 seconds of exposure. It is speculated that the consequent heating up of the flashlight lens close to the leaf surface may have triggered hiding response. To avoid this becoming an extraneous variable, a much shorter, 60-second exposure duration was selected for experimental purposes. It was also confirmed that the chosen tactile stimulus – i.e. dropping a metal bead on a leaf from some height – would trigger leaf closure without exception. Pre-experimental tests were run on specimens from both groups P and C.

A 'target leaf' was selected on every specimen from each group, and marked for easy identification. Leaves that were too new or beginning to wither were excluded from consideration. No leaf used for conducting pre-experimental tests was included in the selection of target leaves.

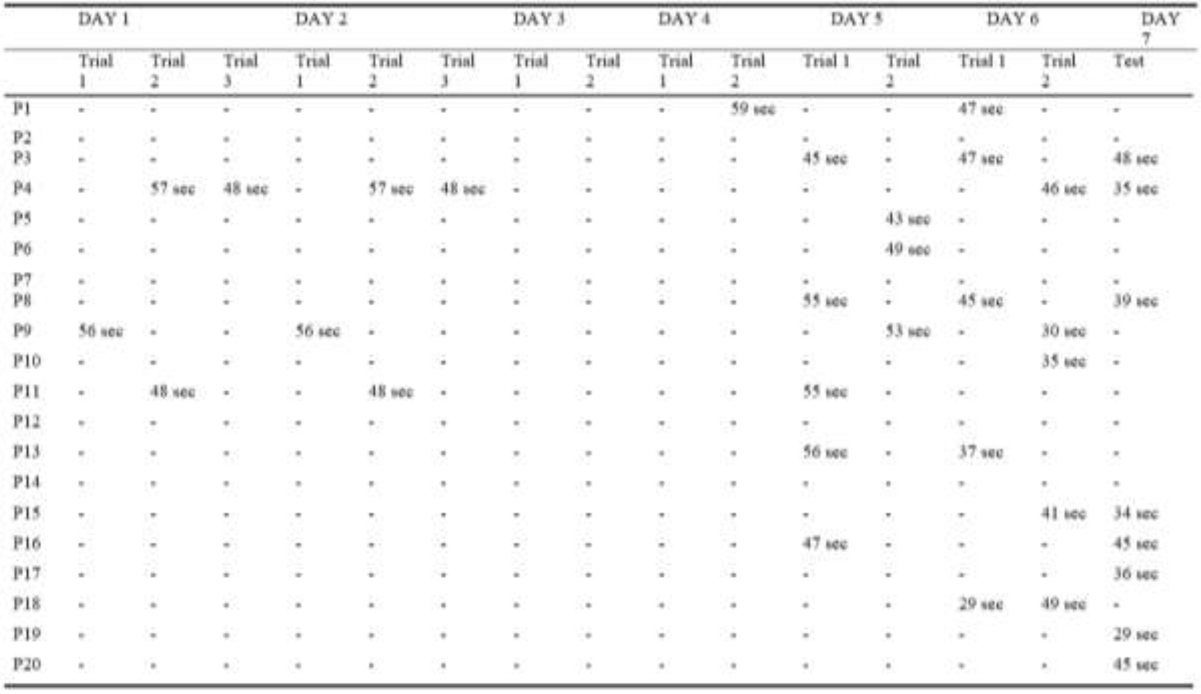
The chosen tactile stimulus (metal bead) was administered to group P specimens, directly following 60 seconds of exposure a beam of light from the chosen light source, in a delay conditioning arrangement, such that the metal bead induced hiding response in the target leaf. The metal bead was dropped from a height of 2 inches from the surface of the target leaf, and the narrow-beam flashlight was held at a distance of 1 inch from the surface of the target leaf. Both the bead and the beam of light were directed at the 4th pair of leaflets, counted from the tip of the target leaf. This intervention was administered to the selected 'target leaf' of every specimen in group P. Each Trial set in this experiment consists of the described intervention being administered to the full set of $n_p$=20 specimens of the experimental group P. There was a 30 second gap between each individual specimen trial to prevent heating up of the light, in order to avoid the accidental introduction of heat as a variable in the intervention. Multiple Trials per day were conducted dependent on leaf reopening. A total of 14 Trials were conducted over 6 consecutive days before the Test trial (Table 1.1). Observed leaf closure is tabulated for the duration of the experiment.

The target leaves of group C specimens ($n_c$=20) were only exposed to the light stimulus for the full 60-second time frame each, without the metal bead being dropped to induce leaf closure. A total of 1 Trial per day was conducted for 6 consecutive days before the Test trial (Table 1.2). Only 1 specimen of group C showed target leaf closure after 57 seconds of light exposure, only on Day 3, as tabulated.

After a 6-day training period, the Test trial was conducted on Day 7. Any spontaneous leaf closure that appeared in response to light-stimulus exposure was noted, in terms of how long the leaf was exposed to the light before it closed completely. Leaf closure was accepted as a 'successful response'. Several P specimens showed some slow movement of leaflets tending towards a hiding response, without the leaf actually closing. These have not been counted as successful responses. Note, that the listed time in seconds for each successful response refers to the moment when the closing movement of the leaf completed, not when such movement started. A few specimens displayed a 3-5 second gap between when the closing movement started and when it completed.

***Statistical Analysis***

***Table 1.1: Time elapsed before light stimulus elicited leaf closure (paired tactile stimulus administered except during Test)***



| | DAY 1 | | | DAY 2 | | | DAY 3 | | DAY 4 | | DAY 5 | | DAY 6 | | DAY 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Trial 1 | Trial 2 | Trial 3 | Trial 1 | Trial 2 | Trial 3 | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Test |
| P1 | - | - | - | - | - | - | - | - | - | 59 sec | - | - | 47 sec | - | - |
| P2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| P3 | - | - | - | - | - | - | - | - | - | - | 45 sec | - | 47 sec | - | 48 sec |
| P4 | - | 57 sec | 48 sec | - | 57 sec | 48 sec | - | - | - | - | - | - | - | 46 sec | 35 sec |
| P5 | - | - | - | - | - | - | - | - | - | - | - | 43 sec | - | - | - |
| P6 | - | - | - | - | - | - | - | - | - | - | - | 49 sec | - | - | - |
| P7 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| P8 | - | - | - | - | - | - | - | - | - | - | 55 sec | - | 45 sec | - | 39 sec |
| P9 | 56 sec | - | - | 56 sec | - | - | - | - | - | - | - | 53 sec | - | 30 sec | - |
| P10 | - | - | - | - | - | - | - | - | - | - | - | - | - | 35 sec | - |
| P11 | - | 48 sec | - | - | 48 sec | - | - | - | - | - | 55 sec | - | - | - | - |
| P12 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| P13 | - | - | - | - | - | - | - | - | - | - | 56 sec | - | 37 sec | - | - |
| P14 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| P15 | - | - | - | - | - | - | - | - | - | - | - | - | - | 41 sec | 34 sec |
| P16 | - | - | - | - | - | - | - | - | - | - | 47 sec | - | - | - | 45 sec |
| P17 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 36 sec |
| P18 | - | - | - | - | - | - | - | - | - | - | - | - | 29 sec | 49 sec | - |
| P19 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 29 sec |
| P20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 45 sec |

*Note*. '-' indicates 'no response' i.e. no leaf closure was elicited by the standardised light stimulus

***Table 1.2: Time elapsed before light stimulus elicited leaf closure (no paired stimulus administered)***

| | DAY 1 | DAY 2 | DAY 3 | DAY 4 | DAY 5 | DAY 6 | DAY 7 |
|---|---|---|---|---|---|---|---|
| | Trial 1 | Trial 1 | Trial 1 | Trial 1 | Trial 1 | Trial 1 | Test |
| C1 | - | - | - | - | - | - | - |
| C2 | - | - | - | - | - | - | - |
| C3 | - | - | - | - | - | - | - |
| C4 | - | - | - | - | - | - | - |
| C5 | - | - | - | - | - | - | - |
| C6 | - | - | - | - | - | - | - |
| C7 | - | - | 57 sec | - | - | - | - |
| C8 | - | - | - | - | - | - | - |
| C9 | - | - | - | - | - | - | - |
| C10 | - | - | - | - | - | - | - |
| C11 | - | - | - | - | - | - | - |
| C12 | - | - | - | - | - | - | - |
| C13 | - | - | - | - | - | - | - |
| C14 | - | - | - | - | - | - | - |
| C15 | - | - | - | - | - | - | - |
| C16 | - | - | - | - | - | - | - |
| C17 | - | - | - | - | - | - | - |
| C18 | - | - | - | - | - | - | - |
| C19 | - | - | - | - | - | - | - |
| C20 | - | - | - | - | - | - | - |

*Note*. '-' indicates 'no response' i.e. no leaf closure was elicited by the standardised light stimulus

***Table 2.1: Independent Samples t-test comparing light-elicited leaf closure in groups P and C during Day 1, Trial 1***

| | | **t-test for Equality of Means** | | | | |
|---|---|---|---|---|---|---|
| | | **t** | **df** | **Sig. (2-tailed)** | **95 % Confidence Interval of the Difference** | |
| | | | | | **Lower** | **Upper** |
| Trial 1 (baseline response) | Equal variances assumed | 1.000 | 38 | .324 | -2.868 | 8.468 |
| | Equal variances not assumed | 1.000 | 19.000 | .330 | -3.060 | 8.660 |

***Table 2.2: Independent Samples t-test comparing light-elicited leaf closure in groups P and C during Day 7, Test Trial***

| | | **t-test for Equality of Means** | | | | |
|---|---|---|---|---|---|---|
| | | **t** | **df** | **Sig. (2-tailed)** | **95 % Confidence Interval of the Difference** | |
| | | | | | **Lower** | **Upper** |
| Test (posttest) | Equal variances assumed | 3.487 | 38 | 0.001 | 6.522 | 24.578 |
| | Equal variances not assumed | 3.487 | 19.000 | 0.002 | 6.216 | 24.884 |

## RESULTS

All statistical procedures were carried out using the IBM® SPSS® statistical software program. It can be seen by comparing the first trial responses (Day 1, Trial 1) of groups P and C that both groups had almost exactly similar responses on being exposed to the light stimulus (Table 1.2 and Table 1.2). In Table 2.1 above, the light-elicited spontaneous leaf closure responses of group P specimens and group C specimens are compared statistically. The 2-tailed p-value of the test for a 0.05 level of significance is seen to be 0.324 and 0.330 respectively, if equal variance is or is not assumed. Thus, for all intents and purposes, there is no significant difference between the two groups at the pre-test phase.

Pre-test here refers to all Day 1, Trial 1 responses elicited *before* the first administration of the tactile stimulus to group P, indicating all such responses occurred before any intervention was administered.

Table 2.2 compares the post-test responses of group P vs group C specimens using an independent samples t-test. The 2-tailed p-value of the test for a 0.05 level of significance is seen to be 0.001 and 0.002 respectively, depending on whether equal variances are assumed or not. This indicates a statistically significant difference in the strength of responses of group P specimens and group C specimens, following the period during which of intervention was administered to P specimens.

Post-test phase responses refer to all responses elicited by the light stimulus *exclusively*, following the 6-day intervention period during which intervention was administered to group P.

## DISCUSSION

The study consisted of a pre-test/post-test quasi-experimental setup in which an intervention was administered to experimental group P, such that if the intervention did have effect on group P's responses, the specimens would show the acquisition of an association between the neutral light stimulus and the tactile stimulus that was administered along with the light stimulus in a delay conditioning pattern. The tactile stimulus was not administered to the control group C. A comparison of group P and C specimens' responses to being exposed to the light stimulus after the treatment phase would highlight whether any change in response pattern could be brought about by the intervention.

Baseline pre-test responses of both groups were similar – mere light stimulus exposure did not trigger significant leaf closure response in either group. If the association of light and tactile stimuli were unable to be acquired by *Mimosa pudica* specimens of group P, then both groups would show analogous response patterns in the post-test phase as well. If such association were indeed acquired by the experimental group, then a statistically significant difference would be seen upon comparing the post-test responses of both groups upon exposure to the light stimulus only.

At a glance, comparing the light-induced leaf closure response of both groups P and C in the post-test phase (Day 7, Test column in *Table 1.1* & *Table 1.2*) shows a noticeable difference. Many group P specimens do show light-induced leaf closure following the intervention treatment – delay conditioned association of induced thigmonastic leaf closure and light-stimulus exposure – while specimens of control group C continue to show no light-induced leaf closure.

This is confirmed in *Table 2.1*, which illustrates the similarity of response between groups P and C, there being no significant difference in their spontaneous responses to the neutral light stimulus in the pre-test phase. *Table 2.2* further confirms the observed difference between post-test responses of groups P and C to be statistically significant.

These indicate that the ability of the neutral light-stimulus to induce leaf closure in *Mimosa pudica* specimens increased significantly when this was paired with a stimulus that was able to reflexively induce leaf closure as a response – which indicates that an association between the light stimulus and the tactile stimulus was formed by group P specimens to a statistically relevant degree. Per the outcomes of the present experiment, some degree of associative learning was indeed demonstrated by *Mimosa pudica* specimens, indicating the ability of this plant to acquire associative learning. The implications of these findings are in line with those of Gagliano et al. (2016) – a demonstration that plant specimens do indeed have the capacity for associative learning.

Gagliano et al. (2014) found variation in environmental variables (such as relative light and darkness exposure) to have a significant effect on the retention of habituated response in *Mimosa pudica* specimens – it is certainly possible that the strength and retention of acquired associations and responses may also be subject to such variation. This presents more uncharted territory for research into plant behaviour.

It is perhaps best clarified that the investigation carried out in the present experiment only concerns the formation of stimulus-response and stimulus-stimulus associations from a purely behavioural perspective – the evidence is insufficient for any claims to be made regarding the cognitive abilities of plants either way.

## CONCLUSION

The present study investigates the possibility of the acquisition of associative learning in plants – specifically, the *Mimosa pudica* plant, selected for its easily observable leaf-closing response. The null hypothesis proposed at the outset has been rejected: that no change of behavioural response would be seen in *Mimosa pudica* specimens, following the administration of an intervention.

Experimental outcomes show a statistically significant increase in light-elicited leaf-closure response in the experimental group specimens. It is concluded that the plant *Mimosa pudica* does indeed show a capacity for acquisition of associative learning. Whether or not such learning is restricted to a limited number of plant species remains to be seen.

### *Limitations*

The present experiment was conducted on a sample of N=40 specimens – repeated testing and similar results being obtained from larger samples would serve to increase scientific confidence in the present findings. Investigation of other influential factors in acquisition of associative learning by *Mimosa pudica* such as ambient light, temperature, stimulus strength etc. remain unknown.

As stated in a previous section, the present experiment only serves to demonstrate the capacity of the *Mimosa pudica* to acquire associative learning. No inherent claims towards plant cognition can be made solely on the basis of this study.

### *Future Implications*

The present study contributes a meagre step towards filling the research gap in finding evidence of associative learning acquisition in plant specimens. Whether or not even this level of learning can be acquired by other plant species remains to be studied. Exploration of pertinent questions such as the relative effectivity of various temporal arrangements of conditioning stimuli, the difference in acquisition of learning mediated by the application of aversive vs appetitive stimuli, the retention of acquired learning etc. also remains. Such study will determine the breadth of generalization possible in the application of fundamental behavioural paradigms to plant species, as well as the possibility of plant learning being expanded into the realm of applied science.

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***Acknowledgment***

This study was carried out in partial fulfilment of a Masters' thesis, and author is grateful to the guidance and support of all who made the study possible.

***Conflict of Interest***

The author declared no conflicts of interests.

***How to cite this article:*** Sengupta, I. & Singh, P. (2024). Associative Learning in Plants: An Investigation of *Mimosa Pudica*. *International Journal of Indian Psychology*, *12*(1), 1749-1758. DIP:18.01.163.20241201, DOI:10.25215/1201.163