**Testing the efficacy of Silver Nanoparticles synthesized from *A. indica as a* Bio-fungicide against Brown Spot Disease in rice.**

**Abstract-** Brown Spot Disease in rice is caused by the fungus *Cochliobolus miyabeanus*. The disease appears first as minute brown dots which later becoming cylindrical or oval spots mainly on the leaf blades and glumes. The spots resemble sesame seeds and, therefore, the disease is also known as 'Sesame Leaf Spot.' Eventually, the spots become larger patches which then coalesce. Once the leaves are covered by the patches, they dry and wither. The dark spots on the glumes result in discoloured and shrivelled grains. Under favourable conditions for the fungus to grow, dark brown conidiophores and conidia develop on the spots attributing to a velvety appearance. Upon penetrating the glume, the fungus affects the endosperm, identified by its discolouration. Severe infection of grains has been reported to prevent germination (Ranganathaiah, 1985) and to cause seed rotting. Fields which are affected by the disease can be identified by their scorched appearances. The infection causes failure of seed germination, seedling mortality and reduces the grain quality and weight. Severe bouts of the disease can lead up to 50% loss in the harvest. Cases of Brown Spot Disease has been reported in all rice-growing countries in the world such as Japan, China, Burma, Sri Lanka, Bangladesh, Iran, Africa, South America, Russia, North America, Philippines, Saudi Arabia, Australia, Malaysia and Thailand (Ou, 1985; Khalili et al., 2012) and is most common in areas with low PH, scarce water supply and nutritional imbalance, especially a lack of nitrogen (Baranwal *et a*l., 2013) and available potassium ($K_2O$). The fungal disease has lots of prevalence in India. It affects all the rice-growing states of India and has severe outbreaks in states where dry/direct-seeded rice is cultivated such as Bihar, Chhatisgarh, Madhya Pradesh, Orissa, Assam, Jharkhand and West Bengal. Cases in the country have been reported to reach a staggering 90% loss in harvest, especially when the leaf-spotting phase gains epiphytotic proportions. Such a case led to one of the harshest agricultural disasters in the history of the nation in the form of the Great Bengal Famine of 1942 (Ghose et al., 1960).

Brown Spot Disease of rice is usually initiated by an infected seed (Bernaux, 1981; Sharma and Maheshwari, 1982; Damicone et al., 2001) as necrotic lesions on the coleoptile and sheath of appearing on the first leaves of the plant. Secondary infection, and lesions, are caused by air-borne spores produced on primary lesions (Ou, 1985). The host epidermis is penetrated through infection pegs arising from the appressoria (Ou, 1985). Aspartic acid, glutamic acid, alanine and methionine in rice the leaf exudates stimulate the

fungus to colonize host leaves (Purkayastha and Mukhopadhyay, 1974). The transmission of *C. miyabeanus* from infected kernels to seedlings varied between 57-77% in sand, blotter and tube agar methods, which produced symptoms on coleoptiles and roots after 7-14 days and on the first leaves after 3-4 weeks, ultimately leading to wilting and death of the infected seedlings.

Various different fungicides have shown efficacy against *C. miyabeanus* . Dithane M-45, Kitazin, Hinosan Thiram, Shield, Foltaf, Ridomil, Bitoxazol, PP 296, triadimenol, tridemorph and edifenphos have proved efficient in controlling mycelial growth (Gowda and Gowda, 1985; Vinay Kumari et al., 1997; Arshad et al., 2013). Research on inhibition of the infection found hexaconazole (EC50 0.11 ppm a.i.) and propiconazole (EC50 0.42 ppm a.i.) to be most effective against the mycelial growth followed by iprobenphos and edifenphos (Sunder et al. 2005). Iprodione (Pereira et al., 2002) and strobilurins viz. azoxystrobin, trifloxystrobin and kresoxim methyl (Thind et al., 2004) have also shown to inhibit mycelial growth. However, excessive use of fungicides causes environment deterioration and its harmful effects spill out to affect plants and animals in the ecosystem. Fungicides create residue problems, resistance development in pathogens and different health hazards to human beings and other living organisms. Many fungicides mentioned above, such Dithane M-45, are known cause harm to aquatic animals and their excessive use has been correlated with an increase in chromosomal defects in humans.

Instead, a more sustainable option for checking the growth of *C. miyabeanus* is biological control. Many plant extracts and botanicals have been found to be effective against Brown Spot Disease of rice. One of these plants which have shown promise in suppressing mycelial growth of the fungi is *Azadirachta indica* (Neem). Water and ethanol extracts from leaves and oil extract from seeds of *A. indica* were found effective in reducing the radial growth of *C. miyabeanus* in culture and controlling the spread of brown spot in rice (Amadioha, 2002).

Nanotechnology has led to the development of novel agricultural products such as nano-biopesticides and nano-bio fungicides which have the potential to manage diseases such as Brown Spot Disease, and, at the same time, increase the shelf life, increase the solubility and reduce the toxicity of conventional drugs. It involves the use of nanoparticles that allow targeted delivery and overcome biological barriers and have, thus, gained prominence as therapeutic and agricultural drug delivery systems. Silver is the most profit-

oriented precious metal used in the preparation of nanoparticles and is known for its antibacterial, antiviral, antifungal, antioxidant, and unusually enhanced physicochemical properties as well as its optical, thermal, electrical, and catalytic properties. Plant-mediated synthesis of nanomaterials has been increasingly gaining popularity due to its eco-friendly nature and cost-effectiveness. An example of this is Silver nanoparticles made from plant extracts that, due to the rich phytochemical composition of the extracts, allow for their complex action, for example, as reducing, stabilizing, and capping agents. This process is known as 'green synthesis' of nanoparticles and is more cost-efficient, has higher yields, and is more environmentally friendly than other methods of producing nanoparticles which require the use of hazardous chemical reagents.

An AgNP (silver nanoparticle) made from the extracts of leaves of *A. indica* would be produced using green synthesis by the bioreduction of $Ag^+$ ions in an aqueous medium containing *A. indica* as the reducing agent and can be used as an inhibitory agent against Brown Spot Disease of rice. The efficient nanoparticle delivery system, as well as the anti-microbial and antifungal properties of silver nanoparticles themselves, can increase the efficacy of the extract and possibly get similar results to chemical fungicides without creating any harm towards the environment.