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Efficacy of silver nanoparticles against rice blast disease and farmers perception about its management in Bangladesh

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Efficacy of silver nanoparticles against rice blast disease and farmers perception about its management in Bangladesh

Effekten av silver nanopartiklar mot risblast sjukdom och bönder uppfattning om sin ledning i Bangladesh

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Foreword

I am from Bangladesh, an agrarian based country where agriculture is the most important sector of economy and providing employment for more than 60 percent of the population. Bangladesh has made notable progress in rice production which has a huge contribution in achieving food security. The agriculture sector is blooming day by day despite frequent natural calamities, various diseases, decreasing agricultural land etc. Although rice is the main cereal crop in Bangladesh, we are trying to incorporate other crops along with rice production to replace monoculture of rice for sustainable agriculture. As I have an association with rice research in the plant pathology department of Bangladesh Rice Research Institute, I always wanted to find out more sustainable ways to deal with rice diseases within the system followed in rice production. Agroecology was a new concept for me for that reason I applied for the agroecology master program at the Swedish University of Agricultural Sciences (SLU) to broaden my knowledge about modern agriculture, environmental perspectives and the socio-economic interaction of stakeholders within the production system. The program was a nice learning experience with excursions, farm visits, case studies, report writing and group discussions. Different courses within the master program made me comprehend suitable approaches of different agro-based production systems including environment, soil health and socioeconomic condition of the farming communities. The agroecology master program provided me a platform to gain vast knowledge on sustainable production system, resource utilization, ecology, plant protection etc. It also enhanced my group as well as independent working abilities, writing and presentation skills. Finally, my background knowledge about agriculture and acquired knowledge from Agroecology master program will help me to broaden my vision and thinking perspective to make contribution to the development of sustainable production system in Bangladesh.

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Abstract

Rice blast caused by *Magnaporthe grisea* is one of the major and recurrent threats for sustainable rice production in Bangladesh. To mitigate this problem, the current study was aimed to investigate the efficacy of silver nanoparticles against rice blast disease and farmers' perception or knowledge about rice blast disease management in Bangladesh. There have been relatively few studies on the applicability of nanoparticles to control blast diseases in Bangladesh. This study was done into two parts: firstly, face to face interviews to understand the farmers' perception as well as to find out their needs and secondly laboratory experimentation to find out the efficacy of silver nanoparticles against *M. grisea* as well as rice blast disease management under controlled conditions. Close-ended questions were prepared for the interview and thirty farmers were interviewed. Farmers' interviews indicated that 73% of the respondent farmers had experienced blast disease in their fields but only 30% of the respondent farmers were aware of rice blast disease and its management. Among the respondents, 89% of the farmers used chemicals in controlling rice blast disease but only 9% of the farmers had an opinion that spraying chemicals worked very well and 21% thought this method worked satisfactorily. Around 59% of respondents maintained close contact with extension workers and adopted new technologies according to their suggestions. *In vitro* assays indicated that silver nanoparticles had a significant inhibitory effect on the mycelial growth of rice blast pathogen. Effective concentration of the silver nanoparticles inhibiting mycelial growth by 50% (EC_{50}) up to 9 days after incubation was 308.1 ppm. However, the inhibitory effect on mycelial growth significantly diminished at 12 days of incubation. To measure leaf blast disease severity, three concentrations (200, 400 and 800 ppm) of silver nanoparticles and a popularly used blast control fungicide Trooper 75WP were sprayed on rice seedlings that were 20-25 days old, 3 days before inoculation for preventive and 3 days after inoculation with spore suspension (10^5 conidia/ml+0.01% Tween 20) for curative measure. Only 1.77% and 3.23% leaf blast disease severity were found in preventive measure at 800 ppm concentration for Trooper 75 WP (standard dose of fungicide) and silver nanoparticles, respectively. Whereas untreated control plot exhibited more than 70.0% disease severity. In greenhouse assay, silver nanoparticles were highly effective in preventative application rather than curative application.

Key words: rice, blast disease, management, mycelial growth, silver nanoparticles.

Contents

List of Figures

List of Tables

1. Introduction

1.1 Rice in Bangladesh

The economy of Bangladesh is basically agrarian with the dominance of rice (*Oryza sativa* L.) crop which considered as a staple food crop in Bangladesh. Bangladesh has a total 14.86 million ha of land and cultivable land is 8.52 million ha with a cropping intensity of 191%. Approximately 80 percent of the total arable land is occupied by rice production in various agroecological zones (Shelley et al., 2016). In Bangladesh, 19.3% of the gross domestic product (GDP) of the country is achieved from agricultural production and employment figure show 47.5% of the total manpower is related to agriculture (Bangladesh Finance Bureau, 2014). The agro-climatic conditions of Bangladesh make it suitable for growing rice year-round throughout the country except for hilly areas. In Bangladesh, rice is grown in three seasons named: 1. Upland Rice/Aus Rice 2. Rainfed lowland Rice/Aman Rice 3. Irrigated lowland Rice/Boro Rice. Among growing seasons, Aman and Boro are the main seasons for rice cultivation and the yield of rice is higher in two seasons than Aus season. Most of the rice varieties grown in Bangladesh are developed by the Bangladesh Rice Research Institute (BRRI). BRRI has done many projects with the collaboration of The International Rice Research Institute (IRRI). Some varieties are grown as season specific due to photosensitivity and climatic requirement and some are cultivated year-round but the difference in yield is observed. Bangladesh has made remarkable progress in rice production and sustaining rice production has become possible due to shifting cultivation from low yielding local to high yielding modern or hybrid varieties (Hossain et al., 2006). Karmokar and Imon (2008) reported that rice production has increased by approximately 1.02% per annum in the period 1971–2010 in Bangladesh and per capita food availability (rice and wheat) has raised from 165.6 kg per annum in 1991–1992 to 225.45 kg per annum in 2009–2010 (Alam and Islam, 2013). Bangladesh Rice Research Institute (BRRI) and Bangladesh Institute of Nuclear Agriculture (BINA) maintain a relentless effort to develop rice cultivars with high yield potential and tolerance against biotic and abiotic stresses. Bangladesh is the 4th top rice producing country and maintaining production stability for a long time. In the year 2013–2014, rice production was 34.3 million tons (Bangladesh Finance Bureau, 2014) and in the year 2016–2017, rice production was 33.8 million tons (Bangladesh Finance Bureau, 2017). After achieving self-sufficiency in rice production, Bangladesh has begun export rice in recent years.

However, various diseases are considered as a major obstacle of rice production for high yielding varieties as well as for national food security.

1.2 Blast disease of rice and its management

Rice plants are infected by ten major fungal, bacterial and viral diseases in Bangladesh. Blast disease caused by the fungus *Pyricularia grisea* [anamorph of *Magnaporthe grisea*], is considered one of the most detrimental rice diseases worldwide (Ou, 1985). Rice blast disease can occur on most of the parts of the plant except the roots and infection occurs from airborne conidia. Symptoms appear as lesion or spots; size, shape and color depend on pathotype, varietal resistance, stage of the lesions and environmental conditions (Ou 1985).

When pathogen infestation occurs at vegetative phase it is called leaf blast and when an infestation occurs on nodes, neck and panicles during reproductive phase it is described as node and neck blast respectively (Bonman, 1992). In the case of leaf blast, lesions are initially whitish to gray with darker borders and with time the lesion turns into eye shaped white/grey color surrounded by red-brown margin. Lesion size varies normally 1-1.5 cm long and 0.3-0.5 cm wide but under favorable environmental conditions, lesions can coalesce and infect almost entire leaf surface. Neck blast is considered the most hazardous phase of the disease and can appear without causing severe leaf blast (Zhu et al., 2005). Neck blast is characterized by the infection of fungus at the panicle base that produces purplish lesions. In severe cases lesion are elongated around the neck or node, dark brown symptoms appear at the base of the panicle causing rot and infected panicles become partly or completely unfilled. In severe infection panicle become white and dead and can easily be confused with an attack of stem borers which also results in a white head symptom. In severe infection reduction of yield could be double when caused by neck blast disease compared to leaf blast (Hwang et al., 1987) and infection in panicles can cause total yield loss (Ou, 1985).

Rice blast disease mostly depends on favorable environmental conditions: cloudy sky with dew for long period of time, high relative humidity and temperature range between 17 to 28°C, temperature fluctuations during day and night favor rice blast development (Webster and Gunnell, 1992). The pathogen overwinters as mycelium or conidia (primary inoculum) on rice stubble, seed or any living plants. Under optimal temperature and dry conditions, conidia can survive for more than one year and mycelium for almost three years (Ou, 1985). Conidia infect rice plants transported by wind or water and under favorable weather conditions will produce conidiophores and release new airborne conidia. Blast is a polycyclic disease including sporulation, germination, and infection phases which can all occur multiple times in a growing season. Sporulation is favored by relative humidity ≥ 90%, temperature between 25 to 28°C, and ≥ 4 hours of leaf wetness (Ou, 1985; Kim, 1994). Under favorable conditions, conidiophores are produced within 4 to 6 hours after dew formation; conidial germination may occur within 3 hours and conidia can be released shortly thereafter (Kim, 1994). Infection of rice by *M. grisea* needs 7 to 14 hours of leaf wetness (Barksdale and Asai, 1965; Kim, 1994).

Blast is the most common and devastating disease in irrigated rice of South-East Asia, especially that of aromatic rice (Khan et al., 2014). In Bangladesh, nearly all varieties of aromatic rice either rainfed or irrigated are highly susceptible to neck blast disease because in their life cycle, there are favorable conditions for disease development at the time of flowering (Khan et. al., 2014). But in recent years, blast pathogen invasion has been reported to new rice cultivars in Bangladesh which were initially considered less susceptible. In 2016 and 2017 the most popular and mega rice variety was BRRI dhan28 followed by BRRI dhan50, BRRI dhan58, BRRI dhan61, BRRI dhan63 etc. These now all have high susceptibility to rice blast. The blast disease causes yield loss of 11% and 46.4% under low and medium disease incidence in Bangladesh (Khan et al., 2014).

The occurrence of different pathogenic races in *M. grisea* was first reported by Latterell et al. (1954) in USA. Since then several pathotypes have been identified worldwide using different rice cultivars as differentials and the number of pathotypes has increased as more isolates were examined (Ou, 1980) as the pathotype differs in different geographical regions. *M. grisea* has different pathotypes with high variability and resistant cultivars become susceptible within a short period of time (Wang et al., 1989). Therefore, breeding for durable rice varieties resistant to blast disease still a major challenge (Barman and Chattoo, 2005). The most common approaches for rice blast disease management include cultural practices like burning stubbles, manipulating planting times, split doses of fertilizers, application of fungicides and planting resistant or partial resistant cultivars (Mbodi et al., 1987; Naidu and Reddy, 1989).

Bangladeshi farmers are now mostly using fungicides to control the disease, but fungicides have long-term profound and often negative impacts on environment as well as on human health and also increase the cost of production. Pesticides arbitrarily kill insect-pests along with natural enemies which are essential to the food web and natural ecosystem which might develop pesticide resistant insect-pest or might cause pest resurgence. Pesticides run into surface water and leach into groundwater and enter into the food chain relentlessly. The consequences include millions of people from developing countries experiencing dermatological problems, reproductive disorders and even cancer. Reduction of natural enemies makes farmers more dependent on pesticides and pollinators are also reduced in number due to massive application of pesticides. Therefore, in the end the use of pesticides can often result in less yield.

1.3 Why Nanotechnology?

From management aspects, the aim of agroecology is to maintain a stable environment, biologically arbitrated soil fertility, sustained productivity and pest management through diversified design and using of low-input technologies in agroecosystems (Altieri, 1994). In the agroecological context, improved technologies are compatible in an aspect of both crop production and crop protection from pests and diseases. However, "rapid conversion towards sustainable agroecosystem from conventional is neither possible nor practical" (Gliessman, 2007). The conversion process could be a long-term process, complex, challenging and requires consideration of both economic and ecological benefits. The reduction of environmentally harmful input costs and practice in substitution management systems for conventional agriculture is one of the principles of conversion to agroecological practice given by Gliessman. The main goal of this principle is to replace resource intensive and environmentally degrading inputs with more ecologically sound products and practices. Nanotechnology can be the new key to agricultural improvement by combating plant disease as nanoparticles have been found to have an inhibitory effect on fungi (Singh et al., 2013).

Nanoparticles can be defined as "the particles having at least one or two dimensions in the range of 1 to 100 nm." (Foldbjerg et al., 2015; Stone et al., 2010). Nanoparticles can be found in two ways: natural i.e. nanoparticles which are present in nature and synthesized by natural process and engineered nanoparticles which are industrially synthesized. Biosynthesis of nanoparticles using leaf extracts has been reported such as tamarind leaf extract using as the reducing agent in synthesis of gold nanotriangles (Ankamwar et al., 2005). Aloe vera (Chandran et al., 2006) and amla (*Emblica officinalis*) fruit extract (Ankamwar et al., 2005) are used for the synthesis of both gold nanotriangles and silver nanoparticles. Huang et al., (2007) demonstrated that for biosynthesis of silver and gold nanoparticles, sun-dried leaves of *Cinnamomum camphora* performed better than aqueous leaf extract.

Silver nanoparticles are the most studied and effective nanoparticles and can have strong bactericidal and fungicidal inhibitory effects. Silver nanoparticles are highly reactive (Morones et al., 2005) and can penetrate efficiently into microbial cells at lower concentrations and result in microbial control (Samuel and Guggenbichler, 2004). Microorganisms that are less sensitive to antibiotics because of less penetration into cells could potentially be made more efficient by treating them with nanoparticles (Samuel et al., 2004).

Silver nanoparticles have been used as a remedy for many diseases in medical science for over 100 years (Morones et al., 2005). Inhibitory effects of different forms of silver nanoparticles have been found on two spore-producing fungal pathogens on rice, *Bipolaris sorokiniana* (Young et al., 2009) and *Magnaporthe grisea* (Young et al., 2009; Elamawi, 2013). Relatively few studies have reported the antifungal effect of silver nanoparticles on sclerotia-forming fungi like *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, and *Sclerotinia minor* (Min et al., 2005). The antifungal effect of zinc oxide nanoparticles against *Penicillium expansum* (He et al., 2011), *Aspergillus niger* (Chitra et al. 2013) and *Helmenthosporium oryza* (Elamawi et al., 2016) were also reported. Nanoparticles are highly reactive and therefore very small amounts can be used effectively as fertilizers and pesticides (Batsmanova et al., 2013 and Elamawi et al., 2013). Biosynthesized silver nanoparticles which are considered more ecofriendly can improve the percentage of seed germination, vigority index and plant health can reduce disease incidence caused by *Fusarium oxysporium* on tomato, barley and Faba bean (Elamawi and Al-Harbi 2014). Silver nanoparticles can be a solution to problematic soil (acidic/ alkaline soil) for crop production. Silver nanoparticles of different forms have an effect on soil pH and also increase the percentage of nitrogen and capability of retaining phosphorus in soil (Tomacheski et al., 2017).

Nanotechnology holds promising potentiality in the agriculture sector, however massive or long-term application of nanoparticles might cause a negative impact on the environment as nanoparticle-based pollutants. Risk assessment of nanoparticles should be evaluated after exposure of nanoparticles to any organism or environment. Exposure of nanoparticles to plants and soil microorganism is still not completely investigated and probability of biotransformation of the remains in the soil cannot be neglected. There might be the possibility of nanoparticles that will create transformations in soil microflora and fauna and alter their mode of action in a positive or negative way (Luo et al., 2008, Schlagenhauf et al., 2014). Microorganisms are an integral part of soil and perform a prime role in the biological process for improving soil health (Judy et al., 2015). Though silver's toxicity for microbes is established, interestingly silver phosphate and silver bionite can improve proliferation of Bacillus, mesophytic bacteria and different fungi (Schlich and Hund-Rinke, 2015). According to Yang et al. (2013), silver ions have a high affinity to interact with inorganic and organic matters and high concentration of phosphorus in silver ion bentonite soil can decrease silver toxicity in soil.

Xue et al., (2014) reported, a residue-free technology with nanoparticles can synergistically increase pesticide efficacy and could control the plant disease rationally along with eliminating its residue successfully; therefore, benefiting society by reducing the pesticidal impact on human health and the environment. Nanotechnology therefore has potential benefits in global food production, food processing and food security but more studies are needed to realize the long-term effects of nanoparticles on agriculture and environment (Phogat et al., 2016).

1.4 Aim and Objectives

In Bangladesh, application of nanoparticles is a totally new concept for rice disease management. My intention behind this research is to find out an ecologically sound technology to reduce fungicide use and understand farmers' opinion about using ecologically sound technology instead of fungicide. Developing a new technology requires a long time so in my master's project, my aim is to find out whether silver can control the race of *M. grisea* responsible for blast disease in Bangladesh successfully or not and farmers knowledge and common practices used in rice blast disease management. Nano sized silver particles are considered as harmless to human (Berger et al., 1976) and silver particles have been randomly used in many pharmaceutical processes and many medical devices are coated with silver compounds (Raad and Hanna, 2002). Antimicrobial efficacy of ionic or nanoparticle silver has a huge potentiality in controlling spore-producing fungi. As the research was conducted in a specific geographical location (Gazipur district, Bangladesh) with specific environmental conditions (temperature, rainfall, humidity, day length etc.), the study result might not be generalized for global aspects, but it might be helpful for further research. As rice is the main crop in Bangladesh and I'm working in rice research institute, my utmost interest and dedication is to improve rice production by incorporating agroecological principles and more environment friendly technologies. However, the long-term effect of silver nanoparticles needs further study.

The objective of this study is to determine the anti-fungal effect of silver nanoparticles on hyphal growth of *M. grisea*, and to evaluate their efficacy of silver compound for rice blast disease management under controlled conditions. In addition, farmers' perception of rice blast disease management will be evaluated by interviewing them in the selected area.

1.5 Research questions

1. Are silver nanoparticle effective against rice the blast pathogen (*Pyricularia oryzae*) under laboratory and greenhouse conditions?

2. What is farmers' perception of rice blast disease management?

1.6 Limitations of the study

In this study, I will evaluate the efficacy of silver nanoparticles only on leaf blast disease. Due to the limitation of time, it is not possible to validate nanoparticles in farmers' fields in neck blast disease management, though it is the major problem among the other two blast diseases (leaf and node blast). Future studies needed to assess the long-term effects of nanoparticles on rice plant and soil health and to validate the use of nanoparticles in farmers' field. The nanoparticles used in this study are engineered nanoparticles. Further investigation is needed in plant-based nanoparticles that are ecologically sound and sustainable.

2. Materials and method

2.1 Study area

The study was carried out in the laboratory and greenhouse at the plant pathology division of Bangladesh Rice Research Institute (BRRI), Bangladesh. Interview of farmers on their perception of rice disease management took place in Gazipur district in Bangladesh.

The Gazipur district is situated in the outskirts of capital city Dhaka, Bangladesh and located between 23.88° - 24.34° latitude and 90.15° - 90.70° longitude.

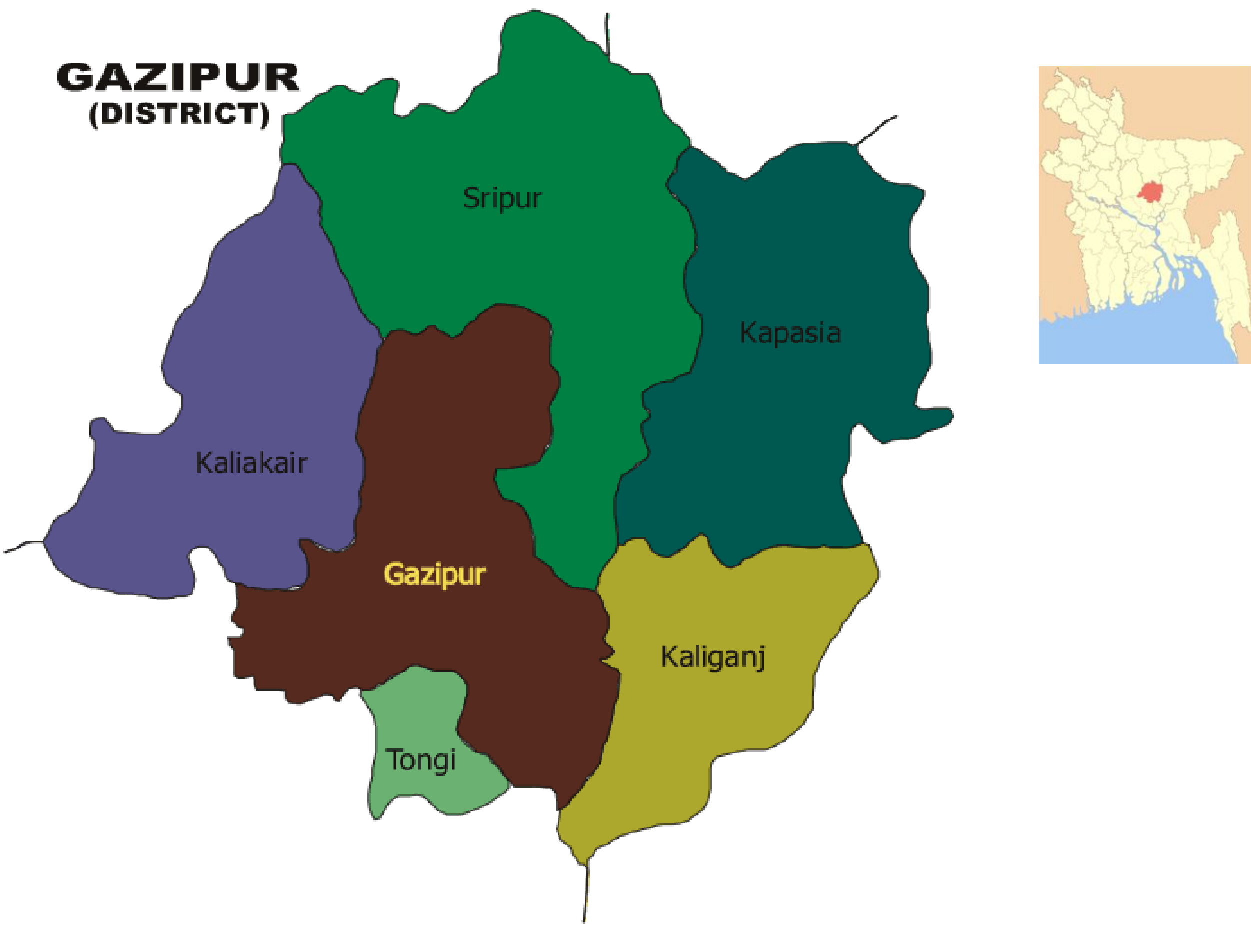


Figure 1: Map of Gazipur district (Akand, 2018)

Gazipur belongs to Old Brahmaputra floodplains and Madhupur tract Agro-Ecological Zone (AEZ). Annual average temperature and rainfall of Gazipur district ranges from 13 to $39^{\circ}C$ and around 240 cm respectively. Total population and population density of Gazipur district is approximately 3.4 million and 1884 km^{-2} respectively with a literacy rate of 62.60% (BBS, 2011). Agricultural products with high economic impact are rice of local, high yielding varieties (HYV) and hybrids, wheat, jute, vegetables, etc. Major agricultural research organizations of Bangladesh like Bangladesh Rice Research Institute (BRRI), Bangladesh Agriculture Research Institute (BARI), Soil Resource Development Institute (SRDI) and an agricultural university situated in Gazipur.

2.2 Qualitative research method

An integration of social science, qualitative research was conducted using face-to-face interview in order to understand farmers' knowledge in rice blast disease management and some data were also collected from secondary sources. According to Taylor (2005), "qualitative research is multi-functional in focus which involves an explanatory, innate accession to its subject matter." As almost all the farmers of Bangladesh are associated with rice production, among them 30 farmers were chosen randomly for interview in Gazipur district. Semi structured close-ended questionnaires were prepared as a tool for the face-to-face interview. In a questionnaire, questions should have a clear objective to avoid confusion for both the interviewer and the interviewee (Bernard, 2006). Each respondent was asked the same questions and each interview started with a soft conversation to establish a good rapport between interviewer and interviewee. Respondents were very co-operative and sometimes they asked questions about related topics. A cordial atmosphere was maintained by giving them satisfactory answers about their quarry throughout the interview.

In a face-to-face interview, interviewer and interviewee can feel a connection, if an interviewee doesn't understand a question, the interviewer can explain it or if the respondent does not answer fully, the interviewer can probe for more information or what she/he desires. While conducting an interview the "probing method" has been narrated as a key for gaining success by Bernard (2006) but also pointed out as a costly method in terms of both time and money (Bernard, 2006).

2.3 Quantitative research method

2.3.1 Nanoparticles and fungicides

Nanoparticles (Si, Cu, Ag and Zn) and silver nitrate were obtained from the applied chemistry and chemical engineering department, Dhaka University. A particle size of below 100 nm was used in this experiment. To prepare nanoparticles, nano-powder were weighed and suspended in deionized sterile water using a sonicator. Nanoparticles of different concentrations (200, 400 and 800 ppm) were prepared by diluting the original stock solution using sterile deionized water and solutions were preserved at 4^{o}C to maintain the quality. A commercial formulation of tricyclazole group fungicide (Trooper 75 WP) for rice blast disease management marketed by Auto Crop Care Bangladesh Ltd. was used as a standard disease control treatment and thus served as a control, to allow us to evaluate how well nanoparticles performed compared to the standard synthetic fungicide.

2.3.2 Fungus preparation

A virulent blast isolate (BD576p) which was selected as differential blast isolate was used as test isolate. A differential isolate is an isolate that was selected for evaluation of blast resistant materials for Bangladesh conditions (Khan et al. 2016). This isolate was collected from the Plant Pathology Division, Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh.

2.3.3 Observation of hyphal growth in the presence of silver nanoparticles

The inhibitory effect of nanoparticles on fungal growth was examined *in vitro* by measuring hyphal growth and sporulation. To measure hyphal growth, pure cultures of *M. grisea* were produced in Potato Sucrose Agar (PSA) medium (200g potato extract, 20g sucrose and 20g agar dissolved in 1000 ml water and autoclaved). PSA media supplemented with different concentrations (200, 400 and 800 ppm) of nano materials (Si, Cu, Zn and Ag) were prepared to measure hyphal growth and three replications were done. From pure cultures, actively growing edge (6 mm in diameter) was obtained and transferred into each plate of both control and nanoparticles treated plates. Plates inoculated with *M. grisea* were incubated at 28^{o}C for 15 days. After colony formation, the radial growth of each culture was measured at every 72 hours interval either in control or nanoparticle treated plates to assess if there was an inhibitory effect on hyphal growth.

2.3.4 Greenhouse assays

The inhibitory effect of only silver nanoparticles against rice blast disease under controlled conditions was measured at the Plant Pathology Division, Bangladesh Rice Research Institute, Bangladesh. As other nanoparticles did not perform satisfactorily in laboratory test. Rice cultivar US-2 was selected as test cultivar, since it is the most susceptible to rice blast disease. Four trays for each treatment were randomly arranged following CRD design. Three concentrations (200, 400 and 800 ppm) of silver nanoparticle suspension and commercial fungicide Trooper 75 WP were sprayed on 20-25 days old rice seedlings 3 days before inoculation with spore suspension (10^5 conidia/ml 0.01% aqueous solution of Tween 20) for preventive measure and 3 days after inoculation with spore suspension (10^5 conidia/ml 0.01% aqueous solution of Tween 20) for curative measure. The inoculated plants were kept in controlled conditions. The inoculated seedlings were incubated in a moist chamber with relative humidity at least 90% and 26 ± 2^o C for 24 hours and then the inoculated plants were kept in controlled conditions in the greenhouse. The reaction was scored at seven to ten days after inoculation using 0-9 scale of Standard Evaluation System (SES) of International Rice Research Institute (IRRI, 2012).

2.4 Statistical analysis

The data were analyzed using Microsoft Excel program and Minitab 17. One-way ANOVA analysis was performed and differences among means were determined through Tukey's multiple comparison tests ($p<0.05$). Means were separated using Tukey's Honest Significant Difference (HSD) Test in disease severity (%) of rice leaf blast. Dose response relationship LC_{50} was calculated based on the reduction of hyphal growth or leaf blast severity over untreated control by Probit Analysis (Finney 1952) using Microsoft Excel program.

2.5 Ethical aspects

While conducting research sometimes ethical issues pose difficulties because it varies from time to time and place to place. Bernard (2006) has concluded ethical aspects as "what is popularly ethical today may become popularly unethical tomorrow." There are some common aspects had taken into consideration while conducting research. To generalize the result, the experiment had enough replication and data were collected very carefully to avoid biases. While conducting interview respondents were informed about the purpose of the interview and ensured that we would maintain confidentiality of their personal data. In semi-structured interviews respondent's consent was taken before starting the interview. I believe there is no perfect project plan, there is always some pros and cons, that's why research is a continuous process.

3. Results

3.1 Rice diseases in Bangladesh

Fungal diseases in rice are common and cause significant yield losses in Bangladesh. Among fungal diseases, rice blast was most severe in Boro season (November to May; irrigated ecosystem) and appeared in Aman season too (Table 1). Sheath blight was dominant in both Aus (January to June; rainfed upland) and Aman season (July to December; rain fed ecosystem). Bacterial blight was seen in all rice season and tungro was found in every season in a small proportion in the field (Table 1).

Table 1: Ranking of the top rice diseases in Bangladesh

Name of the disease	**Causal organism**	**Ecosystem-wise disease ranking (I-V)**		
		Aus	Aman	Boro
Blast	*Pyricularia oryzae*	V	III	I
Sheath blight	*Rhizoctonia solani*	I	I	IV
Bakanae	*Fusarium moniliformae*	III	V	II
Tungro	Rice Tungro Virus (RTV)	IV	IV	V
Bacterial blight	*Xanthomonas oryzae* pv. *oryzae*	II	II	III

Source: BRRI, 2017

3.2 Choice of varieties

In table 2.1, BRRI dhan48 and BRRI dhan28 were the most popular rice varieties and their adoption rate was 17.2% and 15.3% respectively due to their grain quality. The adoption rates of BR26 and BR21 were moderate at 6.9% and 4.3% respectively.

Table 2.1: Ranking of the top varieties based on the cultivation area during Aus 2016-17 season in Bangladesh

Name of variety	Rank	Adoption rate (%)	Reason
BR21	IV	4.3	Medium bold grain, suitable for broadcast
BR26	III	6.9	Fine, long and white grain, low amylose
BRRI dhan28	II	15.3	Medium slender and white grain, most popular in Boro season
BRRI dhan48	I	17.2	Medium bold grain and low amylose
Other BRRI varieties	V	23.2	High yielding
Hybrid varieties	VI	4.5	High yielding

Source: BRRI, 2017

In Aman season (Table 2.2) BRRI dhan48, BR 11 and BRRI dhan34 were the top cultivated varieties and their adoption rate was 10.9%, 7.3% and 3.7% respectively.

Table 2.2: Ranking of the top varieties based on the cultivation area during T. Aman 2016-17 season in Bangladesh

Name of variety	Rank	Adoption rate (%)	Reason
BR11	II	7.3	Medium bold grain
BRRI dhan34	III	3.7	Medium bold and white grain, slightly aromatic
BRRI dhan49	I	10.9	Medium bold, long grain, growth duration 7 days shorter than BR11
BRRI dhan52	IV	3.3	Medium bold grain and submergence tolerant
Other BRRI varieties	V	23.4	High yielding
Hybrid varieties	VI	1.7	High yielding

Source: BRRI, 2017

The Boro season (Table 2.3) was dominated by two mega varieties BRRI dhan28 and BRRI dhan29 and their adoption rate was 35.3% and 26.5% respectively.

Table 2.3: Ranking of the top varieties based on the cultivation area during Boro 2016-17 season in Bangladesh

Name of variety	Rank	Adoption rate (%)	Reason
BRRI dhan28	I	35.3	Medium slender and white grain (most popular variety)
BRRI dhan29	II	26.5	Medium slender and white grain
BRRI dhan58	III	2.2	Medium bold
Other BRRI varieties	V	6.7	High yielding
Hybrid varieties	IV	15.1	High yielding

Source: BRRI, 2017

3.3 Incidence of rice blast disease and awareness of the farmers'

Table 3 showed that in the Boro season serious neck blast infection occurred due to a favorable environment for *M. grisea*. Farmers were interested in cultivating popular rice varieties which are susceptible to blast disease particularly both leaf and neck blast. In Aman season medium neck and node blast symptoms were observed. There was no blast infection in Aus season (dry season); less favorable environmental conditions may be one of the reasons.

Table 3: Ecosystem-wise blast disease incidence in various rice fields of Bangladesh

Ecosystem/Season	**Rate of blast disease incidence (%)**		
	Leaf blast	Node blast	Neck blast
Boro (Irrigated lowland)	+	-	+++
T. Aman (Rainfed lowland)	-	++[1]	++[1]
Aus (Rainfed upland)	-	-	-

Source: BRRI, 2017

Note: [1] *= mostly aromatic rice infected severely by blast during T. Aman season*

- = *no blast infection*

\+ = *low blast infection*

++ = *medium blast infection*

+++ = *high blast infection*

3.4 Socio-demographic characteristics of the respondent farmers

30 rice farmers were interviewed from different age groups of Gazipur district of Bangladesh. Among them all were male farmers because women in Bangladesh are normally not fully engaged with rice farming due to social aspects; sometimes they help their family members in farming.

In figure 2, the respondents' distribution according to different age groups is shown. Most (43%) of the respondents belonged to the age group 31 to 40 years; 27% belonged to the age group up to 30 years, 20% of the respondents were 41 to 50 years and only 10% of the farmer were above 50 years.

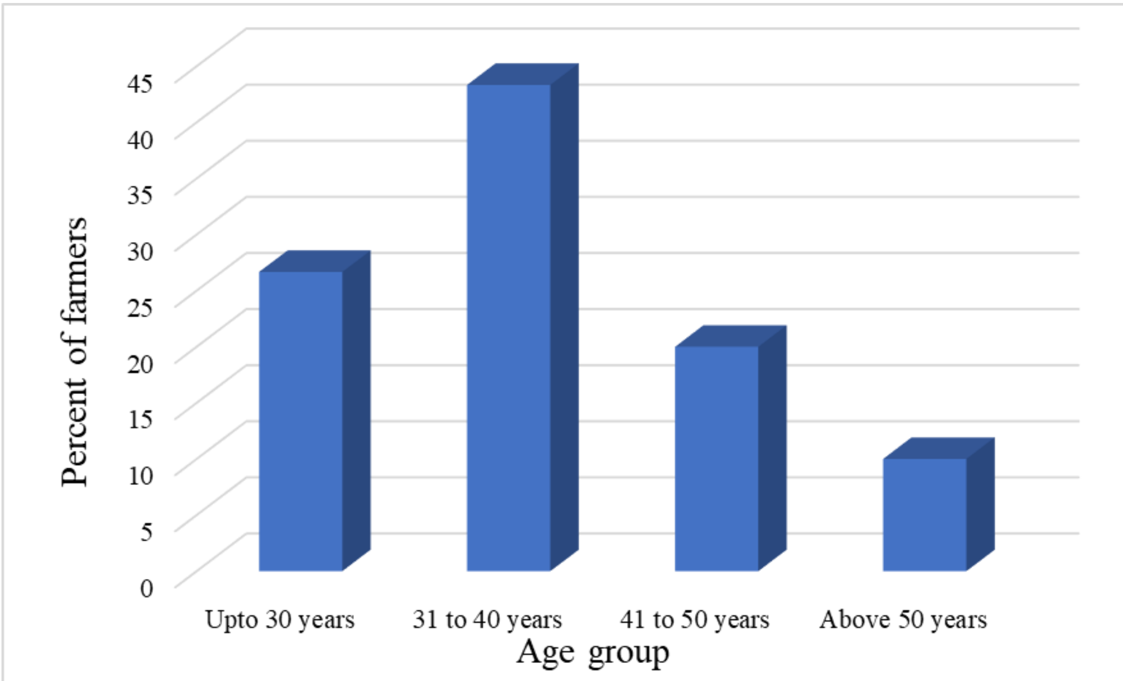


Figure 2: Age distribution of respondents (n=30)

The research study revealed that 33% of the respondents had primary education certificate (PEC, 5 years of education), 27% completed junior school certificate (JSC, 8 years of education), 23% of the farmers did not attend school at all but they can sign their name, 10% had completed secondary school certification (SSC, 10 years of education) and only 7% did higher secondary and above (Figure 3). Education level has a significant influence on crop productivity and adopting new technologies as educated farmers are conscious about upcoming circumstances and trying to keep themselves up to date.

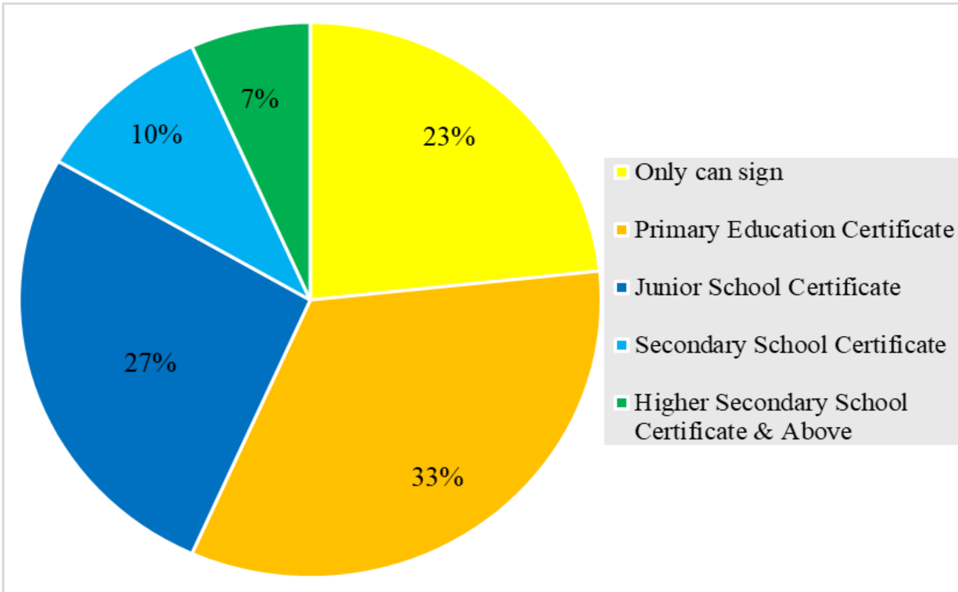


Figure 3: Education level of the farmers

In the study area, the farm size was measured with the total land cultivated or owned by the farmers. The maximum number of the farms were medium in size with 0.4 to 2.02 ha of land (43.33%) followed by small farmers (26.67%) who owned below 0.2 ha of land and large farmers (20%) having more than 2.02 ha of land. Minimum numbers (10%) were observed landless in the study (Figure 4).

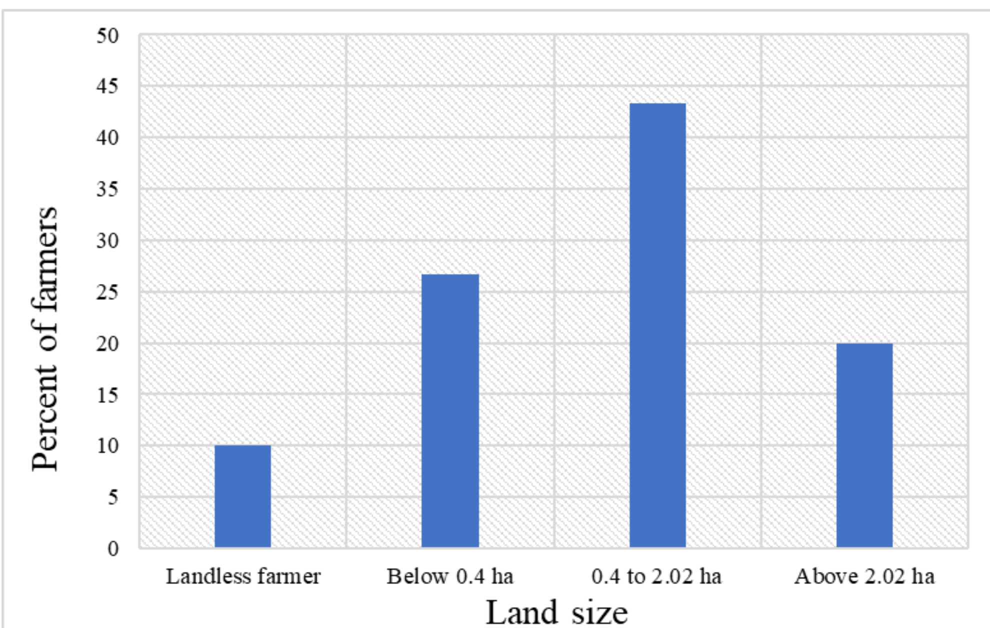


Figure 4: Land size owned by respondents in ha for rice cultivation (n=30)

Though rice blast is one of the major diseases in Bangladesh 27% of the respondent farmers said they had not experienced blast disease in their rice field. Selection of variety, planting time and cultural practices might be a reason for this. Majority of the respondents (73%) had experienced more or less rice blast disease in their fields (Figure 5). Among them only 50% of respondents had aware of rice blast disease, 30% were aware of both rice blast disease and its management and 20% of the farmers were not aware of rice blast disease and its management (Figure 6).

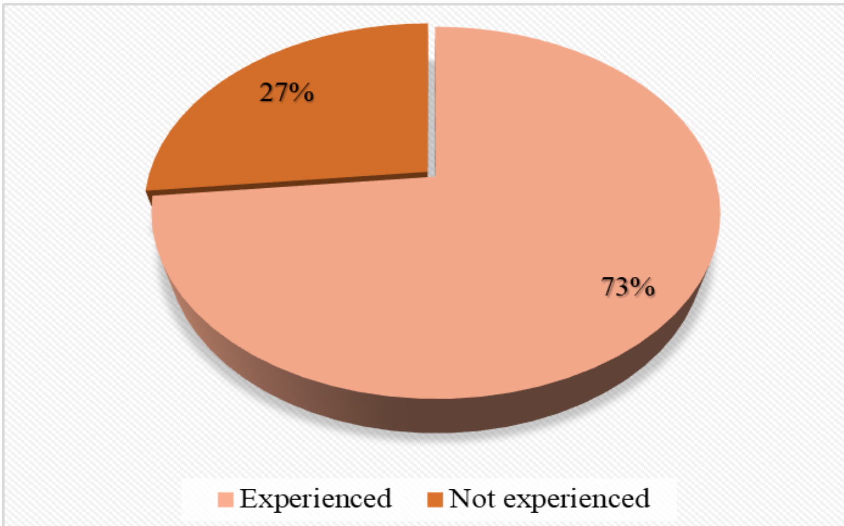


Figure 5: Practical experience on rice blast disease among the respondent farmers (n=30)

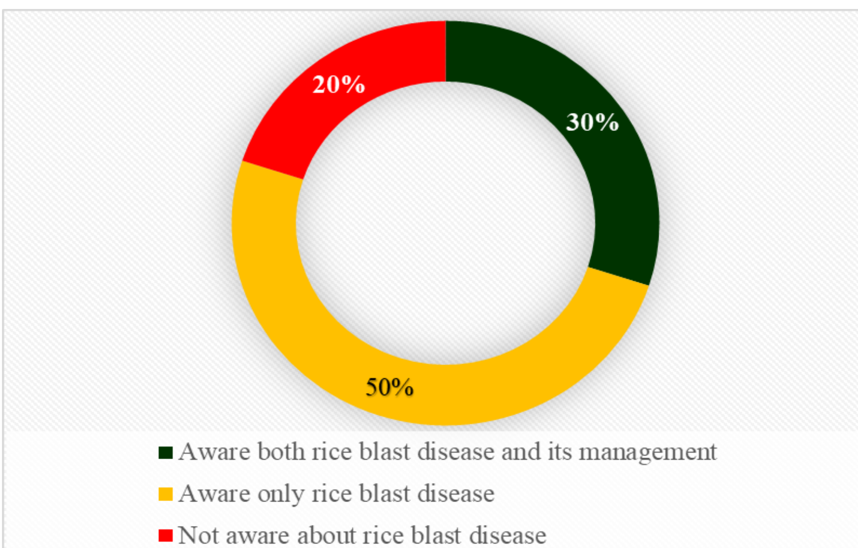


Figure 6: Awareness of rice blast disease of the respondent farmers (n=30)

3.5 Methods adapted by the Farmers

Respondents were asked a range of different methods that could be helpful in controlling blast disease partially or successfully. Among them, chemical spray (89%), split applications of nitrogenous fertilizer (5%), crop rotation (3%) and burning diseased-straw and stubble (1%) were commonly used methods (Table 4). Abandon field, planting resistant cultivars, applications of compost, or changing planting time were strategies that were adopted by less than 1% of questioned farmers (Table 4).

Table 4: Percent usage of various rice blast control methods

Methods of controlling Blast	Percentage (%)
Burning diseased-straw and stubble	1
Plant resistant variety	0
Changing planting time	0
Apply chemicals	89
Apply compost	0
Crop rotation	3
Abandon field	0
Split applications of nitrogenous fertilizer	5
Avoid use of fertilizer	0
Excess use of fertilizer	0
Others (Not using any control method)	2

3.5.1 Farmers perception towards fungicide and source of advice

Around 89% of the respondent farmers used chemicals in controlling rice blast disease but among them only 9% of the farmers had an opinion that spraying chemical worked very well and 21% thought this method worked satisfactorily. Majority of the respondents (53%) thought this method worked but not very well, they wanted some other more effective methods than chemical control. Synthetic pesticide efficacy of chemical is crucial, but dependent on the perfect timing before disease development. Around 14% of the respondent farmers said that spraying chemical did not work well and 3% had no opinion about this management system (Figure 7).

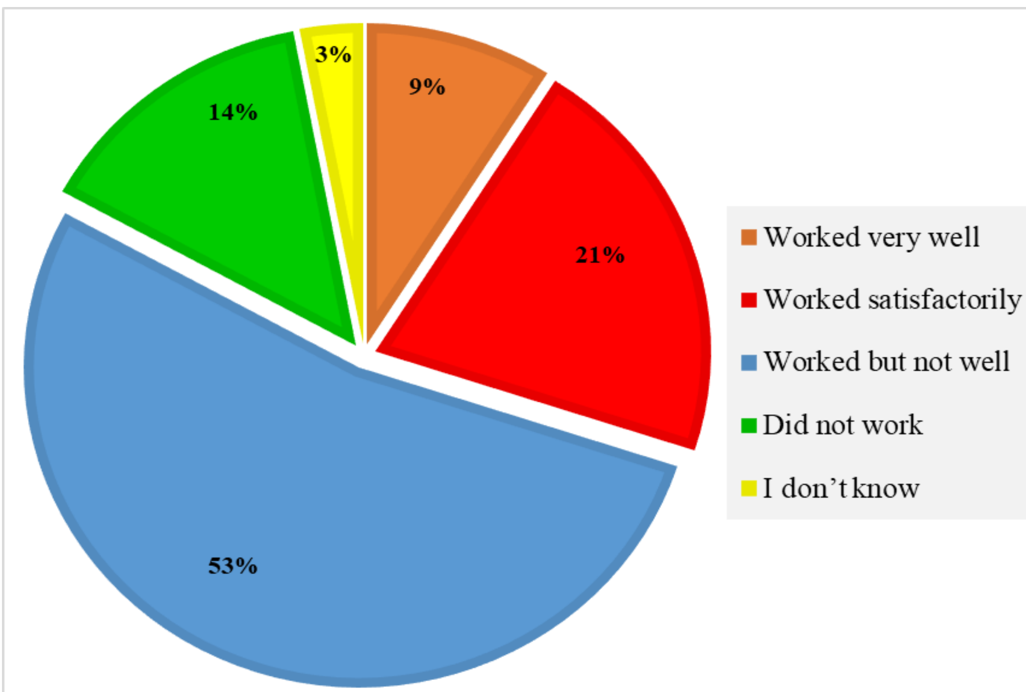


Figure 7: Farmers perception on using chemicals to control rice blast disease (n=30)

Most farmers who participated in this survey obtained rice production related knowledge like new technologies, disease and pest management strategies either from extension workers (59%), fellow farmers (20%) and/or from training or workshops (13%). A small group of farmers received information through visiting or organizing field days with researchers of BRRI (5%) and from the local leader (progressive farmers) (2%) (Table 5).

Table 5: Farmers source of advice on the appropriate method of rice blast disease management

Sources of advice	Percentage
Fellow farmer	20
Extension worker	59
Training or workshops	13
Local leader	2
Visiting researchers (BRRI)	5
Media (Radio/TV)	0
Newspapers/leaflet	0
Other	1

3.6 Laboratory experiments

3.6.1 Inhibition of mycelial growth with different nanoparticles

Study results confirmed that silver nanoparticles had significant inhibitory effects on hyphal growth of *M. grisea*. Silver nanoparticles and silver nitrate successfully controlled mycelial growth and showed significant differences compared with the other nanoparticles. Only silver nanoparticlse at 800 ppm showed a significant difference compared with the other silver nanoparticles and silver nitrate.

Table 6: Comparison studies among different nanoparticles on the mycelial growth of blast pathogen (*M. grisea*)

Treatments	Concentration(ppm)	Mycelial growth (mm)
Si nanoparticle	200	64.6 bcd*
Si nanoparticle	400	76.4 ab
Si nanoparticle	800	69.4 abc
Cu nanoparticle	200	55.4 d
Cu nanoparticle	400	59.9 cd
Cu nanoparticle	800	60.1 cd
Zn nanoparticle	200	67.3 abc
Zn nanoparticle	400	65.1 bcd
Zn nanoparticle	800	78.7 a
Ag nanoparticle	200	28.7 ef
Ag nanoparticle	400	17.2 fg
Ag nanoparticle	800	14.8 g
$AgNO_3$	200	33.2 e
$AgNO_3$	400	24.8 efg
$AgNO_3$	800	17.7 fg
Control	-	70.9 abc

**letters indicating significance of differences*

3.6.2 Measuring disease severity (%) of rice leaf blast

Leaf blast disease severity (%) when treatments were applied as a preventive measure was lower (1.77) at 800 ppm concentration of Trooper 75 WP which had no significant difference with silver nanoparticles at 800 ppm (3.23). Both of these treatments showed a significant difference from other concentration (200 ppm and 400 ppm). Silver nanoparticles had no significant difference in leaf blast disease severity (%) between 400 ppm and 800 ppm but showed significant difference at 200 ppm. Trooper 75 WP and silver nanoparticles both had significant difference at 200 ppm concentration. Both Trooper 75 WP and silver nanoparticles had significant difference with control for preventive measure.

Leaf blast disease severity (%) when treatment was applied as a curative measure was lower at 800 ppm for both Trooper 75 WP and silver nanoparticles and it had no significant difference. Leaf blast disease severity (%) showed no significant difference at 400 ppm of Trooper 75 WP and silver nanoparticles but showed significant difference at 200 ppm concentration (Table 7).

Table 7: Disease severity (%) of leaf blast of rice caused by *Magnaporthe grisea* treated with silver nano-particles and fungicide

Treatments	Concentration (ppm)	Leaf blast disease severity (%)	
		Preventive[A]	Curative[B]
Control[C]	0	73.47a	71.90 a
Trooper 75%[D]	200	33.20 b	41.30 b
Trooper 75%	400	8.33 d	20.67 d
Trooper 75%	800	1.77 f	11.87 e
Silver nano (AgN)[E]	200	28.43 c	32.50 c
Silver nano (AgN)	400	5.90 de	20.47 d
Silver nano (AgN)	800	3.23 ef	9.60 e
HSD[F] ($p=0.05$)		3.69	4.53

**letters (small) indicating significance of differences*

[A] *Chemicals (Trooper 75% and AgN) were sprayed on plants 3 days before inoculation with spore suspension (10^5 conidia/ml) for preventive measure.*

[B] *Chemicals (Trooper and AgN) were sprayed on plants 3 days after inoculation with spore suspension (10^5 conidia/ml) for curative measure.*

[C] *Only water was sprayed in control plot.*

[D] *A commercial formulation of tricyclazole group fungicide for rice blast disease management marketed by Auto Crop Care Bangladesh Ltd.*

[E] *Size of the nano particles was below 100 nm.*

[F] *Means were separated using Tukey's Honest Significant Difference (HSD) Test.*

3.6.3 Assessment of LC_{50}

Table 8 shows that leaf blast disease severity was reduced by 50% at 136.5 ppm concentration of silver nanoparticle before inoculation where commercial formulation of tricyclazole group fungicide Trooper 75WP showed 50% leaf blast disease reduction at 175.6 ppm concentration before inoculation. Trooper 75WP showed 50% leaf blast disease reduction at 166.3 ppm after inoculation but silver showed the same at higher concentration than Trooper 75WP (226.2 ppm).

Table 8: Effective concentration of silver nano particles (AgP) to cause 50% leaf blast disease reduction (LC_{50})[a]

Treatment	Concentration of LC_{50} (ppm)	
	Preventive[b]	Curative[c]
Silver nano (AgP)	136.5	226.2
Trooper 75WP	175.6	166.3

[a] *Dose response relationship LC_{50} was calculated based on the reduction of leaf blast disease severity over untreated control by Probit Analysis (Finney 1952).*

[b] *Chemicals (Trooper 75% and AgP) were sprayed on plants 3 days before inoculation with spore suspension (10^5 conidia/ml) for preventive measure.*

[c] *Chemicals (Trooper and AgP) were sprayed on plants 3 days after inoculation with spore suspension (10^5 conidia/ml) for curative measure.*

Data from three replication were averaged for each treatment and used to calculate EC_{50} value (Table 9). The range of EC_{50} was calculated at 3 to 15 days after incubation on *M. grisea* conidia and the concentration of silver nanoparticle was ranging from 227.0 to 46415888.3 ppm and the concentration of trooper was ranging from 105.4 to 120.7 ppm.

Table 9: Effective concentration of Silver nano particles (AgP) and Trooper 75WP causes 50% mycelial growth reduction (LC_{50})[a] of *M. grisea*

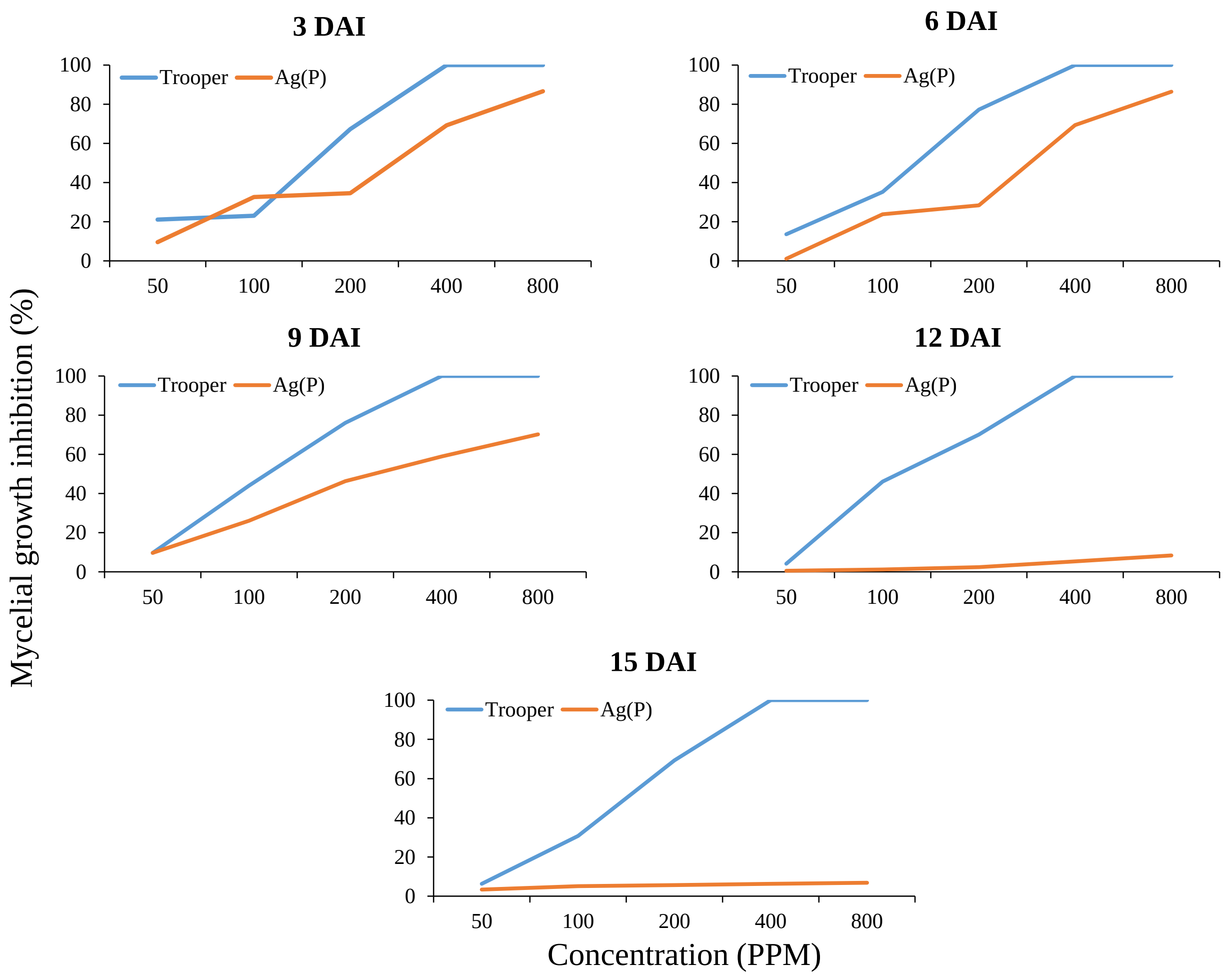
Treatment	Concentration of EC_{50} (ppm)				
	3 DAI[b]	6 DAI	9 DAI	12 DAI	15 DAI
Silver nano (AgP)	227.0	285.5	301.8	42751.6	46415888.3
Trooper 75%	111.8	105.4	106.6	118.1	120.7

[a] *Dose response relationship LC_{50} was calculated based on the reduction of mycelial growth over untreated control by Probit Analysis (Finney 1952).*

[b] *DAI=Days after incubation.*

At 3 days after incubation, mycelial growth was inhibited by 13.50% at both 50 ppm and 100 ppm concentration of Trooper 75WP. Mycelial growth was almost completely inhibited (0%) at 400 ppm and 800 ppm of Trooper 75WP. Silver nanoparticles inhibited mycelial growth by 15.67% at 100 ppm silver nanoparticles which were almost similar to control (17.33%). Silver nanoparticle inhibited mycelial growth notably 5.33% and 2.33% at higher concentration 400 ppm and 800 ppm respectively.

After 6 days of incubation, Trooper 75WP successfully inhibited mycelial growth at 400 ppm and 800 ppm and 6.67% mycelial growth reduced at 200 ppm concentration. Mycelial growth of *M grisea* was not inhibited desirably at 50 ppm and 100 ppm. Silver nanoparticle showed satisfactory inhibition at 400 ppm and 800 ppm and inhibited mycelial growth 9% and 4% respectively and mycelial growth increased 22.33% and 30% at 50 ppm and 100 ppm.



**DAI=Days after incubation*

Figure 8: Mycelial growth inhibition of *M. grisea* treated by different concentrations of Silver nanoparticle [Ag(P)] and Trooper over the time

After 9 days of inoculation, Trooper 75WP was steadily inhibiting mycelial growth at 400 ppm and 800 ppm but silver nanoparticle could not inhibit mycelial growth satisfactorily and growth increased 13.33% and 18.33% at 800 ppm and 400 ppm respectively.

After 12 days of inoculation, Trooper 75WP was steadily inhibiting mycelial growth at 400 ppm and 800 ppm. Mycelial growth increased 14.67% at 200 ppm of Trooper 75 WP and lower doses than 200 ppm could not inhibit mycelial growth. In this stage silver nanoparticle could not inhibit mycelial growth even at 800 ppm concentration. After 15 days of inoculation Trooper 75WP and silver nanoparticle showed almost similar inhibitory performance like 12 days after inoculation.

4. Discussion

In Bangladesh rice is affected by various fungal, bacterial, viral and nemic diseases. Among them, the top rice diseases are fungal diseases, and these causes significant yield loss. Naturally, Bangladesh is a warm humid country, for that reason rice crops suffer from several fungal diseases. Fungal disease management is economically important for sustainable yield and approximately 73% of respondent rice farmers experienced blast disease in this study. Among the respondent farmers, 50% were aware of rice blast disease, 30% were aware of both rice blast disease and its management. Management of blast disease is also crucial. Farmers should observe fields frequently and when climatic conditions favorable to disease development occur there are two outcomes: either disease will appear in the field or not and then the farmer should spray fungicides for preventive measure. Neck blast is visible to the naked eye when severe infestation has occurred during the time of harvesting. Once disease is visible by naked eye that means it is already too late to secure yield. Among the respondent farmers 20% of the farmers were not aware of rice blast disease and its management. The reasons behind these might be that farmers were either ignorant about blast disease or could not identify the symptoms as it is very crucial to identify blast disease at an early stage in the field. Perhaps they were unconsciously practicing some cultural practices like earlier the planting time, using partially resistant variety etc. that would lead to a reduction in disease symptoms.

In the recent world, it is a timely to develop a safe and ecologically sound disease management method which is economically feasible in rice. The major challenge in the management of rice blast disease in Bangladesh is most of the farmers prefer to grow susceptible or partially susceptible rice varieties due to their market value. The people of Bangladesh prefer non-sticky medium slender to medium bold grain rice. Farmers are growing different varieties according to their preferences. Farmers mostly consider the variety that gives the highest yield and fetches good market prices than disease resistance and for that reason, BRRI dhan28, BRRI dhan29, BRRI dhan58 were the dominating cultivated variety despite their high susceptibility to blast disease. The adoption rate of BRRI dhan28 and BRRI dhan29 was 35.3% and 26.5% respectively which are highly susceptible in Boro season. Disease incidence of the blast was higher in BRRI dhan47 (58.2%) followed by BRRI dhan29 (39.8%), BRRI dhan28 (20.3%) in Boro season regardless of place and cropping sequence (Hossain et al., 2017). In Boro season, farmers were experienced with all types of blast diseases viz. leaf, node and neck blast due to favorable environmental conditions for pathogens. Incidence and severity of rice blast disease were recorded higher in Boro (21.19%) than transplanted Aman season (11.98%) (Hossain et al., 2017). In Aman season BRRI dhan48, BR11 and BRRI dhan34 were the top cultivated variety and their adoption rate was 10.9%, 7.3% and 3.7% respectively (Table 2.2). Disease incidence was observed higher in BRRI dhan34 ranging from 50.7 to 59.8% and lower in BRRI dhan49 (4.9 to 9.3%) in Aman season (Hossain et al., 2017).

Durable blast resistant varieties have yet not been developed for Bangladesh, and thus this is still a big challenge for the breeder in Bangladesh. Most of the rice farmers are using different fungicides of tricyclazole group. Around 89% of the respondents' farmer used synthetic fungicides; among them, only 9% farmers thought to spray fungicide worked very well and the majority of the farmers thought fungicides worked but not satisfactorily. The study results found that application of fungicide prior infection (prophylaxis) was much more effective than application to an already infected field. When the climate is favorable for disease formation farmers should take preventive measures to control the disease successfully. However, the adoption rate of using resistant varieties and other cultural practices were very low. The majority of rice farmers were not interested in cultural practices in controlling blast disease, they found this ineffective and laborious. Most of the farmers' found that hiring labor was expensive. Rice straw is used as a cattle feed and some places used as a building material of roofs of the houses. As Bangladesh is a densely populated country for that reason farmers find it as a luxury in abandoning the field for one season. Though Boro season is considered as most blast disease prone season, farmers cultivate rice due to higher productivity than other rice growing seasons.

Nanotechnology is emerging as a revolutionary edge of rapidly developing fields such as pharmaceuticals, biosensing, disease diagnosis, disease remediation, biotechnology, food industry, agriculture, etc. Nanotechnology is opening a new horizon in agricultural research viz., diagnosis of plant diseases, inhibition of pathogen growth, increasing the efficiency of fertilizer and pesticide usage, enhancement of plant growth and improvement of soil health and structure, etc. (Ghormade et al., 2011). Nanostructured materials as a pesticide can enhance the efficacy of active ingredients of pesticide and control the release in the environment. This will reduce the environmental pollution and lower the overall amount of pesticide use.

This study results confirmed that other nanoparticles like zinc, copper, and silicon have no effective impact in controlling spore producing fungal plant pathogen *M. grisea*. Recent investigations reported that silver nanoparticles have a broad spectrum of anti-microbial properties with low toxicity and great antifungal potentiality to control spore-producing microorganisms (Young et al., 2009). Silver nanoparticles have high antimicrobial effects due to their large surface area and high fraction of surface atoms (Sharon et al., 2010). Therefore, they are highly reactive as they can produce Ag^+ ions compared to metallic or bulk silver (Morones et al., 2005). Nanoparticles are applied at low concentrations to control microbial activity since the large surface areas increase the opportunity for microbial contact and enhances their ability to penetrate into microbial cells (Lamsal et al., 2011). Efficacy of silver nanoparticles was influenced greatly by the time of application. Preventative measures with nanoparticles performed better than curative applications. Leaf blast disease severity (%) for both fungicide (Trooper 75WP) and silver nanoparticles was significantly lower at 800 ppm when used as preventive measures. The performance of the chemical fungicide (Trooper 75 WP) and silver nanoparticles were not satisfactory after inoculation of fungal spores. *M. grisea* produces asexual conidia, under favorable conditions conidia germinate and produce germ tubes to penetrate in plant cells within 24 hours cause disease (Tucker and Talbot, 2001). Jo et al. (2009) reported that after inoculation of 24 hours antifungal activity of silver was reduced. It was mentioned earlier that once infection occurred then it is very critical to inhibit disease development.

Disease severity percentage of leaf blast indicates that application of 800 ppm silver nanoparticles was highly effective before inoculation (3.23%) and worked satisfactorily after inoculation (9.60%). Elamawi et al., 2013 reported that application of 100 ppm silver nanoparticles (20-30 nm) was very effective in both preventive and curative measure. This study was the first to demonstrate antifungal activity of silver nanoparticles against *M. grisea* in Bangladesh. In Bangladesh Rice Research Institute (BRRI) had no facility to measure the accurate size of the nanoparticle (below 100 nm). Nanoparticle size might be the probable cause that the study result differs from the study done by Elamawi et al. (2013). It has been reported that nanoparticles have size specific microbial activity against plant pathogens like silver nanoparticles ranging 10 to 20 nm with spherical shape can effectively control spot blotch disease in wheat (Mishra et al., 2014). Similarly zinc oxide nanoparticles have antifungal activity against *Aspergillus flavus* and *Aspergillus niger* ranging 27±5 nm in size. Zinc oxide nanoparticles ranging from 16 to 20 nm have antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, Proteus mirabilis, Pseudomonas aeruginosa, Escherichia coli* etc. However, the maximum inhibition to *Staphylococcus aureus* was observed in particle size ranges from 23.8 ± 0.76 nm as compared to others (Elumalai et al., 2015).

Namasivayam and Chitrakala (2011) observed that silver nanoparticles have non-toxic or poor phyto and ecotoxic effect on plant growth, seedling emergence, soil properties, microflora and fauna in the phyllosphere. This study result showed that EC_{50} of mycelial growth reduction after 3 to 15 days of incubation of *M. grisea* conidia ranges from 227.0 to 46415888.3 ppm of silver nanoparticles and the concentration of trooper was ranging from 105.4 to 120.7 ppm. The higher EC_{50} value indicates that suppression power of silver nanoparticles on mycelial growth was reduced very quickly in a short period of time which might be the indication of lower toxicity of silver nanoparticles in rice. Jo et al. (2009) reported that antifungal efficacy of silver compounds like silver nitrate or silver nanoparticles is notably influenced by sodium chloride. Silver cations reacted with chloride ions and form silver chloride which has less antifungal efficacy than silver ion. Trooper performed steadily after 15 days of incubation. However, the fate of nanoparticles in plant and ecosystem are largely unknown. Many studies reported that nanoparticles promote plant growth and improve soil health with a focus on using at lower concentration but owing to limited research and documentation it can be argued that it may cause insignificant damage on human health and environment (Colvin, 2003).

Further research should focus on the development of silver compounds from natural sources. This is named green technology because in this method nanoparticles are synthesized by using biological entities which are part of the nature such as fungi (Hemath et al., 2010), bacteria (Navazi et al., 2010), different plant parts, are completely nonhazardous and ecologically sound compared to chemical method. This method consumed very little energy and was inexpensive as it uses plant-based products or microorganisms and operated under normal temperature and pressure (Bhat et al., 2013; Nazeruddin et al., 2014). Synthesis of silver nanoparticles from plants can be the deliverance way from the hidden risk of bioavailability of nanoparticles as this method was recognized to be stable and non-hazardous than other synthesis methods (Baker et al., 2013). It is proved that natural extracts act as reducing agents in the time of synthesis and plays a pivotal role in influencing the morphology of synthesized nanoparticles (Mukunthan &

Balaji, 2012) because naturally different plant parts of different species have different biochemical reducing agents (Li et al., 2011). That is how green technology or synthesis of nanoparticles become friendly to environment. It is very important to assess the efficacy of silver nanoparticles in controlling neck blast disease in the field and impact of silver on the environment and food chain.

5. Conclusion

Rice is the most important cereal crop in South-East Asia and fulfills the food requirement of large parts of the world's population. Every year a huge yield loss occurs due to fungal diseases and rice blast is one of them. A systemic fungicide from the tricyclazole group has been widely used to control rice blast disease and is considered as a hazardous fungicide by the World Health Organization (WHO, 2004). Indiscriminate use of fungicide promotes the risk of ecological imbalance, environmental pollution, pathogen resurgence or development of resistant pathotypes. Silver nanoparticles have great potentiality in controlling *M. grisea* than other nanoparticles. Silver might have less or poor toxicity to animals and human beings and development of resistance might not possible owing to multiple modes of action (Jo et al., 2009). However, nanotechnology opens up the new era in improving existing crop protection techniques, metal-based nanomaterials might be questionable due to long term effect on the environment that is still not fully understood. Many studies suggest that silver nanoparticles do not have long term effects. Nanoparticles prepared from natural sources can be the best solution for escaping from toxicity. Biodegradable, non-toxic and chitosan-based nanoparticles getting preference worldwide (Saharan et. al., 2013). Further research is needed in field application of silver nanoparticles against neck blast disease and impact on environment and human health.

References

Akand, R. S., (2018). Gazipur city polls: Four candidates warned for violating code of conduct. Dhaka Tribune, p. D_2.

Alam, M. S. and Islam, M. A. (2013). Rethinking the measurement of food security: from first principles to best practice. *Food security,* 5(3), 327–343.

Altieri, M. A. (1994). *Biodiversity and pest management in agroecosystems*. New York, NY: Haworth Press.

Amkamwar, B., Damle, C., Ahmad, A. and Satry, M. (2005). Biosynthesis of gold and Ag nanoparticles using *Emblica officinalis* fruits extract, their phase transfer and transmetallation in an organic solution. *Journal of Nanoscience and Nanotechnology,* 5, 1665–1671. doi: 10.1166/jnn.2005.184.

Ankamwar, B., Chaudhary, M. and Sastry, M. (2005). Gold nanotriangles biologically synthesized using tamarind leaf extract and potential application in vapor sensing. *Synthesis and Reactivity in Inorganic Metal-Organic and Nano Metal Chemistry*, 35(1), 19-26. doi.org/10.1081/SIM-200047527.

Baker, S., Rakshith, D., Kavitha, K. S., Santosh, P., Kavitha, H. U. and Rao, Y. (2013). Plants: emerging as nanofactories towards facile route in synthesis of nanoparticles. *Bio Impacts*, 3, 111-117.

Bangladesh Bureau of Statistics (2011). Population census. Ministry of Planning, Government of the People's Republic of Bangladesh.

Bangladesh Finance Bureau (2014). Agricultural Statistics. Ministry of Agriculture, Government of the People's Republic of Bangladesh.

Bangladesh Finance Bureau. (2017). Agricultural Statistics. Ministry of Agriculture, Government of the People's Republic of Bangladesh. Retrieved from http://bbs.portal.gov.bd/sites/default/files/files/bbs.portal.gov.bd/page/1b1eb817_9325_4354_a756_3d18412203e2/Yearbook-2016-Final-19-06-2017.pdf

Bangladesh Rice Research Institute (2017). Annual research review report for 2016-17, 335p. Gazipur 1701, Bangladesh.

Barksdale, T. H., and Asai, G. N. (1965). Minimum values of dew period and temperature required for infection by *Pyricularia oryzae*. *Phytopathology*, 55, 503.

Barman, S. R. and Chattoo, B. B. (2005). Rice blast fungus sequenced. *Current science*, 89(6), 930-931.

Batsmanova, L. M., Gonchar, L. M., Taran, N. Y. and Okanenko, A. A. (2013). Using a colloidal solution of metal nanoparticles as micronutrient fertilizer for cereals. *Proceedings of the International Conference Nanomaterials*, 2(4), 2.

Berger, T. J., Spadaro, J. A., Chapin, S. E., and Becker, R. O. (1976). Electrically generated silver ions: Quantitative effects on bacterial and mammalian cells. *Antimicrobial Agents Chemotherapy,* 9,357-358.

Bernard, H. R. (2006). *Research methods in anthropology, qualitative and quantitative approaches* (4th ed.). UK: Altamiara Press.

Bhat, R., Ganachari, S., Deshpande, R., Ravindra, G. and Venkataraman, A. (2013). Rapid biosynthesis of silver nanoparticles using areca nut Areca catechu extract under microwave assistance. *Journal of Cluster Science*, 24, 107-114.

Bonman, J. M. (1992). Blast. In: R. K. Webster and P. S. Gunnell (Eds.), *Compendium of Rice Diseases* (pp. 14-17). Saint Paul, USA, MN: American Phyto pathological Society Press.

Chandran, S. P., Chaudhary, M., Pasricha, R., Ahmad, A. and Sastry, M. (2006). Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract. *Biotechnology Progress.* 22(2), 577-83.

Chitra K, and G. Annadurai (2013). Antimicrobial activity of wet chemically engineered spherical shaped ZnO nanoparticles on food borne pathogen. *International Food Research. Journal*, 20(1), 59-64.

Colvin, V. L. (2003). The potential environmental impact of engineered nanomaterials. *Nature of Biotechnology*, 21, 1166-1170.

Elamawi, R. M. and Al-Harbi, R. E. (2014). Effect of biosynthesized silver nanoparticles on *Fusarium oxysporum* fungus the cause of seed rot disease of faba bean, tomato and barley. *Journal of Plant Protection and Pathology*, 5 (2), 225 – 237.

Elamawi, R. M., Bassiouni, S. M, Elkhoby, W. M. and Zayed, B. A. (2016). Effect of zinc oxide nanoparticles on brown spot disease and rice productivity under saline soil. *Journal of Plant Protection and Pathology,* 7 (3), 171– 181.

Elamawi, R. M., Rabab M. A. and EL-Shafey R. A. S. (2013). Inhibition effect of silver nanoparticles against rice blast disease caused by *Magnaporthe grisea*. *Egyptian Journal of Agricultural Research,* 91(4).

Elumalai, K., Velmurugan, S., Ravi, S., Kathiravan, V., Ashokkumar, S. (2015). Green synthesis of zinc oxide nanoparticles using Moringa oleifera leaf extract and evaluation of its antimicrobial activity. *Spectrochimica acta. Part A, Molecular and biomolecular spectroscopy*, 158–164.

Finney, D.J., (1952). *Probit Analysis*. Cambridg: Cambridge University Press.

Foldbjerg, R., Jiang, X., Miclaus, T., Chen, C., Autrup, H. and Beer, C. (2015). Silver nanoparticles – wolves in sheep's clothing? *Toxicology Research,* 3. doi: 10.1039/C4TX00110A

Ghormade, V., Deshpande, M. V. and Paknikar, K. M. (2011). Perspectives for nano-biotechnology enabled protection and nutrition of plants. *Biotechnology Advances*, 29, 792-803.

Gliessman, S.R. (2007). *Agroecology: the ecology of sustainable food systems.* New York, NY: CRC Press.

He L., Liu, Y., Mustapha, A. and Lin, M. (2011). Antifungal activity of zinc oxide nanoparticles against *Botrytis cinerea* and *Penicillium expansum. Microbiological Research*, 166, 207-215.

Hemath, N. K., Kumar, G., Karthik, L. and Bhaskara R. K. (2010). Extracellular biosynthesis of silver nanoparticles using the filamentous fungus Penicillium sp. *Archives of Applied Science Research*, 2, 161-167.

Hossain, M., Ali, M. A. and Hossain, M. D. (2017). Occurrence of blast disease in rice in Bangladesh. *American Journal of Agricultural Science,* 4 (4), 74-80.

Hossain, M., Bose, M. L., and Mustafi B. A. A. (2006). Adoption and productivity impact of modern rice varieties in Bangladesh. *The Developing Economics,* 44(2), 149–166.

Huang, J., Li, Q., Sun, D., Lu, Y., Su, Y., Yang, X., Wang, H., Wang, Y., Sha, W., He, N., Hong, J. and Chen, C. (2007). Biosynthesis of silver and gold nanoparticles by novel sun dried *Cinnamomum camphora* leaf. Nanotechnology, 18, doi.org/10.1088/0957-4484/18/10/105104

Hwang, B. K, Koh, Y. J., and Chung, H. S. (1987). Effects of adult-plant resistance on blast severity and yield of rice. *Plant Disease Journal*, 71, 1035–1038.

International Rice Research Institute (2012). *Standard Evaluation System for Rice*. Manila Philippines: IRRI press.

Jo, Y. K., Kim, B. H., and Jung, G. (2009). Antifungal activity of silver ions and nanoparticles on phytopathogenic fungi. Plant Disease, 93, 1037-1043.

Judy J. D., Kirby K. K., Creamer, C., Mclaughlin, M. J., Fiebiger, C., Wright, C., Cavagnaro, T. R. and Bertsch, P. M. (2015). Effects of silver sulfide nanomaterials on mycorrhizal colonization on tomato plants and soil microbial community bio-solid-amended soil. *Environmental Pollution*, 206, 256-263.

Karmokar, P. K. And Imon, A. H. M. R. (2008). Trend analysis of the production of rice in Bangladesh. *International Journal of Statistical Science*, 8, 103–110.

Khan, M. A. I., Ali, M. A., Monsur, M. A., Kawasaki-Tanaka, A., Hayashi, N., Yanagihara, S., Obara, M., Mia, M. A. T., Latif, M. A. and Fukuta, Y. (2016). Diversity and distribution of rice blast (*Pyricularia oryzae* Cavara) races in Bangladesh. *Plant Disease*, 100(10), 2025-2033.

Khan, M. A. I., Sen, P. P., Bhuiyan, R., Kabir, E., Chowdhury, A. K., Fukuta, Y., Ali, A. and Latif, M. A. (2014). Phenotypic screening and molecular analysis of blast resistance in fragrant rice for marker assisted selection. *Comptes Rendus Biologies*, 337, 318–324.

Kim, C. K. (1994). Blast management in high input, high yield potential, temperate rice ecosystems. In: Zeigler, R. S. and Leong, S. A. (eds.), *Rice Blast Disease*. Wallingford, UK: CAB International.

Lamsal, K., Kim, S. W., Jung, J. H., Kim, Y. S., Kim, K. S., and Lee, Y. S. (2011). Inhibition effects of silver nanoparticles against powdery mildews on cucumber and pumpkin. *Mycobiology,* 39(1), 26-32. doi: 10.4489/MYCO.2011.39.1.026

Latterell, F. M., Tullis, E. C., Otten, R. J. and Gubernick, A. (1954). Physiologic races of *Pyricularia oryzae*. *Phytopathology,* 44, 495-496.

Li, X., Xu, H., Chen, Z. S. and Chen, G. (2011). Biosynthesis of nanoparticles by microorganisms and their applications. *Journal of Nanomaterials*, 270-274.

Luo, J., Wang, L.; Mott, D., Njoki, P. N., Lin, Y., He, T., Xu, Z., Wanjana, B. N., Lim, I.-Im S. and Zhong, C. J. (2008). Core/Shell nanoparticles as electrocatalysts for fuel cell reaction. *Advanced materials*, 20(22), 4342. doi.org/10.1002/adma.200890107.

Mbodi, Y., Gaye, S., and Diaw, S. (1987). The role of tricyclazole in rice protection against blast and cultivar improvement. *Parasitica*, 43, 187-198.

Min, J. S., Kim, K. S., Kim, S. W., Jung, J. H., Lamsal, K., Kim, S. B., Jung, M. and Lee Y. S. (2005). Effects of colloidal silver nanoparticles on sclerotium-forming phytopathogenic fungi. *The Plant Pathology Journal*, 25(4), 376-380.

Mishra, S., Singh, B. R., Singh, A., Keswani, C., Naqvi, A. H., Singh, H. B. (2014). Biofabricated silver nanoparticles act as a strong fungicide against Bipolaris sorokiniana causing spot blotch disease in wheat. *PLOS one*, doi.org/10. 1371/journal.pone.0097881.

Morones, J. R., Elechiguerra, J. L., Camacho, A., Holt, K., Kouri, J. B., Ramirez, J. T., and Yacaman, M. J. (2005). The bactericidal effect of silver nanoparticles. *Nanotechnology*, 16, 2346-2353. doi :10.1088/0957-4484/16/10/059.

Mukunthan, K. and Balaji, S. (2012). Cashew apple juice (Anacardium occidentale L.) speeds up the synthesis of silver nanoparticles. *International Journal of Green Nanotechnology*, 4, 71-79.

Naidu, V. D., and Reddy, G. V. (1989). Control of blast (BI) in main field and nursery with some new fungicides. *International Rice Research Newsletter*, 14(4), 35-36.

Namasivayam, S. K. R. and Chitrakala, K. (2011). Ecotoxicological effect of Lecanicillium lecanii (Ascomycota: Hypocreales) based silver nanoparticles on growth parameters of economically important plants. *Journal of Biopesticide*, 4, 97-101.

Navazi, Z. R., Pazouki, M. and Halek, F. S. (2010). Investigation of culture conditions for biosynthesis of silver nanoparticles using Aspergillus fumigates. *Iran Journal of Biotechnology*, 8, 56-60.

Nazeruddin, G., Prasad, N., Prasad, S., Shaikh, Y., Waghmare, S. and Adhyapak, P. (2014). Coriandrum sativum seed extract assisted in situ green synthesis of silver nanoparticle and its anti-microbial activity. *Industrial Crops and Products*, 60, 212-216.

Ou, S. H. (1980). Pathogen variability and host resistance of the rice blast fungus, *Pyricularia oryzae. Phytopathology*, 18, 167-187.

Ou, S. H. (1985). *Rice Diseases* (2nd ed.). Kew, UK: CAB publications.

Phogat, Khan, N. S. A, Shankar, S., Ansary, A. A. and Uddin, I. (2016). Fate of inorganic nanoparticles in agriculture. *Advanced Materials Letters,* 7(1), 03-12.

Raad, I. I., and Hanna, H. A. (2002). Intravascular catheter-related infections: New horizons and recent advances. *Archives of International Medicine,* 162, 871-878.

Saharan, V., Mehrotraa, A., Khatik, R., Rawal, P., Sharma, S. S. and Pal, A. (2013). Synthesis of chitosan-based nanoparticles and their in vivo evaluation against phytopathogenic fungi. International Journal of Biological Macromolecules, 62, 677-683.

Samuel, U. and Guggenbichler J. P. (2004). Prevention of catheter related infections: the potential of a new nano-silver impregnated catheter. *International Journal of Antimicrobial Agents*, 23(1), 75–78.

Schlagenhauf, L., Nüesch, F. and Wang, J. (2014). Release of carbon nanotubes from polymer nanocomposites. *Fibers, 2*(2), 108-127. doi.org/10.3390/fib2020108.

Schlich, K. and Hund-Rinke, K. (2015). Influence of soil properties on the effect of silver nanomaterials on microbial activity in five soil. *Environmental Pollution*, 196, 323-333.

Sharon, M., Chowdhary, A. and Kumar, R. (2010). Nano technology in agricultural disease and food safety. *Journal of Phytopathology*, 2(4), 83-92.

Shelley, I. J., Nosaka, M. T., Nakata, M. K., Haque, M. S., and Inukai, Y. (2016). Rice cultivation in Bangladesh: present scenario, problems, and prospects. *Journal of International Cooperation for Agricultural Development*, 14, 20–29.

Singh, D., Kumar, A., Singh, K. and Tripathi, H. S. (2013). Induction of resistance in field pea against rust disease through various chemicals/ micronutrients and their impact on growth and yield. *The Plant Pathology Journal*, 12, 36-49.

Stone, V., Nowack, B., Baun, A., Brink, N. V. D., Kammer, F. V. D., Dusinska, M., Handy, R., Hankin, S., Hassellöv, M., Joner, E. and Fernandes, T. F. (2010). Nanomaterials for environmental studies: classification, reference material issues, and strategies for physio-chemical characterization. *Science of the Total Environment,* 408(7), 1745. doi: 10.1016/j.scitotenv.2009.10.035

Taylor, G. R. (2005) *Integrating quantitative and qualitative methods in research*. USA: University Press of America.

Tomacheski, D., Pittol, M., Simões, D. N., Ribeiro, V. F., and Santana, R. M. C. (2017). Impact of silver ions and silver nanoparticles on the plant growth and soil microorganisms. *Global Journal Environmental Science Management,* 3(4), 341-350. doi: 10.22034/gjesm.2017.03.04.001.

Tucker, S. L., and Talbot, N. J. (2001). Surface attachment and pre-penetration stage development by plant pathogenic fungi. *Annual Review of Phytopathology,* 39, 385-417.

Wang, Z., Mackill, D. J and Bonman, J. M. (1989). Inheritance of partial resistance to blast in India rice cultivars. *Crop Science,* 29, 848-853.

Webster, R. K., and Gunnell, P. S. (Eds.). (1992). *Compendium of Rice Diseases*. St. Paul, MN: American Phyto pathological Society.

World Health Organization (2004). The WHO recommended classification of pesticides by hazards and guideline to classification.

Xue, J., Luo, Z., Li, P., Ding, Y., Cui, Y. and Wu, Q. (2014). A residue-free green synergistic antifungal nanotechnology for pesticide thiram by ZnO nanoparticles. *Scientific Reports*, 4, 5408. DOI: 10.1038/srep05408.

Yang, I., Wang, J., Xiu, Z. and Alverez, P. J. J. (2013). Impacts of silver nanoparticles on cellular and transcriptional activity of nitrogen-cycling bacteria. *Environmental Toxicology and Chemistry*, 32(7), 1488-1494.

Young, K. J., Byung, H. Kim and Jung G. (2009). Antifungal activity of silver ions and nanoparticles on phytopathogenic fungi. *Plant Disease,* 1037-1043.

Zhu, Y. Y., Fang, H., Wang, Y. Y., Fan, J.X., Yang, S. S., Mew, T. M. and Mundt, C. C. (2005). Panicle blast and canopy moisture in rice cultivar mixtures. *Phytopathology*, 95, 433–438.

Appendix

Efficacy of silver nanoparticles against rice blast disease (c.o. *Magnaporthe grisea*) and farmers' perception about rice blast disease management in Bangladesh

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Abstract

Rice blast caused by *Magnaporthe grisea* is one of the major and recurrent threats for sustainable rice production in Bangladesh. To mitigate this problem, the current study was aimed to investigate the efficacy of silver nanoparticles against rice blast disease and farmers' perception or knowledge about rice blast disease management in Bangladesh. This study was done into two parts: firstly, face to face interviews to understand the farmers' perception as well as to find out their needs and secondly laboratory experimentation to find out the efficacy of silver nanoparticles against *M. grisea*. Close-ended questions were prepared for the interview and thirty farmers were interviewed. Farmers' interviews indicated that 73% of the respondent farmers had experienced blast disease in their field but only 30% of the respondent farmers were aware of rice blast disease and its management. Among the respondent, 89% of the farmers used chemicals in controlling rice blast disease but only 9% of the farmers had an opinion that spraying chemicals worked very well and 21% thought this method worked satisfactorily. Around 59% of respondents maintained close contact with extension workers and adopted new technologies according to their suggestions. *In vitro* assays indicated that silver nanoparticles had a significant inhibitory effect on the mycelial growth of rice blast pathogen. Effective concentration of the silver nanoparticles inhibiting mycelial growth by 50% (EC_{50}) up to 9 days after incubation was 308.1 ppm. However, the inhibitory effect on mycelial growth significantly diminished at 12 days of incubation. To measure leaf blast disease severity, three concentrations (200, 400 and 800 ppm) of silver nanoparticles and a popularly used blast control fungicide Trooper 75WP, were sprayed on rice seedlings that were 20-25 days old, 3 days before inoculation for preventive and 3 days after inoculation, with spore suspension (10^5 conidia/ml+0.01% Tween 20) for curative measure. Only 1.77% and 3.23% leaf blast disease severity were found in preventive measure at 800 ppm concentration for Trooper 75 WP (standard dose of fungicide) and silver nanoparticles, respectively. Whereas untreated control plot exhibited more than 70.0% disease severity. In greenhouse assays, silver nanoparticles were highly effective in preventative application rather than curative application.

Key words: rice, blast disease, management, mycelial growth, silver nanoparticles.

Introduction

The economy of Bangladesh is basically agrarian with the dominance of rice (*Oryza sativa* L.) crop which considered as a staple food crop in Bangladesh. Bangladesh has a total 14.86 million ha of land and cultivable land is 8.52 million ha with a cropping intensity of 191%, approximately 80 percent of the total arable land is occupied by rice production in various agroecological zones (Shelley et al., 2016). In Bangladesh, 19.3% of the gross domestic product (GDP) of the country is achieved from agricultural production. Employment figures show 47.5% of the total manpower is related to agriculture (Bangladesh Finance Bureau, 2014). In the year 2016–2017, rice production was 33.8 million ton (Bangladesh Finance Bureau, 2017). After achieving self-sufficiency in rice production, Bangladesh has begun export rice in recent years. However, various diseases are considered as a major obstacle of rice production for high yielding varieties as well as for national food security. Rice plants infected by ten major fungal, bacterial and viral diseases in Bangladesh and blast disease caused by the fungus *Pyricularia grisea* [anamorph of *Magnaporthe grisea*], is considered as one of the most detrimental rice diseases worldwide (Ou, 1985). Rice blast disease can occur most of the parts of the plant except root and infection occurs from airborne conidia. Symptoms appear as lesion or spots; size, shape and color depend on pathotype, varietal resistance, stage of the lesions and environmental conditions (Ou 1985). Blast is the most common and devastating disease in irrigated rice of South-East Asia, especially that of aromatic rice (Khan et al., 2014). But in recent years, blast pathogen invasion has been reported in new rice cultivars in Bangladesh which were initially considered less susceptible. The blast disease causes yield loss of 11% and 46.4% under low and medium disease incidence in Bangladesh (Khan et al., 2014).

Bangladeshi farmers are now mostly using fungicides to control the disease, but fungicides have long-term profound and often negative impacts on environment as well as on human health and also increase the cost of production. Pesticides arbitrarily kill insect-pests along with natural enemies which are essential to the food web and natural ecosystem which might develop pesticide resistant insect-pest or might cause pest resurgence. It is high time to replace resource intensive and environment degrading inputs with more ecologically sound products and practices. Nanotechnology can be the new key to agricultural improvement by combating plant disease as nanoparticles have been found inhibitory effect to fungi (Singh et al., 2013). Nanoparticles can be defined as “the particles having at least one or two dimensions in the range of 1 to 100 nm.” (Foldbjerg et al., 2015; Stone et al., 2010). Silver nanoparticles are the most studied and effective nanoparticles and can have strong bactericidal and fungicidal inhibitory effects. Silver nanoparticles have high antimicrobial effects due to their large surface area and high fraction of surface atoms (Sharon et al., 2010). Therefore, they are highly reactive as they can produce Ag^{+} ions compared to metallic or bulk silver (Morones et al., 2005). Nanoparticles are applied at low concentrations to control microbial activity since the large surface areas increase the opportunity for microbial contact and enhances their ability to penetrate into microbial cells (Lamsal et al., 2011).

Nanotechnology holds promising potentiality in the agriculture sector and massive or long-term application of nanoparticles might cause negative impact on environment as nanoparticle-based pollutant. Risk assessment of nanoparticles should be evaluated after exposure of nanoparticles to any organism or environment. Xue et al., (2014) reported, a residue-free technology with nanoparticles can synergistically increase pesticide efficacy and could control the plant disease rationally along with eliminating its residue successfully; therefore, benefit the society by reducing the pesticidal impact on human health and environment. Nanotechnology has the potential benefit in global food production, food processing and food security but more study needed to realize long term effect of nanoparticles on agriculture and environment (Phogat et al., 2016).

Materials and method

Study area

The study was carried out in the laboratory and greenhouse at the plant pathology division of Bangladesh Rice Research Institute (BRRI), Bangladesh. Interview of farmers on their perception of rice disease management took place in Gazipur district in Bangladesh.

Farmers’ interview

As almost all the farmers of Bangladesh are associated with rice production, among them 30 farmers were chosen randomly for interview in Gazipur district. Semi structured close-ended questionnaire was prepared as a tool for the face-to-face interview. Each respondent was asked the same questions and interview started with a soft conversation to establish a good rapport between interviewer and interviewee. Respondents were very co-operative and sometimes they asked questions about related topic. A cordial atmosphere was maintained by giving them satisfactory answers about their quarry throughout the interview. In a face-to-face interview, interviewer and interviewee can feel a connection, if an interviewee doesn't understand a question, the interviewer can explain it or if the respondent does not answer fully, the interviewer can probe for more information or what she/he desires.

Silver nanoparticles and fungicides

Silver nanoparticles were obtained from the applied chemistry and chemical engineering department, Dhaka University. A particle size of below 100 nm was used in this experiment. To prepare the silver nanoparticles, silver nano-powder was weighed and suspended in deionized sterile water using a sonicator. Silver nanoparticles of different concentrations (200, 400 and 800 ppm) were prepared by diluting the original stock solution using sterile deionized water and solutions were preserved at 4^oC to maintain the quality. A commercial formulation of tricyclazole group fungicide (Trooper 75 WP) for rice blast disease management marketed by Auto Crop Care Bangladesh Ltd. was used as a standard disease control treatment and thus served as a control, to allow us to evaluate how well silver nanoparticles performed compared to the standard synthetic fungicide.

Fungus preparation

A virulent blast isolate (BD576p) which was selected as differential blast isolate was used as test isolate. A differential isolate is an isolate that was selected for evaluation of blast resistant materials for Bangladesh conditions (Khan et al. 2016). This isolate was collected from the Plant Pathology Division, Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh.

Observation of both hyphal growth and sporulation in the presence of silver nanoparticles

The inhibitory effect of nanoparticles on fungal growth was examined *in vitro* by measuring hyphal growth and sporulation. To measure hyphal growth, pure cultures of *M. grisea* were produced in Potato Sucrose Agar (PSA) medium (200g potato extract, 20g sucrose and 20g agar dissolved in 1000 ml water and autoclaved). PSA media supplemented with different concentrations (200, 400 and 800 ppm) of nano materials (Si, Cu, Zn and Ag) were prepared to measure hyphal growth and three replications were done. From pure cultures, actively growing edge (6 mm in diameter) was obtained and transferred into each plate of both control and nanoparticles treated plates. Plates inoculated with *M. grisea* were incubated at 28^oC for 15 days. After colony formation, the radial growth of each culture was measured at every 72 hours interval either in control or nanoparticle treated plates to assess if there was an inhibitory effect on hyphal growth.

Greenhouse assays

The inhibitory effect of silver nanoparticles against rice blast disease under controlled conditions was measured at the Plant Pathology Division, Bangladesh Rice Research Institute, Bangladesh. Rice cultivar US-2 was selected as test cultivar, since it is the most susceptible to rice blast disease. Four trays for each treatment were randomly arranged following CRD design. Three concentrations (200, 400 and 800 ppm) of silver nanoparticle suspension and commercial fungicide Trooper 75 WP were sprayed on 20-25 days old rice seedlings 3 days before inoculation with spore suspension (10^5 conidia/ml 0.01% aqueous solution of Tween 20) for preventive measure and 3 days after inoculation with spore suspension (10^5 conidia/ml 0.01% aqueous solution of Tween 20) for curative measure. The inoculated plants were kept in controlled conditions. The inoculated seedlings were incubated in a moist chamber with relative humidity at least 90% and 26 ± 2^o C for 24 hours and then the inoculated plants were kept in controlled conditions in the greenhouse. The reaction was scored at seven to ten days after inoculation using 0-9 scale of Standard Evaluation System (SES) of International Rice Research Institute (IRRI, 2012).

Results

Incidence of rice blast disease and awareness of the farmers'

Table 1 showed that, in Boro season high neck blast infection occurred due to favorable environment for *M. grisea*. In Aman season medium neck and node blast symptoms were observed. There was no blast infection in Aus season (dry season); less favorable environmental conditions may be one of the reasons.

Table 1: Ecosystem-wise blast disease incidence in various rice fields of Bangladesh

Ecosystem/Season	Rate of blast disease incidence (%)		
	Leaf blast	Node blast	Neck blast
Boro (Irrigated lowland)	+	-	+++
T. Aman (Rainfed lowland)	-	++[1]	++[1]
Aus (Rainfed upland)	-	-	-

Source: BRRI, 2017

Note: [1] = mostly aromatic rice infected severely by blast during T. Aman season

- = no blast infection

+ = *low blast infection*
++ = *medium blast infection*
+++ = *high blast infection*

Though rice blast is one of the major diseases in Bangladesh 27% of the respondent farmers said they had not experienced blast disease in their rice field. Selection of variety, planting time and cultural practices might be a reason for this. Majority respondents (73%) had experienced rice blast disease more or less in their fields (Figure 1). Among them only 50% of respondents were aware about rice blast disease, 30% were aware about both rice blast disease and its management and 20% of the farmers were not aware about rice blast disease and its management (Figure 2).

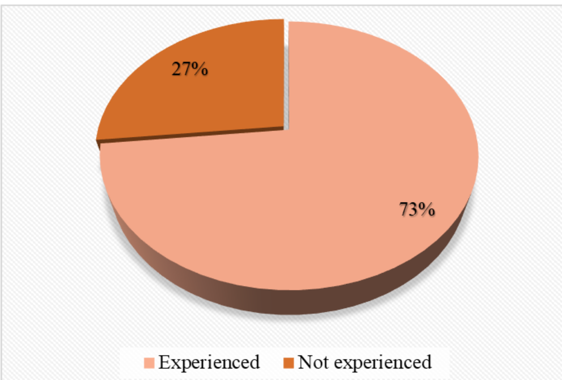


Figure 1: Practical experience on rice blast disease among the respondent farmers (n=30)

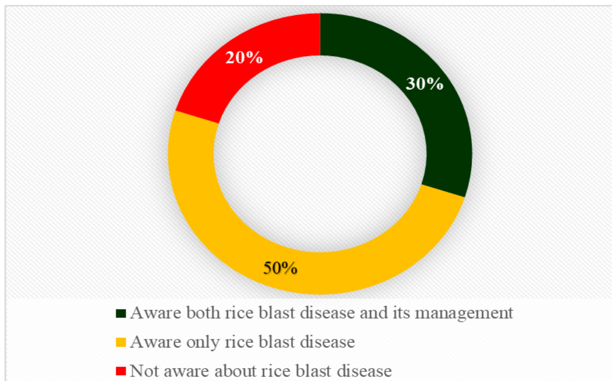


Figure 2: Awareness of rice blast disease of the respondent farmers (n=30)

Methods adapted by the Farmers

Respondents were asked a range of different methods that could be helpful in controlling blast disease partially or successfully. Among them chemical spray (89%), split applications of nitrogenous fertilizer (5%), crop rotation (3%) and burning diseased-straw and stubble (1%) were commonly used methods (Table 4). Abandon fields, planting resistant cultivars, applications of compost, or changing planting time were strategies that were adopted by less than 1% of questioned farmers (Table 2).

Table 2: Percent usage of various rice blast control methods

Methods of controlling Blast	Percentage (%)
Burning diseased-straw and stubble	1
Plant resistant variety	0
Changing planting time	0
Apply chemicals	89
Apply compost	0
Crop rotation	3
Abandon field	0
Split applications of nitrogenous fertilizer	5

Avoid use of fertilizer	0
Excess use of fertilizer	0
Others (Not using any control method)	2

Farmers' perception towards fungicide and source of advice

Around 89% of the respondent farmers used chemicals in controlling rice blast disease but among them only 9% of the farmers had opinion that spraying chemicals worked very well and 21% thought this method worked satisfactorily. Majority of the respondents (53%) thought this method worked but not very well, they wanted some other more effective methods than chemical control. Synthetic pesticide efficacy is crucial, but dependent on the perfect timing before disease development. Around 14% of the respondent farmers said that spraying chemicals did not work well and 3% had no opinion about this management system (Figure 3).

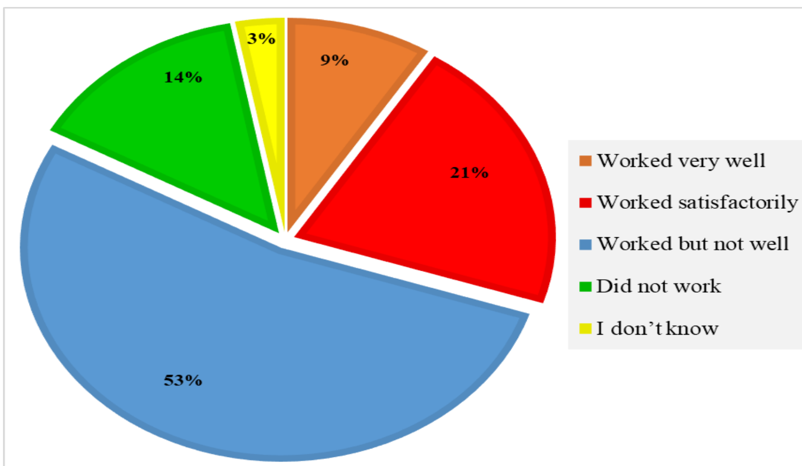


Figure 3: Farmers perception on using chemicals to control rice blast disease (n=30)

Most farmers participated in this survey obtained rice production related knowledge like new technologies, disease and pest management strategies either from extension workers (59%), fellow farmers (20%) and/or from training or workshop (13%). A small group of farmers received information through visiting or organizing field days with researchers of BRRI (5%) and from the local leaders (2%) (Table 3).

Table 3: Farmers source of advice on the appropriate method of rice blast disease management

Sources of advice	Percentage
Fellow farmer	20
Extension worker	59
Training or workshops	13

Local leader	2
Visiting researchers (BRRI)	5
Media (Radio/TV)	0
Newspapers/leaflet	0
Other	1

Inhibition of mycelial growth with different nanoparticles

Study results confirmed that silver nanoparticles had significant inhibitory effects on hyphal growth of *M. grisea*. Silver nanoparticles and silver nitrate successfully controlled mycelial growth and showed significant differences compared with the other nanoparticles. Only silver nanoparticles at 800 ppm showed a significant difference compared with the other silver nanoparticles and silver nitrate. (Table 4).

Table 4: Comparison studies among different nano-particles on the mycelial growth of blast pathogen (*M. grisea*)

Treatments	Concentration(ppm)	Mycelial growth (mm)
Si nanoparticle	200	64.6 bcd*
Si nanoparticle	400	76.4 ab
Si nanoparticle	800	69.4 abc
Cu nanoparticle	200	55.4 d
Cu nanoparticle	400	59.9 cd
Cu nanoparticle	800	60.1 cd
Zn nanoparticle	200	67.3 abc
Zn nanoparticle	400	65.1 bcd
Zn nanoparticle	800	78.7 a
Ag nanoparticle	200	28.7 ef
Ag nanoparticle	400	17.2 fg
Ag nanoparticle	800	14.8 g
$AgNO_3$	200	33.2 e
$AgNO_3$	400	24.8 efg
$AgNO_3$	800	17.7 fg
Control	-	70.9 abc

**letters indicating significance of differences*

3.2.3 Assessment of LC_{50}

Table 5 shows that leaf blast disease severity was reduced by 50% at 136.5 ppm concentration of silver nanoparticle before inoculation where commercial formulation of tricyclazole group fungicide Trooper 75WP showed 50% leaf blast disease reduction at 175.6 ppm concentration before inoculation. Trooper 75WP showed 50% leaf blast disease reduction at 166.3 ppm after inoculation but silver showed the same at higher concentration than Trooper 75WP (226.2 ppm).

Table 5: Effective concentration of silver nano particles (AgP) to cause 50% leaf blast disease reduction (LC_{50})[a]

Treatment	Concentration of LC_{50} (ppm)	
	Preventive[b]	Curative[c]
Silver nano (AgP)	136.5	226.2
Trooper 75WP	175.6	166.3

[a] *Dose response relationship LC_{50} was calculated based on the reduction of leaf blast disease severity over untreated control by Probit Analysis (Finney 1952).*

[b] *Chemicals (Trooper 75% and AgP) were sprayed on plants 3 days before inoculation with spore suspension (10^5 conidia/ml) for preventive measure.*

[c] *Chemicals (Trooper and AgP) were sprayed on plants 3 days after inoculation with spore suspension (10^5 conidia/ml) for curative measure.*

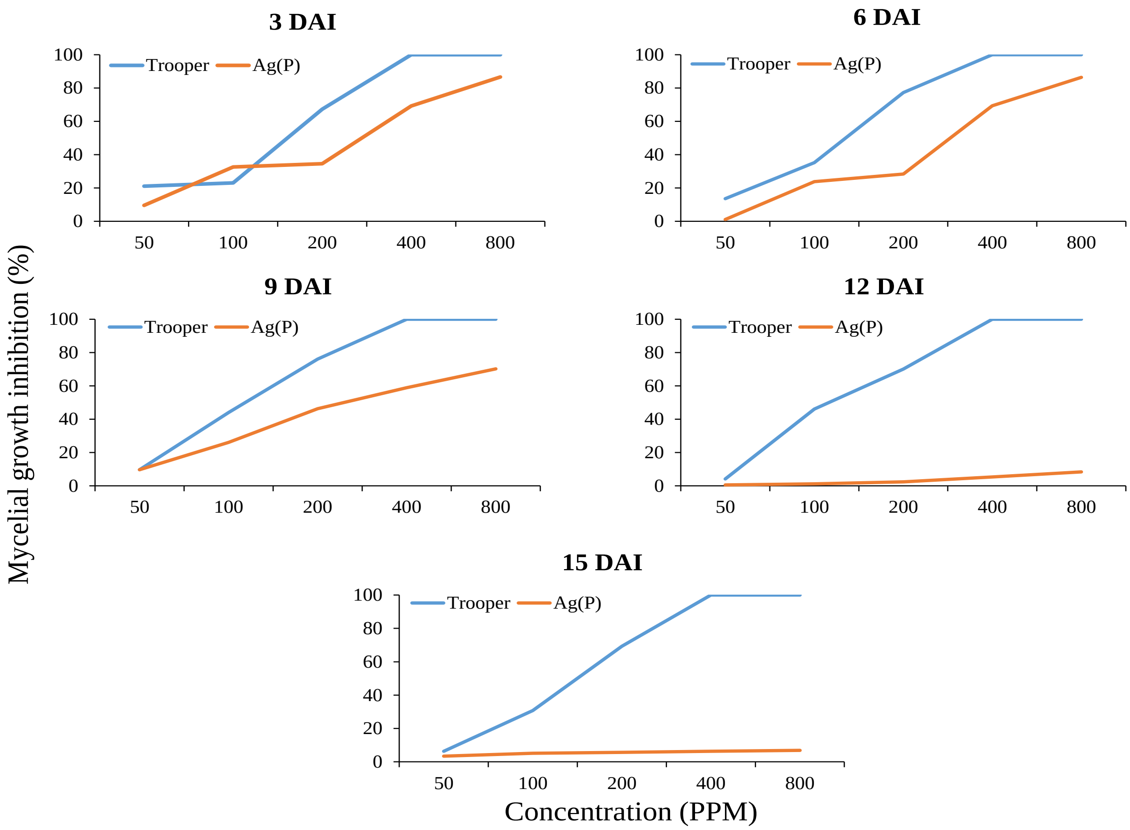
Data from three replication were averaged for each treatment and used to calculate EC_{50} value. The range of EC_{50} was calculated at 3 to 15 days after incubation on *M. grisea* conidia and the concentration of silver nanoparticle was ranges from 227.0 to 46415888.3 ppm and the concentration of trooper was ranges from 105.4 to 120.7 ppm (Table 9). Effectiveness of silver nanoparticles against mycelial growth inhibition of *M. grisea* was reduced after 9 days after incubation while Trooper performed successfully until 15 days after incubation (Figure 4).

Table 9: Effective concentration of Silver nano particles (AgP) and Trooper 75WP causes 50% mycelial growth reduction (LC_{50})[a] of *M. grisea*

Treatment	Concentration of EC_{50} (ppm)				
	3 DAI[b]	6 DAI	9 DAI	12 DAI	15 DAI
Silver nano (AgP)	227.0	285.5	301.8	42751.6	46415888.3
Trooper 75%	111.8	105.4	106.6	118.1	120.7

[a] *Dose response relationship LC_{50} was calculated based on the reduction of mycelial growth over untreated control by Probit Analysis (Finney 1952).*

[b] *DAI=Days after incubation.*



DAI*=Days after incubation

Figure 4: Mycelial growth inhibition of *Magnaporthe grisea* affected by different concentrations of Silver nano particle [Ag(P)] and Trooper over the time

Discussion

In Bangladesh, rice is affected by various fungal, bacterial, viral and nemic diseases. Among them, top rice diseases are fungal diseases and cause significant yield loss. Naturally, Bangladesh is a warm humid country, for that reason crop suffers from several fungal diseases. Fungal disease management is economically important for sustainable yield and approximately 73% respondent rice farmers experienced blast disease in this study. Management of blast disease is very crucial. Farmers should observe field frequently especially, when favorable climatic condition to disease development occurred either disease appeared in the field or not farmer should take preventive measure. Neck blast visible to naked eye when severe infestation had occurred during the time of harvesting. Once disease visible by naked eye that means it is already late to secure yield. Among the respondent farmers 20% of the farmers were not aware of rice blast disease and its management. The reason behind these might be farmers were either ignorant about blast disease or could not identify the symptoms as it is very crucial to identify blast disease at early stage in the field. Perhaps they were unconsciously practice some cultural practices like earlier the planting time, using partially resistant variety etc.

In the recent world, it is a demand of time to develop a safe and ecologically sound management method which is economically feasible. The major challenge in the management of rice blast disease in Bangladesh is most of the farmers prefer to grow susceptible or partially susceptible rice varieties due to market value. People of Bangladesh prefer non-sticky medium slender to medium bold grain rice. Farmers are growing different varieties according to their preferences. Farmers mostly considered the variety that gives high yield and fetches good market prices than disease resistance and for that reason, BRRI dhan28, BRRI dhan29, BRRI dhan58 were the dominating cultivated variety despite their high susceptibility to blast disease. Disease incidence of the blast was higher in BRRI dhan47 (58.2%) followed by BRRI dhan29 (39.8%), BRRI dhan28 (20.3%) in Boro season regardless of place and cropping sequence (Hossain et al., 2017). In Boro season farmers were experienced with all types of blast diseases viz. leaf, node and neck blast due to favorable environmental conditions for pathogens. Incidence and severity of rice blast disease were recorded higher in Boro (21.19%) than transplanted Aman season (11.98%) (Hossain et al., 2017).

Durable blast resistant variety yet not developed and still a big challenge for the breeder in Bangladesh. Most of the rice farmers are using different fungicides of tricyclazole group. Around 89% of the respondents' farmer used synthetic fungicides; among them, only 9% farmers thought to spray fungicide worked very well and majority of the farmers thought fungicides worked but not satisfactorily. Study result found that application of fungicide prior infection was much effective than an already infected field. When the climate is favorable for disease formation farmers should take preventive measures to control the disease successfully. However, the adoption rate of using resistant variety and other cultural practices were very low. Majority of rice farmers were not interested in cultural practices in controlling blast disease they were found this ineffective and laborious. Most of the farmers’ found that hiring labor was expensive. Rice straw is used as a cattle feed and some places used as a building material of roofs of the houses. As Bangladesh is a densely populated country for that reason farmers find it as a luxury in abandoning the field for one season. Though Boro season is considered as most blast disease prone season, farmers cultivate rice due to higher productivity than other rice growing season.

Nanostructured materials as a pesticide can enhance the efficacy of active ingredients of pesticide and control the release in the environment will reduce the environment pollution and amount of pesticide use. Recent investigation reported that silver nanoparticles have a broad spectrum of anti-microbial properties with low toxicity. Many reports confirmed that silver ion or nanoparticle has great antifungal potentiality to control spore-producing microorganisms like *M. grisea*. Silver nanoparticles are highly reactive (Morones et al., 2005) and can penetrate efficiently into microbial cells at lower concentrations and result in microbial control (Samuel and Guggenbichler, 2004). Study results confirmed that other nanoparticles like zinc, copper, and silicon have no impact in controlling spore producing fungal plant pathogen *M. grisea* (isolates BD 576p).

Efficacy of silver nanoparticles was influenced greatly by the time of application. Leaf blast disease severity (%) for both fungicide (Trooper 75WP) and silver nanoparticles was significantly lower at 800 ppm for preventive measure. The performance of chemical fungicide (Trooper 75 WP) and silver nanoparticles were not satisfactory after inoculation of the spore. M. grisea produces asexual conidia, under favorable conditions conidia germinate and produces germ tube to penetrate in plant cells within 24 hours cause disease (Tucker and Talbot, 2001). Jo et al. (2009) reported that after inoculation of 24 hours antifungal activity of silver was reduced. Disease severity percentage of leaf blast indicates that application of 800 ppm silver nanoparticles was highly effective before inoculation (3.23%) and worked satisfactorily after inoculation (9.60%). Elamawi et al., 2013 reported that application of 100 ppm silver nanoparticles (20-30 nm) was very effective in both preventive and curative measure. The study was the first to demonstrate antifungal activity of silver nanoparticles against *M. grisea* in Bangladesh. In Bangladesh Rice Research Institute (BRRI) had no facility to measure the accurate size of the nanoparticle (below 100 nm). Nanoparticle size and pathotype of *M. grisea* might the probable cause that the study result differs from the study done by Elamawi et al. (2013).

Namasivayam and Chitrakala (2011) observed that silver nanoparticle has non-toxic or poor phyto and ecotoxic effect on plant growth, seedlings emergence, soil properties, microflora and fauna in the phyllosphere. The study result showed that EC_{50} of mycelial growth reduction after 3 to 15 days of incubation of *M. grisea* conidia ranges from 227.0 to 46415888.3 ppm of silver nanoparticles and the concentration of trooper was ranging from 105.4 to 120.7 ppm. The higher EC_{50} value indicates that suppression power of silver nanoparticles on mycelial growth was reduced very quickly in a short period of time which might be the indication of lower toxicity of silver nanoparticles in rice. Jo et al. (2009) reported that antifungal efficacy of silver compounds like silver nitrate or silver nanoparticles is notably influenced by sodium chloride. Silver cations reacted with chloride ions and form silver chloride which has less antifungal efficacy than silver ion. Trooper performed steadily after 15 days of incubation. However, the fate of nanoparticles in plant and ecosystem are largely unknown. Many studies reported that nanoparticles promote plant growth and improve soil health with a focus on using at lower concentration but owing to limited research and documentation it can be argued that it may cause insignificant damage on human health and environment (Colvin, 2003). Further research should focus on the development of silver compounds from natural sources. It is very important to assess the efficacy of silver nanoparticles in controlling neck blast disease in the field and impact of silver on the environment and food chain.

Conclusion

Nanotechnology opens up the new era in improving existing crop protection techniques, metal-based nanomaterials might be questionable due to long term effect on the environment that is still not fully understood. Many studies suggest that silver nanoparticles do not have long term effects. Nanoparticles prepared from natural sources can be the best solution for escaping from toxicity. Biodegradable, non-toxic and chitosan-based nanoparticles getting preference worldwide (Saharan et. al., 2013). Further research needed in field application of silver nanoparticle against neck blast disease and impact on environment and human health.

References

Bangladesh Finance Bureau (2014). Agricultural Statistics. Ministry of Agriculture, Government of the People's Republic of Bangladesh.

Bangladesh Finance Bureau. (2017). Agricultural Statistics. Ministry of Agriculture, Government of the People's Republic of Bangladesh. Retrieved from http://bbs.portal.gov.bd/sites/default/files/files/bbs.portal.gov.bd/page/1b1eb817_9325_4354_a756_3d18412203e2/Yearbook-2016-Final-19-06-2017.pdf

Bangladesh Rice Research Institute (2017). Annual research review report for 2016-17, 335p. Gazipur 1701, Bangladesh.

Bhat, R., Ganachari, S., Deshpande, R., Ravindra, G. and Venkataraman, A. (2013). Rapid biosynthesis of silver nanoparticles using areca nut Areca catechu extract under microwave assistance. *Journal of Cluster Science*, 24, 107-114.

Colvin, V. L. (2003). The potential environmental impact of engineered nanomaterials. *Nature of Biotechnology*, 21, 1166-1170.

Elamawi, R. M., Rabab M. A. and EL-Shafey R. A. S. (2013). Inhibition effect of silver nanoparticles against rice blast disease caused by *Magnaporthe grisea*. *Egyptian Journal of Agricultural Research,* 91(4).

Finney, D.J., (1952). *Probit Analysis*. Cambridg: Cambridge University Press.

Foldbjerg, R., Jiang, X., Miclaus, T., Chen, C., Autrup, H. and Beer, C. (2015). Silver nanoparticles – wolves in sheep's clothing? *Toxicology Research,* 3. DOI: 10.1039/C4TX00110A

Hemath, N. K., Kumar, G., Karthik, L. and Bhaskara R. K. (2010). Extracellular biosynthesis of silver nanoparticles using the filamentous fungus Penicillium sp. *Archives of Applied Science Research*, 2, 161-167.

Hossain, M., Ali, M. A. and Hossain, M. D. (2017). Occurrence of blast disease in rice in Bangladesh. *American Journal of Agricultural Science*, 4 (4), 74-80.

Jo, Y. K., Kim, B. H., and Jung, G. (2009). Antifungal activity of silver ions and nanoparticles on phytopathogenic fungi. Plant Disease, 93, 1037-1043.

Khan, M. A. I., Sen, P. P., Bhuiyan, R., Kabir, E., Chowdhury, A. K., Fukuta, Y., Ali, A. and Latif, M. A. (2014). Phenotypic screening and molecular analysis of blast resistance in fragrant rice for marker assisted selection. *Comptes Rendus Biologies*, 337, 318–324.

Khan, M. A. I., Ali, M. A., Monsur, M. A., Kawasaki-Tanaka, A., Hayashi, N., Yanagihara, S., Obara, M., Mia, M. A. T., Latif, M. A. and Fukuta, Y. (2016). Diversity and distribution of rice blast (*Pyricularia oryzae* Cavara) races in Bangladesh. *Plant Disease*, 100(10), 2025-2033.

Lamsal, K., Kim, S. W., Jung, J. H., Kim, Y. S., Kim, K. S., and Lee, Y. S. (2011). Inhibition effects of silver nanoparticles against powdery mildews on cucumber and pumpkin. *Mycobiology,* 39(1), 26-32. doi: 10.4489/MYCO.2011.39.1.026

Morones, J. R., Elechiguerra, J. L., Camacho, A., Holt, K., Kouri, J. B., Ramirez, J. T., and Yacaman, M. J. (2005). The bactericidal effect of silver nanoparticles. *Nanotechnology,* 16, 2346-2353. doi :10.1088/0957-4484/16/10/059.

Navazi, Z. R., Pazouki, M. and Halek, F. S. (2010). Investigation of culture conditions for biosynthesis of silver nanoparticles using Aspergillus fumigates. *Iran Journal of Biotechnology*, 8, 56-60,

Nazeruddin, G., Prasad, N., Prasad, S., Shaikh, Y., Waghmare, S. and Adhyapak, P. (2014). Coriandrum sativum seed extract assisted in situ green synthesis of silver nanoparticle and its anti-microbial activity. *Industrial Crops and Products*, 60, 212-216.

Namasivayam, S. K. R. and Chitrakala, K. (2011). Ecotoxicological effect of Lecanicillium lecanii (Ascomycota: Hypocreales) based silver nanoparticles on growth parameters of economically important plants. *Journal of Biopesticide,* 4, 97-101.

Ou, S. H. (1985). *Rice Diseases* (2nd ed.). Kew, UK: CAB publications.

Phogat, Khan, N. S. A, Shankar, S., Ansary, A. A. and Uddin, I. (2016). Fate of inorganic nanoparticles in agriculture. *Advanced Materials Letters,* 7(1), 03-12.

Saharan, V., Mehrotraa, A., Khatik, R., Rawal, P., Sharma, S. S. and Pal, A. (2013). Synthesis of chitosan-based nanoparticles and their in vivo evaluation against phytopathogenic fungi. International Journal of Biological Macromolecules, 62, 677-683.

Samuel, U. and Guggenbichler J. P. (2004). Prevention of catheter related infections: the potential of a new nano-silver impregnated catheter. *International Journal of Antimicrobial Agents,* 23(1), 75–78.

Sharon, M., Chowdhary, A. and Kumar, R. (2010). Nano technology in agricultural disease and food safety. *Journal of Phytopathology*, 2(4), 83-92.

Shelley, I. J., Nosaka, M. T., Nakata, M. K., Haque, M. S., and Inukai, Y. (2016). Rice cultivation in Bangladesh: present scenario, problems, and prospects. *Journal of International Cooperation for Agricultural Development*, 14, 20–29.

Singh, D., Kumar, A., Singh, K. and Tripathi, H. S. (2013). Induction of resistance in field pea against rust disease through various chemicals/ micronutrients and their impact on growth and yield. *The Plant Pathology Journal,* 12, 36-49.

Stone, V., Nowack, B., Baun, A., Brink, N. V. D., Kammer, F. V. D., Dusinska, M., Handy, R., Hankin, S., Hassellöv, M., Joner, E. and Fernandes, T. F. (2010). Nanomaterials for environmental studies: classification, reference material issues, and strategies for physio-chemical characterization. *Science of the Total Environment*, 408(7), 1745. doi: 10.1016/j.scitotenv.2009.10.035

World Health Organization (2004). The WHO recommended classification of pesticides by hazards and guideline to classification.

Xue, J., Luo, Z., Li, P., Ding, Y., Cui, Y. and Wu, Q. (2014). A residue-free green synergistic antifungal nanotechnology for pesticide thiram by ZnO nanoparticles. *Scientific Reports*, 4, 5408. DOI: 10.1038/srep05408.