



# *Saprolegnia parasitica*, A Lethal Oomycete Pathogen: Demands to be Controlled

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**Abstract** | Fungal infections of fishes with Oomycetes, commonly recognized as water molds, are well-known in fresh water and symbolize the most important fungal group distressing wild and cultured fish. The *Saprolegniaceae*, in particular members of the genus *Saprolegnia*, are conscientious for significant infections, involving both living and dead fish and eggs, predominantly in aquaculture amenities. Saprolegniosis, a disease that is characterized by evident white or grey patches of filamentous mycelium on top of the body or fins of fresh water fishes are caused by the members of genus *Saprolegnia*. Malachite green (an organic dye that is extremely efficient in killing the *Saprolegnia* pathogen) is used to stay *Saprolegnia* infection in aquaculture under control. Though the use of malachite green has been barred worldwide because of its carcinogenic as well as toxicological effects and this has resulted in a remarkable re-appearance of *Saprolegnia* infections in aquaculture. Genus *Saprolegnia* is at the moment economically a very important aquatic fungus, pathogenic to fishes, in particular to catfish, salmon and trout species and necessitate advance study to build up new substitute control strategies. This review focuses on the impact of *Saprolegnia parasitica* on aquaculture, and different methods used for its control.

**Keywords** | Fungal infection, Oomycetes, *Saprolegniaceae*, *Saprolegnia*, Saprolegniosis

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## INTRODUCTION

The present need to increase the world supply of food for the mounting human population has resulted in the development of exhaustive production of aquatic life under controlled conditions. In intensive fish culture the fishes are reserved at high densities in inadequate space and provided with supplementary feeding. Hard work have also been in steps forward to culture the fish in sewage-fed ponds or other extraordinary conditions such as to bring up them in warm water used for cooling atomic reactors at optimum temperature all through the year. Rearing the fish in high compactness in limited water with supplementary feeding, keeping it at relatively high temperature level and the risks of pollution are all factors predisposing to the increase of serious disease problems in fishes. Among the freshwater mycotic fish diseases Saprolegniosis disease

is one of the most economical diseases. Saprolegniaceous fungi are usually known as water molds, are ubiquitous constituent of aquatic environment and are capable of growth and reproduction throughout the year. Oomycetes are traditional saprophytic opportunists, multiplying on fish that are physically injured, stressed or infected (Pickering and Willoughby 1982a). Nevertheless there are more than a few reports of Oomycetes as primary infection agents of fish (Willoughby 1978, Pickering and Christie 1980). These water molds are the most significant fungi affecting cultured fish and are measured by some next only to bacterial disease in terms of economic importance to aquaculture (Meyer, 1991). Numerous species of Saprolegniales infecting fish, fish eggs, amphibians and crustaceans have a major impact on freshwater ecosystem (Densmore and Green, 2007; Fernandez-Beneitez et al., 2008; Kiesecker et al., 2001b; McAllister and Robison 2016). *Saprolegnia par-*

*asitica* and *Saprolegnia diclina* are closely related and are the best studied aquatic oomycetes of the order Saprolegniales (Dieguez-Urbeondo et al., 2007). The Salmon fish farming of countries with temperate climate, such as North-west Europe, Chile, Japan and Canada are mostly reported to be infected by *S. parasitica* and *S. diclina*. (Hussain & Hatai, 2002, Gordon Ritchie, personal communication) stated that there are losses estimated at tens of pounds annually in the salmon aquaculture business in Scotland, Scandinavia, Chile, Japan, Canada and USA. 50% “winter kill” in catfish was caused by *S. parasitica* in USA resulted in economic loss of 25 million (Bruno and Wood, 1999). The rapid increase in the aquaculture production of salmonids has been followed by a rise in several diseases. In particular, Saprolegniosis can account for at least 10% of the annual economic loss in salmonids. (Sandoval-Sierra et al., 2014), also Sandoval-Sierra et al. (2014); represents the first detailed molecular characterization of *Saprolegnia* species involved in Saprolegniosis in Chile, and the first study showing specific association of different *Saprolegnia* species with different stages in the salmonid life cycle.

## TAXONOMY

**Early identification:** The taxonomic position of *S. parasitica* and other oomycetes remains uncertain. The taxonomy of oomycetes has been largely based on morphology of reproductive structures and certain biochemical and physiological characteristics; therefore historically there has been a problem in their identification and classification (Dick, 1973; Dick et al., 1984; Beakes et al., 1994a). Saprolegniales are distinguished by mode of zoospore release and their asexual sporangia (Daugherty et al., 1998; Seymour, 1970); oogonia, oospores, and antheridia are used for their identification at species level (Johnson jr. et al., 2002; Seymour, 1970). Taxonomic confusion regarding isolates of genus *Saprolegnia* that are pathogenic to freshwater animals has been there as identification to species level is often difficult or even not possible, as many isolates do not build up sexual structures *in vitro* and therefore information regarding these are inadequate.

**Modern identification:** Exact species identification is critical for most biological disciplines. The lack of a vigorous classification in the genus, *Saprolegnia* is principal to the presence of wrongly named isolates in culture collections and of growing number of misassigned sequences in DNA database. GeneBank represents the major source of errors for identifying *Saprolegnia* species as it possesses sequences with misassigned names and also sequencing errors. A recently proposed approach to solve species delimitation (taxonomic diagnostic system) of difficult organisms is the definition of Molecular Operational Taxonomic Units (MOTUs). (Sandoval-Sierra et al., 2014) used 961 sequences of nrDNA ITS from culture collections (461 sequences) and GeneBank (500 sequences), to perform

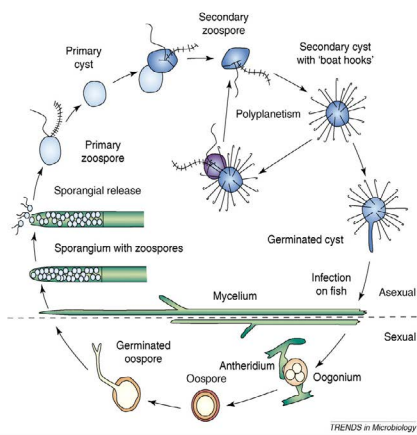
phylogenetic and clustering optimization analysis; as a result identified 29 DNA-based MOTUs in agreement with phylogenetic studies. The resulting molecular clusters supported the validity of 18 species of *Saprolegnia* and identified 11 potential new ones, also listed a number of incorrectly named isolates in culture collections, misassigned species names to GeneBank sequences, and reference sequences for the species. Study from (Dieguez-Urbeondo et al., 2007) showed that within the *S. diclina*-*S. parasitica* complex, six clades could be distinguished. (Beakes et al., 1994) amusingly grouped all *Saprolegnia* species pathogenic to fish in one group. These analyses recommend that a new method of classification will be required upon conclusion of sequencing projects; such as scheme could possibly result in a number of species being renamed. Transient gene silencing through RNAi is a feasible method to functionally characterize genes in *S. parasitica* (Saraiva et al., 2014). The analysis of ITS region sequence inconsistency can make possible genus- and species-level recognition of unknown samples from aquaculture amenities and give helpful information on species composition (De la Bastide et al., 2014).

## BIOLOGY (LIFE CYCLE) OF SAPROLEGNIA SPP.

*Saprolegnia* lifecycle is characterized by having asexual production of sporangia and zoospore and sexual reproduction, resulting in the formation of morphologically distinct oospores as that of other species of Oomycetes, as presented in (Figure 1). The mycelia of *Saprolegnia* are relatively broad, thick hyphae compared to mycelium of Ascomycetes and Basidiomycetes (Dieguez-Urbeondo et al., 2004). The hyphae are mostly aseptate, i.e. coenocytic. Septa are only produced in sexual structures or occasionally in germlings (Dieguez-Urbeondo et al., 2007).

**Asexual reproduction:** The asexual life stages of *S. parasitica* are responsible for saprolegniosis (Andersson & Cerenius 2002; Robertson et al., 2009). Asexual reproduction may take place by the production of chlamydospores or gametes, but usually zoospores are produced. Asexual phase is mostly responsible for dispersing free swimming zoospores in order to find new hosts. Zoospores are produced in sporangia, specific factors recognized to set off *Saprolegnia* zoospore discharge are lack of nutrients (Fuller and Jaworski, 1987) or it may be due to abrupt fall in temperature. In channel catfish (*Ictalurus punctatus*) a drop of temperature by 10°C for 24 hours results in release of elevated number of zoospores (Bly et al., 1992). Amusingly zoospore discharge as a result of cold shock coincides with lowered immune response by the catfish; rising the chance of successful infections by *Saprolegnia* (Bly et al., 1992). Ribeiro (1983) has also reported release of zoospores as a result of cold shock in some Oomycetes. The asexual spore of *Saprolegnia* release motile, primary zoospores (Bruno and Wood, 1999; Willoughby, 1994). Primary zoospores are poor swimmers

and are active only for few minutes before they encyst, germinate and release a secondary zoospore (Seymour, 1970; Willoughby, 1994). Secondary zoospores are motile for a longer period of time than primary zoospores and are considered the main dispersion and infection spore of *Saprolegnia* (Willoughby, 1994; Hatai & Hoshiai, 1994). These secondary zoospores have a riniform shape with two lateral flagella. Secondary zoospores of several species of *Saprolegnia* including *S. parasitica*, undergo a process, Repeated Zoospore Emergence “(RZE)” or “polyplanetism” in which there is repeated cycles of encystment and zoospore release (Cerenius and Soderhall, 1984a,b; Dieguez-Urbeondo et al., 1994a, 2007). RZE may represent an adaptation to the parasitic mode of life in the Saprolegniales (Beakes et al., 1994; Cerenius and Soderhall, 1985; Dieguez-Urbeondo et al., 2007, 2009).



**Figure 1:** The life cycle of *Saprolegnia parasitica*. The life cycle consists of both sexual and asexual stages. The figure is not drawn to scale, have been copied from, Andrew J. Phillips.

**Sexual reproduction:** In saprolegniales sexual reproduction involves the production of antheridium and oogonium gametangia, which unite for fertilization (Pickering and Willoughby, 1982; Seymour, 1970). In most of Oomycetes species male and female sexual reproductive structures appear on a single mycelium (homothallic). In *Saprolegnia parasitica* fertilization occurs with the fusion of antheridium and oogonium, oospores released are characterized by a thick wall as a protective covering from harsh environmental conditions (e.g. dry or extreme temperature) and enabling them to germinate when conditions are favorable (Beakes and Bartinicki-Garcia, 1989). Oospores produced sexually differ in size, number and shape from one species to other. As there is a variation in oospores, antheridium and oogonia, this makes them only source of morphological variation for species classification and identification (Coker, 1923). The identification of *Saprolegnia* species has been mostly hindered, as sexual reproduction appears to be difficult to initiate *in vivo*, and is sometimes impossible *in*

*vitro*. It is always possible to identify species based on morphological characteristics of their sexual structures (Beakes et al., 1994; Dieguez-Urbeondo et al., 2007).

**DISEASES CAUSED BY *SAPROLEGNIA PARASITICA***

Saprolegniosis, observed in numerous fresh water fishes is characterized mostly by white or greyish cotton-wool like tuft found on infected skin, represented in (Figure 2) and gills of infected adult fish, fry, fingerlings or on eggs,. This disease has received less study than most of the other important diseases of freshwater fish, most likely because fungal infections are usually considered to be the result of an opportunistic, mostly caused by mechanical injury after handling, exposure to extreme pH levels, prolonged exposure to low water temperatures, lack of food, and presence of other microbial infections (Pickering and Willoughby, 1977). In the case of salmonid fish the Saprolegniosis is almost always caused by a single taxon, *Saprolegnia parasitica* (Willoughby & Roberts. 1992). As skin is mainly affected in case of Saprolegniosis diseases so it is also known as dermatomycosis (Hatai, 1980). The chances of predation of infected fishes are more, as being lethargic due to destruction of epidermis and underlying tissue. There is impaired osmoregulation, respiratory failure and in some cases organ failure of diseased fish in the final stage of infection (Pickering and Willoughby, 1982). There is predation (in the wild) or culling (in aquaculture) in the late stages of Saprolegniosis. It is mostly observed that healthy fishes, with no wounds or parasites are at low risk of being infected by *Saprolegnia parasitica*.



**Figure 2:** Showing white cotton wool like tuft on dorsal portion of fungal infected gold fish.

Eggs of a large variety of freshwater fishes have been reported to be infected by some *Saprolegnia* species, whose vulnerability is thought to be dependent of egg size. Eggs of common carp (*Cyprinus carpio*) and catfish (*Pelteoba-*



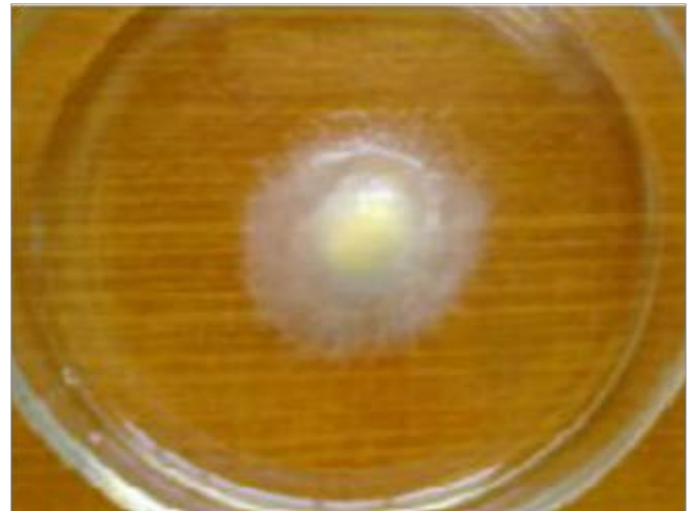
*grus fulvidraco*) were found to be infected within days as are small in size, in contrast to eggs of salmonid eggs of much larger size, in which infection took much longer to establish (Chukanhom and Hatai, 2004; Cao et al., 2012). At present it is seen that *Saprolegnia* zoospores cannot infect the salmonid species eggs (Kitancharoen et al., 1997; Thoen et al., 2011). Dead or unfertilized eggs are the places where from infection originates, suggested from reports of fish farmers, so dead eggs serve as a substrate for zoospore colonization, from where mycelia mats engulf and kill nearby eggs (hyphal infection). Hyphal infection is considered to be devastating in aquaculture hatcheries and can spread quickly between neighbouring eggs (Smith et al., 1985).

### ISOLATION

In case of a suspected *Saprolegnia* infection, isolation of fungi from infected fish is carried out by taking small pieces of infected mussel about 2mm in diameter from different portions of body and thoroughly washed with sterilized distilled water to remove unwanted microorganisms adhered on surface and growing them on selected media (potato dextrose agar Figure 3) and repeatedly transferring growing mycelia tips to new media. Some specific antibiotics, such as streptomycin and streptopencillin are used in the culture medium to exclude the growth of other microbes (bacteria and true fungi). *Saprolegnia parasitica* can be grown on baits like sterilized hempseeds or rice for the microscopic examination of mycelia, sexual reproductive structures and zoospores (Willoughby and Wood, 1986) (Figure 4). The type of the species isolated is however influenced by type of bait used. The formation of white, cotton-like growth on seeds determines the colonization of zoospores, which are then transferred to selective media plates. Further development in this method was done to enable quantification of zoospore number in water with the use of microstate plates (Thoen et al., 2010).



**Figure 3:** Showing culture of fungus on potato dextrose agar isolated from fungal infected fish.



**Figure 4:** Showing culture of aquatic fungi on soybean seed.

### IDENTIFICATION

Several techniques can be used for the identification of the species isolated. Generic identification of Saprolegniales can be done by microscopic examination of asexual sporangia and mode of zoospore release (Daugherty et al., 1998; Seymour, 1970); species identification is done by examination of sexual structures, oogonia, oospores and antheridia (Jhonson Jr. et al., 2002; Seymour, 1970). Identification to species level is often difficult or even not possible, as many isolates do not build up sexual structures *in vitro* and therefore information regarding these is inadequate, which has led to the progress of molecular identification methods that are providing new insights into oomycete classification. The use of the Internal Transcribed Spacer (ITS) region, located between the 18S and the 28S rDNA, is commonly used method for identification and includes the 5.8S rDNA sequence. *Saprolegnia* species (ITS) is approximately 700 bp in length and is considered very suitable for intra-species analysis (Dieguez-Urbeondo et al., 2007). Oomycete species can also be identified by using variable sections from 18S rDNA, 28S rDNA and *cox2*-gene (Levesque and De Cock, 2004). A recently proposed approach to solve species delimitation (taxonomic diagnostic system) of difficult organisms is the definition of molecular operational taxonomic units (MOTUs). (Sandoval-Sierra et al., 2014) used 961 sequences of nrDNA ITS from culture collections (461 sequences) and GeneBank (500 sequences), to perform phylogenetic and clustering optimization analysis, as a result identified 29 DNA-based MOTUs in agreement with phylogenetic studies. The resulting molecular clusters supported the validity of 18 species of *Saprolegnia* and identified 11 potential new ones, also listed a number of incorrectly named isolates in culture collections, misassigned species names to GeneBank sequences, and reference sequences for the species.

Monoclonal antibodies (MAB) (Fregeneda-Grandes et al.,

2007a) or SEM (Beakes et al., 1994; Stueland et al., 2005) provided information for the identification of zoospores of strains of *S. parasitica*. But these techniques are found to be less suitable for the identification of other *Saprolegnia* species as *S. diclina* that has much less pronounced cyst morphology (Beakes, 1983). The techniques of highly desirable to better discriminate between various *Saprolegnia* strains and species, are the development of fingerprinting techniques, such as Multi Locus Sequence Typing (MLST), micro-satellites or one of several polymorphism (Molina et al., 1995; Whisler, 1996).

### CONTROL MEASURES

Fish constitutes cheapest and richest source of protein of good quality, easily digestible. Preventing Saprolegniosis is certainly a near-impossible task, and the use of drugs to both prevent and control the disease has been predictable. The easiest and most lucrative way to control the fungal disease is by good husbandry techniques. Fish health management should focus on to prevent the onset of disease in fish farms Meyer (1991). Minimizing stressful environmental and physiological conditions, maintaining water supply standards and nutritionally complete diets, and isolating infected fish from healthy stocks are some important steps for fungal fish control. Malachite green, an organic drug was used to control Saprolegniosis (Bruno and Wood, 1999; Willoughby and Roberts, 1992). However, it has been banned all over the world, because of its teratogenic properties (Fitzpatrick et al., 1995); Mutagenic properties (Bruno and Wood, 1999). This has resulted in recent resurgence of Saprolegniosis in the aquaculture industry, leading to financial losses world-wide. So it is the demand of the time to find substitute methods to control this mycotic disease. Many alternative control measures for Saprolegniosis have been tested, such as glutaraldehyde; hydrogen peroxide; iodine; the salts potassium permanganate and copper sulphate; and the herbicides diquat, simazine, Three profitable products that contain the chemical formalin, or formaldehyde, have been granted approval from the United States Food and Drug. Formalin is currently used in aquaculture to control Saprolegniosis. However, its use is also likely to be banned in the future. It can control *Saprolegnia* infection effectively in both fish and their eggs (Marking et al., 1994b; Gieseke et al., 2006); also useful to control *Saprolegnia* infection in Catfish, but its concentration used must be monitored carefully Bly et al. (1996). High concentrations of sodium chloride (NaCl) can be used for the inhibition of *Saprolegnia* growth (Marking et al., 1994b; Ali, 2005). Amphotericin B can be used to control infections on channel catfish Bly et al. (1996).. The modified chitosans used for control of *Saprolegnia parasitica* are regarded as nontoxic Muzzarelli et al. (2001). Bronopol (2-bromo-2-nitropropane-1,3-diol), microbial drug has an efficacy in controlling *Saprolegnia* infection in rainbow trout and when used at a higher concentration it

could also prevent infection in fertilized rainbow trout ova (Pottinger and Day, 1999; Branson 2002). KMnO<sub>4</sub> has a defensive role against oxidative stress response; in addition there is a role of FCA in modulating the oxidative stress response and enhancing fish immune response against infections (Zahran and Risha, 2013). Apart from these some other chemicals used for Saprolegniosis control are; copper products (Alderman, 1994; Straus et al., 2009b, 2011), Formaline (Marking et al., 1994; Taylor Francis et al., 1994); UV radiations (J. Heikkinen et al., 2013; Sako, 1985); and Ozone treatment (Sako, 1985). Different concentration of copper sulfate can affect both vegetative and zoospore stages of water molds, *Achlya* spp., (Panchai et al., 2016). A concentration of formaldehyde of 500 mg/l or greater, was found to be effective in controlling egg mortality, when administered as disinfection baths, for a period of 15 min, twice a week., without apparent harming the female broods (Benedetto Sicuro, 2016).

Biocontrol of infectious diseases is very important and gaining ground in agriculture (Hofte and Altier, 2010; Qi et al., 2009; Verschuere et al., 2000) and quite a lot of formulations are being used in aquaculture in China but scientific proof is very inadequate. Actinobacteria, of genus *Frondehabitans* (Microbacteriaceae) effectively inhibit attachment of *Saprolegnia* to salmon eggs. Low incidence of Saprolegniosis is strongly correlated with a high richness and abundance of this Actinobacteria, so microbial landscapes of fish eggs may provide new sustainable means to alleviate up-and-coming diseases. Live inocula of aquatic *Pseudomonas* strains, as a replacement for their bioactive compound, can present new (micro) biological and sustainable means to diminish oomycete diseases in aquaculture (Liu Y et al., 2015). Among the fungal community associated with salmon eggs, *Trichoderma* species may play a role in *Saprolegnia* suppression in aquaculture (Yiyang Liu, 2016). Most recently it has been found that, the complex sterol synthesis pathway for *S. parasitica* may lead to the identification of some specific targets for inhibition, since sterols are vital for oomycete survival, so there is importance of these molecules as potential drug targets in control strategies (Dahlin et al., 2017). There are few more studies regarding the antifungal effect of probiotics. Lategan et al. (2004) isolated *Aeromonas media* (strain A199) from eel (*Anguilla australis*) culture water and were observed to have a strong inhibitory activity against *Saprolegnia* sp. In a separate study, *Pseudomonas* ssp. M162, *Pseudomonas* sp. M174 and *Janthino bacterium* sp. M169 enhanced immunity against Saprolegniosis in rainbow trout. Atira et al., (2012) demonstrated that *L. plantarum* FNCC 226 exhibited inhibitory activity against *Saprolegnia parasitica* A3 in catfish (*Pangasius hypophthalmus*).

### CONCLUSION AND FUTURE RESEARCH

In recent years, aquaculture has attained a key economic



revolution. However infectious diseases of bacterial, viral, mycotic and parasitic origin are the most notable preventive agents in the upgrading of intensified aquaculture. 54.9% bacterial pathogens, 22.6% viruses, 3.1% mycotic agents, and 19.4% parasitic agents are accountable for periodical disease outbreaks in fish cultures (Dhar et al., 2014). *S. parasitica* represents a demanding problem in the aquaculture development industry (Molina et al. 1995; Van West 2006; Phillips et al., 2008). Saprolegniosis caused by *S. parasitica* affects aquaculture brood fish and incubating eggs. It is expected that 10% of all hatched salmon succumb to Saprolegniosis, causing major economic loss in an industry accounting for around 30% of the universal fish production for consumption (Molina et al. 1995; Van West 2006; Fregeneda-Grandes et al., 2007; Phillips et al., 2008). The occurrence of Saprolegniosis extends to Asian humid aquaculture systems where more than 80% of fish produced by aquaculture comes from the area (Karunasagar et al. 2003). Given that the international ban of Malachite green in 2002, the need to build up substitute methods for the control of Saprolegniosis has become increasingly urgent. Genomic and proteomic study of *S. parasitica* and additional pathogenic oomycetes have provided an exceptional source for studying the molecular processes underlying Saprolegniosis. In addition, the complementary identification of genes and proteins concerned in the immune response of diseased host fish infected through Saprolegniosis will present an understanding of how to put off Saprolegniosis and eventually control the spread of *S. parasitica*. Future studies of the molecular processes underlying Saprolegniosis in *S. parasitica* – host fish relations will without a doubt boost our information and understanding of the pathology of *S. parasitica*, enabling the progress of successful fish vaccines and early recognition of *S. parasitica* creating a substitute method for controlling Saprolegniosis. Controlling Saprolegniosis is compulsory to make certain sustained development in the aquaculture industry, particularly in Asian steamy aquaculture systems where over 80% of fish produced by aquaculture come from the area. For an industry that accounts for roughly 30% of the worldwide production of fish for consumption, it is vital to prolong studying the fundamental molecular processes of Saprolegniosis in *S. parasitica* – host fish relations.

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## CONFLICT OF INTEREST

The authors have no conflict of Interest.

Showkat Aziz Lone prepared the manuscript while Susan Manohar checked the article for any mistakes.

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