International Journal of Research and Review
Vol. 9; Issue: 7; July 2022
Website: www.ijrrjournal.com
Review Paper *E-ISSN: 2349-9788; P-ISSN: 2454-2237*

Review on the Ecological, Economical, Physiological Phytochemical and *In-Vitro* Studies in the Moss *Hyophila involuta* (Hook.) A. Jaeger

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DOI: https://doi.org/10.52403/ijrr.20220735

ABSTRACT

Mosses are one of the major plant groups on which taxonomic studies, as well as various experimental aspects, has been tried on. These promising group of plants has been used against Ecological, Economic as well as Anti-Pollution objectives as well. The present literature reviews all the experimental aspects which have been studied on the particular moss *Hyophila involuta* (Hook.) A. Jaeger.

Keywords: *Hyophila involuta* (Hook.) A. Jaeger., Physiology, Ecology, Economic importance, Protonema, Gemmae.

INTRODUCTION

Bryophytes are one of the most ancient lineages of terrestrial plants; dating to the Ordovician period (488 - 444 mya). In the1600s, some scientists considered mosses to be aborted plant foetuses (Crum, 2001). This group has been broadly divided into Marchantiophyta, Anthocerophyta and Bryophyta, of which members of Bryophyta could be regarded as more advanced among the group. Hyophila involuta (Hook.) A. Jaeger. Is a moss under the family Pottiaceae of Pottiales order. It has an erect habit with its mature sporangium being erect and dark brown on maturation. It is characterized by the leaves being involute while drying, hence the name came. Various aspects of the moss have been evaluated so far including its Ecology, Economic uses, Physiological and Phytochemical aspects as well as *in-vitro* studies also. The present review aims to bring all the data which has been drawn out using all the applied aspects studied about this particular moss.

Ecology

It is one of the most light tolerant moss (Kariyappa et al., 2015). In a study conducted in Karst rocky desertification area of China, *H. involuta* was one the most drought-resistant mosses. In a long run, physiologically and morphologically it had attained resistance. The results also recommend the use of *H. involuta* in bio-crust cultivation for restoration (Cao et al., 2020). From being known over a few locations, *H. involuta* has been disperses too far away locations by humans, especially in Canada (Ireland and Shchepanek, 1993).

Poikilohydry and desiccation tolerance in mosses are some of the strategies of morphological adaptation to smaller size and growth on limited moisture (Proctor & Tuba, 2002). For maintenance of moisture content inside the plant body and to enhance water absorption, *H. involuta* contains hyaline cells, which makes this moss poikilohydric. The involute nature of leaves when dry is another moisture retention strategy (Printarakul and Jampeetong, 2021).

Due to the close proximity of opposite sex organs and also among the same population, sexual reproduction is frequent in monoecious species (Gemmell, 1950; Rohrer, 1982; Longton, 1992; Oliveira & Pôrto, 1998). Most of the Pottiaceae family members are able to withstand as well as establish over high environmental constrains like extreme temperatures and anthropogenic activities (Zander, 1996). Every population of *H. involuta* may not exhibit perfect 1:1 sex ratio. Population analysis shows that, micro climatic variations cannot be the reason for this bias in sex ratio (Oliveira & Cavalcanti, 2005).

On the locations where the gametophytes have been never reported forming sporophytes, they completely rely on the multicellular gemmae developing from leaf bases (Glime, 2006). Studies conducted in southwest Nigeria proves that gametangial development requires an ample amount of water- begins in the rainy season (Fatoba, 1998). *H. involuta* can propagate both by means of spores and propagules. It may be the reason for its cosmopolitan distribution, especially in low lands (Printarakul and Jampeetong, 2021).

Economic and Ecological Uses

Daily use of leaf decoction of *H. involuta* with a pinch of ground pepper is used against symptoms of cough, sore throat and cold (Chandra et al., 2017). The plants of *H. involuta* is highly recommended for aquarium culture and it lasts over one year in a closed terrarium with no fertilizers but provided with tap water also inside a fully air-conditioned room (Benl, 1958; Tan et al., 2004).

Dye-sensitized solar cell (DSSC) is one of the renewable energy sources which can potentially fulfil future energy needs (Shanmugam et al., 2013). In a DSSC electricity is generated by conducting the photo-excited electrons from an artificial dye through a semiconductor and refilling the void by passing the electron through the counter electrode (Somsongkul et al., 2011). As the dyes used usually are not environment friendly, scientists nowadays are using plant pigments as a photosensitizer for making them cheaper as well as eco-friendly. Hassan and team in 2016 were successful in making a DSSC with high photoelectric conversion efficiency using chlorophyll dye extracted from *Hyophila involuta*.

Uses against Pollution

Mosses can be used effectively to monitor atmospheric pollution and heavy metal accumulation. Mosses can easily accumulate gaseous atmospheric pollutants because they lack a well-developed root system and they act as a reservoir of chemicals absorbed in their life time. The air quality in natural ecosystems can be determined by quantitative analysis of PAHs in mosses, especially in *Hyophila involuta* (Fong, 2010)

Polycyclic aromatic hydrocarbons (PAHs) in the atmosphere results in incomplete combustion of fossil fuels or non-fossil fuels, which is hazardous for humans (Maliszewska-Kordybach, 1999; Abdel-Shafy et al., 2016). The levels of atmospheric deposition of PAHs in monthly and annual basis using moss *Hyophila involuta* was done around two metropolitan areas in Sri Lanka; Sapugaskanda oil refinery and Kelanitissa powerplant by Jayalath and team in 2020. Atmospheric deposition of PAHs in moss *Hyophila involuta* around Sapugaskanda oil refinery and Kelanitissa power plant was found to be much higher than of the area where the pollutant concentration was minimum.

Mosses on heavy accumulation of metal ions, some exhibits serious physiological threats. When *H. involuta* was exposed to 0.75 ppm of Zinc sulphate solution, on the 14th day, the plant turned brown. The same results were obtained with lead nitrate in 0.65 ppm concentration (Tyagi et al., 2007). This moss was found to be inhabiting in highly desiccated regions and is recommended to be used as bio crust cultivation for restoration purposes (Cao et al., 2020).

Physiology

The chlorophyll accumulation was found to be greater in forest-dwelling ones than those belonging to Savanna (Aroyehun & Makinde, 2016). This might be due to the presence of empty non-chlorophyllous hyalocysts protecting the chlorocysts in leaves from photo-oxidation (Fisher, 2006). Chlorophyll a or b concentration is 1.695 in 50 klux intensity (Deora and Chaudhary, 1991).

Absorption spectrophotometry shows the absorption maxima of the particular pigments in the sample as peaks in the spectral range. Chlorophyll from *H. involuta* dissolved in ethanol when subjected to spectrophotometry, under the visible range (400nm-700nm), it showed 3 peaks. The absorption maxima were at 436, 470, and 664 nm (Hassan et al., 2016).

Phytochemical Studies

Hyophila involuta was tested qualitatively positive for the presence of phytochemicals like Amino acids, Carbohydrates, fats, flavonoids, anthraquinone, cardiac glycosides, tannins, proteins, steroids and alkaloids (Singh et al., 2016). Similarly, in another work, acetone extract of *H. involuta* tested positive for alkaloids, cardiac glycosides and flavonoids while ethanol extract showed the presence of alkaloids, cardiac glycosides and saponins (Makinde & Fajuyigbe, 2015). Flavonoid content of 198.6 mg/g (Sakanaka et al., 2005) and phenolic content of 161 mg/g (Singleton & Rossi, 1965) were quantified from *H. involuta*.

In spite of greater humidity in the habitat, mosses are very resistant to microbial attacks. It's due to their ability to produce antimicrobials (Asakawa, 2001; Xie and Lou, 2009; Bodade et al., 2008) or the presence of certain bioactive compounds (Nweze et al., 2004). The resistance is due to the presence of flavonoids, biflavonoids, and isoflavonoids (Hahn et al., 1995; Basile et al., 1999).

Extracts of *H. involuta* has exhibited antibiotic properties (Kumar et al., 2007; Singh et al., 2016). Extracts showed antibacterial activity against three bacterial species. *E. coli* and *B. subtilis* died at all three concentrations of plant extract- 10mg/ml, 20mg/ml and 30mg/ml, but *B. cereus* at concentrations of 10mg/ml and 30mg/ml (Singh et al., 2016).

in vitro studies show the production of active antibiotic substances by bryophytes (Ilhan et al., 2006; Isa et al., 2014). Their range and activity depend upon the age of the gametophyte (Subramoniam and Subhisha, 2005). In a series of experiments done with alcohol extract of this moss against 4 bacterial species, *Baccilus subtilis* and *Staphylococcus aureus* (Gram-positive bacteria) were found to be more sensitive than *E. coli* and *Pseudomonas aeruginosa*, a Gram-negative bacteria (Olasoji et al., 2019). It may be due to the less complexity of Gram-positive cell wall than Gram-negative one (Lamikanra, 1999).

The oil palm wine and schnapp extracts showed high resistance against these bacterial species, which may be due to the low minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) or minimum fungicidal concentrations (MFC) values (Olasoji et al., 2019), because reactivity and MIC, MBC/MFC counts are inversely proportional. *Candida albicans* and *Candida pseudotropicalis* were found to be sensitive to schnapp extracts of *Hyophila involuta* (Olasoji et al., 2019).

Aspergillus flavus and *Candida albicans* were fully resistant to Acetone extracts, while *Escherichia coli* was slightly resistant. The growth of *Staphylococcus aureus* was checked when treated with Acetone extract. *Escherichia coli* and *Staphylococcus aureus* were resistant to Ethanol extracts, while *Candida albicans* were slightly resistant. *Aspergillus flavus* was very sensitive to treatment with Ethanol extract (Makinde & Fajuyigbe, 2015).

in-vitro studies

MS Medium successfully induces callus on *H. involuta* protonemal filaments. An alternative of NBM with all vitamins of MS

Medium + Ammonium nitrate also shows results. A lower concentration of 2,4-D (10-7 and 10-6 M) positively influenced callus growth. Kinetin showed an inhibitory effect in 10-4 M concentration, while it had no effects on further lower concentrations. A combination of 10-6M 2,4-D + 10-5M Kinetin is optimal for callus growth. Ammonium nitrate, chelated iron, vitamins and sucrose are vital for callus induction. MS medium devoid of chelated iron and vitamin failed to induce callus growth. Kinetin(10-4M) + IAA on Nitsch's medium was able to induce buds in *H. involuta*, but the same combination with kinetin (10-5) failed to induce in some of the colonies. A slight improvement in protonemal growth, but inhibition of callus was found in 1, 2.5, 5 and 10 mg L-l concentrations of Peptone (Rahbar and Chopra 1982).

During the course of callus induction, elongated chlorenchyma cells become smaller and rounded to become a bead-like appearance. They get separated and most of the separated units are divided forming brownish, rounded friable callus in 9 weeks. Even a 4-month culture on basal medium with altering temperatures and illumination failed bud induction, but protonemal growth was affected at 25 ± 2 °C in 3500 to 4000 lux of continuous illumination. Basal medium is not sufficient for bud induction even though the caulonemal growth was normal. It needed a threshold level of bud-inducing substances. Cytokinin-induced gemmae and interaction of IAA with DMAAP/Kinetin-induced buds. Protonemal growth was a little better at all concentrations of EDTA (Rahbar and Chopra, 1982).

Sood, 1975 was also able to conclude that buds are induced only by a synergetic activity of kinetin and IAA. Younger protonemata benefit from older nurse protonemata in *Hyophila involuta*. The protonemal diffusate (from the gemmae) as well as kinetin, positively contributed in gemmae formation collectively, whereas protonemal diffusate and ABA inhibited. (Mehta, 1990). Variation in light intensity, duration or temperature has no effect on bud induction in *Hyophila involuta* (Rahbar and Chopra 1982).

Coconut milk and yeast extract has negligible results, while a positive effect when 2,4-D(10-7 M) + 10% coconut milk was used. Usage of casein hydrolase at 600 mg/L caused callus to change its colour from brown to greenish and optimum growth of callus in a combination of 10-7 M of 2,4-D + Casein hydrolase. Urea had inhibition effects, while a little callus initiation was found at 10-8 M concentration after 6 weeks. Not only the inhibitory effect of activated charcoal was covered when it was used with NAA (10-7 M) + BAP (10-6 M), but with 1% AC, callus was induced on 4 week old protonema. *H. involuta* exhibits a heterotrichous protonemal stage with an erect and prostrate system (Rahbar and Chopra, 1982).

Genetic relationships among 24 genotypes of *Hyophila involuta* collected from five different natural populations of Mount Abu (Rajasthan) was analysed using RAPD and SSR markers. The efficiency parameters were calculated for each marker system such as polymorphic information content (RAPD = 0.34; SSR = 0.66), marker index (RAPD = 2.78; SSR = 2.62) and resolving power (RAPD = 8.13; SSR = 2.23). The RAPD marker system showed higher values for some indices but microsatellites are more accurately reproducible than RAPD. Moreover, in case of the SSR, the average number of alleles was almost twice as compared to RAPD. Mean coefficient of genetic differentiation between population with RAPD was Gst = 0.269, while with SSR marker was Fst = 0.224 (Pandey & Alam, 2021).

The UPGMA cluster analysis assembled genotypes into two main clusters with diverse levels of sub-clustering within the clusters. Also, the Mantel test showed no significant correlation between geographical and genetic distances. The observed moderately high genetic variability can be explained by efficient spore dispersal. Other factors such as reproductive mode, somatic mutation, continuous propagule recruitment and high degree of intermingling have great impact on the level of genetic variability in moss populations (Pandey & Alam, 2021).

CONCLUSION

Poikilohydry or desiccation tolerance has been proven to be exhibited by mosses in drought habitats. As well as the asexual reproductive strategy like gemmae for better colonization. Apart from some of these takeaways from an ecological point of view, it was drawn that H. involuta has been used in several ways from an ecological point of view. The presence of Amino acids, Carbohydrates, fats, flavonoids, anthraquinone, cardiac glycosides, tannins, proteins, steroids, biflavonoids, isoflavonoids has been proved by various research outcomes, which can be the potential reason for the resistance of the moss against herbivory. Various in-vitro studies provide information regarding the different concentrations of growth regulators needed for the development of protonemal filaments as well as the gemmae.

Acknowledgement: None
Conflict of Interest: None
Source of Funding: None

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How to cite this article: Kalathil Sethumadhavan Abhilash, Alex Philip Alen. Review on the ecological, economical, physiological phytochemical and *in-vitro* studies in the moss *Hyophila involuta* (hook.) a. jaeger. *International Journal of Research and Review*. 2022; 9(7): 313-319. DOI: *https://doi.org/10.52403/ijrr.20220735*

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