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Evaluation of micronutrients i.e. silicon, copper and zinc applications for management of sheath blight disease on rice

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Abstract
Application of micronutrients as fertilizer can play an important role in the management of various fungal and bacterial diseases. Therefore, the study was undertaken to manage the rice sheath blight disease of rice by application of the micro nutrients in soil under flooded conditions. In the present investigation, the nutrients *i.e.* Cu as Copper sulphate, Zn as Zinc sulphate and Silicon as Silicon dioxide (SiO_2) were mixed into pot soil in required quantity. The experiment was carried out *in vitro* and pot conditions in completely randomized design (CRD) with four replications during the kharif seasons 2016 and 2017. Effect of application of micronutrients under *in vitro* and pot study showed that the application of copper sulphate, zinc sulphate and silica at 500 ppm and *in vitro* significantly reduced the growth of *R. solani*. Whereas, in pot study, the mixing of copper sulphate in soil prior to transplanting effectively reduced the sheath blight PDI (38.34%) as compared to control treatment (55.56%) PDI.

Keywords: Rice, sheath blight, management, micronutrients, SiO_2, Cu, Zn

Introduction
Rice crop suffers due to number of diseases accounting for severe losses. Of the several factors known to destabilize rice yields, pests and diseases account for 30-40 percent crop losses. Most parts of the country regularly encounter complete crop failure due to epidemics of pests and diseases. In Chhattisgarh, rice production is comparatively smaller than the national average production. A lot of fungal, bacterial, nematode, and viral diseases are attacked on rice. Serious incidences of diseases such as blast, sheath blight and bacterial blight have been reported from rice growing areas in Chhattisgarh regions. Sheath blight is one of India's widespread and harmful rice diseases. Rice sheath blight disease is causing significant loss, particularly in areas where high yielding varieties are cultivated. *Rhizoctonia solani* (Perfect stage-*Thanatephorus cucumeris*) which causes rice sheath blight in both soil and water borne. The presence of sheath blight disease in rice from several parts of India and beyond has been confirmed by workers of different parts of India. Butler made reference to the Indian disease as early as 1918. The presence of this disease has been confirmed by Andhra Pradesh, Assam, Jammu and Cashmir, Kerala, Tamil Nadu (Anonymous, 1971) [1], Orissa and West Bengal (f, 1970) [24], Madhya Pradesh (Anonymous, 1975; Verma *et al.*, 1979) [2, 24].
The systematic search of higher plants for antifungal activity has shown that plant have the ability to impede spore germination and mycelia development in many fungal species. The antifungal activity used for systematic search of higher plants has shown in many fungal species, plants have the capacity to disrupt spore germination and mycelial growth. Kannaiyan and Prasad (1979) [11] reported that two leaf sprays (10 days apart) of seven micronutrients at a concentration of 0.5% reduce the infection of *Rhizoctonia solani* and increase the grain yield of the ADT 31 variety. Good results have been obtained with borax, copper sulfate. Iron sulfate and zinc sulfate. Lakpale (1992) [14] concluded that the drastic reduction in *Rhizoctonia solani* mycelium growth in boron and zinc supplemented the media. The formation of sclerotia was completely inhibited by both elements. Zinc (Zn) is essential for a few biochemical processes in the rice plant, *e.g.* B. cytochrome and nucleotide amalgamation, auxin metabolism, chlorophyll production, enzyme activation and membrane integrity (IRRI, 2000) [9]. Marchner (1995) [15] found that copper (Cu) fertilization reduced the incidence of a variety of bacterial and fungal diseases related to cell wall integrity and plant lignification. Copper plays a crucial role in the formation of chlorophyll and the increase in yields (Heitholt *et al.* 2002) [8]. Somani (2008) [23] reported that micronutrient deficits deteriorate dramatically due to

intensive harvesting, loss of fertile top soil, and nutrients from surface runoff. Datnoff *et al.* (2005) [6] concluded that the disease management approach concentrated here is to manipulate the cultural practices of resistance to host plants and apply micronutrients to disease management. The effectiveness of micronutrients against sheath blight disease was very good against macronutrients (Bhattacharya *et al*., 2001, Ganguli *et al*., 2003 and Salam *et al.*, 2010) [3, 7, 19]. Bhattacharya and Roy (2001) [3] investigated the field performance of some chemicals against R. solani and found that sodium selenite, zinc sulfate, lithium sulfate, calcium nitrate, sodium fluoride and iron chloride most effectively reduced the disease length and lesion number of the sheath blight disease in rice. These chemicals showed no *in vitro* fungitoxicity at appropriate concentrations. Ganguli and Sinha (2003) [7] examined the effect of a dilute solution of seven metal salts on the occurrence of rice sheath blight disease in pots and fields and reported that copper chloride, barium sulfate, iron chloride, lithium sulfate and zinc sulfate had a moderate to strong effect. The lower concentration (10–4 M) had a strong influence on the symptoms of the disease, which led to a reduction in the severity of the disease by 41.4 to 65.5%. The use of silicon fertilizer not only increases rice yield, but also reduces fungal diseases in rice (Rodrigues and Datnoff, 2005 and Wang Meiqin, 2005) [6, 26]. Khaing *et al.* (2014) [12] investigated that the use of micronutrients that reduced the incidence and incidence of sheath blight disease affected varieties. Silicon fertilization was significantly more effective in minimizing the loss of yield due to sheath blight disease compared to Cu and Zn treatments.

Khaing *et al.* (2014) [12] showed that the micronutrients reduced the incidence and severity of sheath blight. The study was carried out to compare rice varieties MR219 and MR253 against sheath blight disease and micronutrient applications and to determine the influence of the disease on rice yield. The epidemics of sheath blight disease in pots were triggered in April 2013 by inoculation at the stage of maximum tillering under greenhouse conditions. Silica gel, copper sulfate and zinc sulfate were applied to the soil at a rate of 360 g, 0.30 g, 0.45 g per 15 kg of soil before planting. Inoculation significantly increased the severity and incidence of sheath blight disease, resulting in 11% loss of yield for the moderately resistant variety MR219 and 50% for the moderately susceptible variety MR253. The use of micronutrients reduced the incidence and severity of sheath blight regardless of the variety. Fertilization with Si, Cu and Zn treatments was effective for both strains to minimize the loss of yield due to sheath blight disease. Numerous studies (Nand R., 1996; Sagwal *et al.* 1994, Selvi S., 1995) [16, 20, 21] have been carried out with Zn applications in rice plants in order to increase the grain yield. Zn has been found to increase the phenol content of plants and reduce the severity of rice blight (Singh *et al*., 2010) [22].

Material and Methods

Application of micronutrients as fertilizer can play an important role in the management of various fungal and bacterial diseases. Therefore, the study was undertaken to manage the rice sheath blight disease of rice by application of the micro nutrients in soil under flooded conditions. In the present investigation, the nutrients *i.e.* Cu as Copper sulphate, Zn as Zinc sulphate and Silicon as Silicon dioxide (SiO_2) were mixed into pot soil in required quantity. The experiment was carried out *in vitro* and pot conditions in completely randomized design (CRD) with four replications during the kharif seasons 2016 and 2017.

Under the study the rice seeds were soaked in water for 24 h and dried for another 24 h at room temperature to hasten germination. 100 pre-germinated healthy seeds were planted into plastic seedling boxes (22.6cm x 18.6cm x 6.9 cm). Twelve-day seedlings were carefully uprooted from the nursery tray and three rice seedlings were transplanted into well-prepared experimental pots (35 cm in diameter and 38 cm in depth) containing 15 kg of paddy soil and placed under glasshouse conditions. Plants were regularly watered everyday and kept under flooded conditions until the end of the experiment. All the pots were applied nitrogen (N), phosphorus (P) and potassium (K) fertilizers according to the recommended rate. Applications of Nitrogen fertilizers (150 kg N ha-1) as urea split into 3 times at 15 days after planting (DAP) (25%), 35 DAP (30%) and 55 DAP (45%). Phosphorus (90 kg P_2O_5 ha-1) as rock phosphate and Potassium (150 kg K_2O ha-1) as murate of potash applied at basal and at panicle initiation stage at 55 DAP.

Application of silicon, copper and zinc nutrients

Granular silica gel with a minimum SiO_2 content of 95% and particle size ranging from (0.6-2 mm), Cu source as copper sulphate ($CuSO_4.5H_2O$) with 99% purity and Zn source as zinc sulphate ($ZnSO_4.7\ H_2O$) were used in this study. Silica gel, copper sulphate and zinc sulphate were applied to the soil prior to planting at the rates of 360g, 0.30 g, 0.45g per 15 kg soil respectively, as compared to the inoculated and un-inoculated (control) treatment without incorporation of these supplements. There were no modifications were made to the pots to allow the drainage and the rice plants were kept under flooded conditions till the end of the experiment.

Disease assessment and yield determination:

The rice seedlings in each treated pot were inoculated with the well prepared seven days inoculum of R. solani in rice stem bits at maximum tillering stage. Diseased rice plants of pots were evaluated on 21 days of inoculation for sheath blight development and the disease intensity was determined by the Highest Relative Lesion Length (HRLH percent) using the scale of Standard Evaluation System for rice by International Rice Research Institute.

Highest Relative Lesion Height (%) = (Vertical height of uppermost lesion on sheath or stem) / (Plant height) x 100

At the same time, disease severity was recorded in SES scale IRRI (2014) [14] and PDI were calculated with above formula. At maturity stage, grain yield was also recorded.

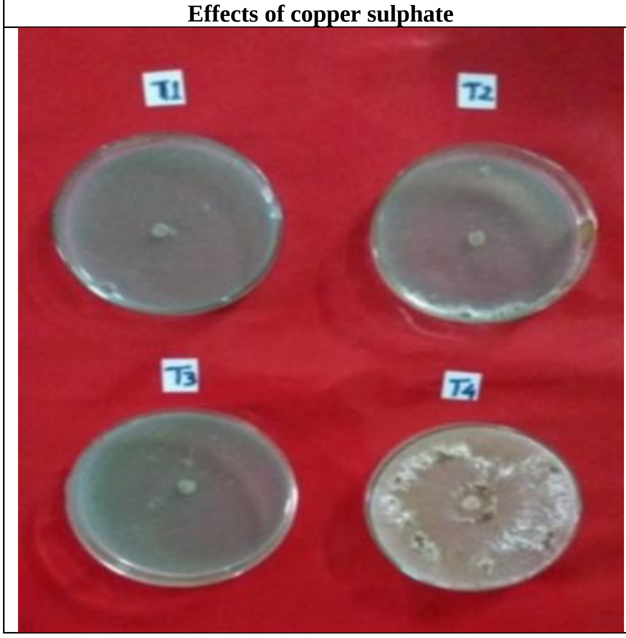
Results and Discussion

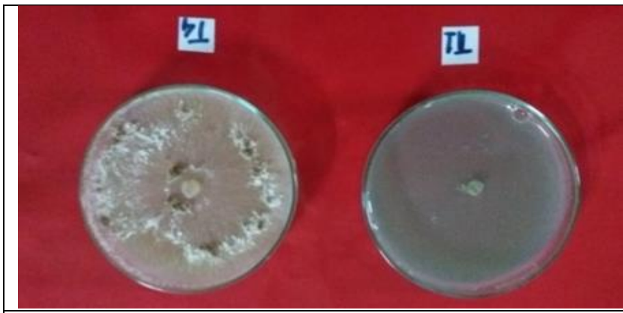
Rice sheath blight disease is the main disease that is difficult to control. An extreme shortage of soil micronutrients and a high dose of nitrogen fertilizers that lead to sheath blight have become an obstacle to rice production. A study was undertaken to understand the impact of micronutrient use on sheath blight and to determine the effects of the disease severity and yield of rice. The experiment was carried out under *in vitro* and pot conditions in a completely randomyzed design with four replications during kharif 2016 and kharif 2017. The nutrients included in the study were Cu as copper sulphate, Zn as zinc sulphate and silicon as silicon dioxide (SiO_2). Silicon (Si) is the second most common mineral element in the soil and plays an important role in reducing various environmental impacts and maximizing plant resistance to pathogens through the accumulation of Si in epidermal leaf cells that serve as a mechanical obstacle to fungal attack (Cai *et al*., 2008) [4]. Zinc (Zn) is important for the biochemical reactions of many rice plants, such as the formation of cytochrome and nucleotides, auxin synthesis, the formation of chlorophyll, enzyme stimulation and membrane integrity (IRRI, 2000) [9]. Numerous experiments (Nand R., 1996; Sagwal *et al.* 1994, Selvi S., 1995) [16, 20, 21] showed that the incorporation of Zn in rice plants improves the grain yield. Copper plays a crucial role in the development of chlorophyll and the increase is directly linked to an improvement in yield and fruit production (Heitholt *et al*., 2002) [8]. Under glasshouse conditions, sheath blight disease in pots were initiated by inoculation at maximum tillering stage of rice. Silica gel, copper sulphate and zinc sulphate were applied to the soil prior to planting at the rates of 360 g, 0.30 g, 0.45 g per 15 kg soil respectively. Micronutrients application reduced sheath blight severity and HRLH%. Fertilization with Cu was significantly more effective than Zn and Si treatments in minimizing yield loss due to sheath blight.

In vitro study

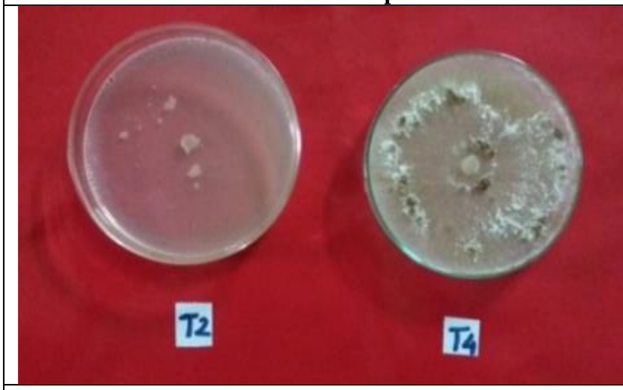
Table 1 and Fig. 1 and Fig. 2 and 3 indicated that all micronutrients were significantly inhibit the mycelial growth of R. solani as compared to the control. Copper sulphate and zinc sulphate (with 0.0 mm mycelial growth and 100% decrease over control) significantly reduced the mycelial growth followed by silicon dioxide (with 73.24 mm mycelial growth and 18.61% decrease over control) compared to the control treatment. Mycelium growth was recorded to the maximum in control treatment (73.24 mm). No sclerotial formation was observed in copper sulphate and zinc sulphate while 53.74 sclerotia per plate was observed in silica compared to the control (61.08 per plate) treatment. Small sized sclerotia were generally formed in silica, but normal sclerotia were formed in the control.

Effects of copper sulphate





Effects of Zinc sulphate



Effects of Silicon dioxide

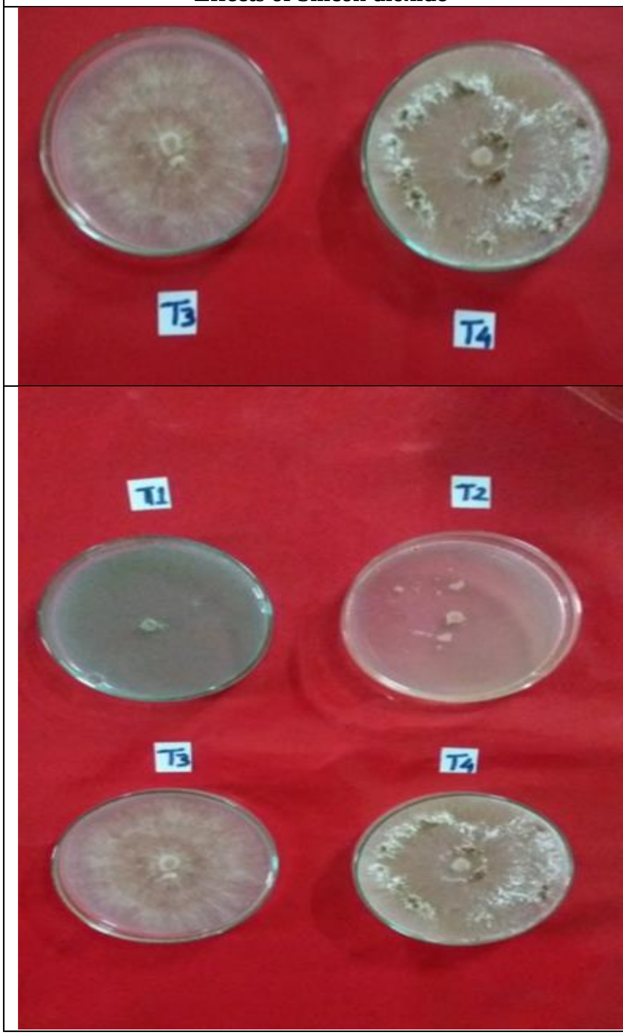


Fig 1: Effects of Silicon, Copper and Zinc Applications on Sheath Blight Disease Severity on Rice

Table 1: Effects of Silicon, Copper and Zinc on mycelial growth and sclerotia formation of *R. solani*

Micronutrient (500 ppm)	Mycelial growth (in mm)	No. of sclerotia per plate	% decrease over control
T1 - Copper sulphate	0.0 (0.71)	0.0 (0.71)	100.0 (10.03)
T2 – Zinc sulphate	0.0 (0.71)	0.0 (0.71)	100.0 (10.03)
T3 – SiO_2	73.24 (8.58)	53.74 (7.30)	18.61 (4.34)
T4 – Control	90.0 (9.51)	61.08 (7.84)	-
S.Em±	0.06508	0.120651	
CD (5%)	0.20053	0.371764	

*Figures in parenthesis are square root transformed value

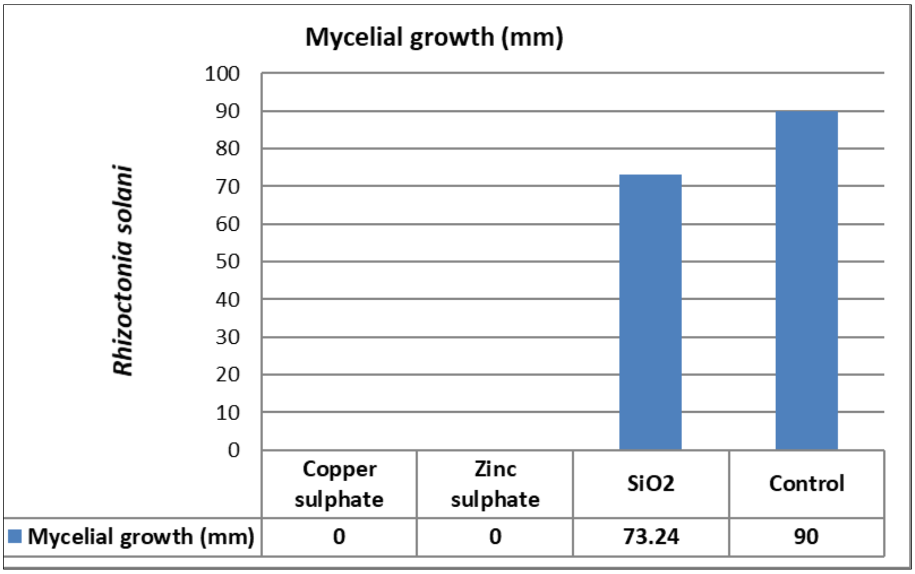


Fig 2: Effects of Silicon, Copper and Zinc on mycelial growth of *R. solani*

4.10.2 Pot study

During kharif 2016, the data presented in table 2 showed that copper sulphate significantly reduced the sheath blight incidence (37.06%) over all treatments is followed by zinc sulphate (40.41%) and SiO_2 (46.99%) over the incoulated control treatment (52.30%). The HRLH % (Highest Relative Lesion Height percent) of sheath blight disease ranged from 27.43 to 36.17 per cent and found significant difference over the control among the micronutrients. Minimum HRLH% was also observed in copper sulphate (27.43%) is followed by zinc sulphate (31.24%) and SiO_2 (36.17%) treatment. The copper sulphate treatment significantly reduced the sheath blight disease (34.45% PDI) which is followed by zinc sulphate (45.56% PDI) and silica (54.44% PDI) treatment over control. Whereas 11.11% PDI was noted in the uninoculated control and 55.56% PDI for the inoculated control. The treatment of copper sulphate also significantly increased the yield (4469.60 kg/ha) over all treatments are followed by zinc sulphate (4160kg/ha) and SiO_2 (3856kg/ha) over the inoculated control treatment (3480.0 kg/ha).

Table 2: Effect of micronutrients on sheath blight disease development under pot condition (Year-2016)

Treatments	Disease incidence (%)	HRLH (%) (Highest Relative lesion height %)	PDI (%)	Grain yield (Kg/ha.)
T1 - Copper sulphate	37.06 (37.49)	27.43 (31.56)	34.45 (35.93)	4469.60
T2 - Zinc sulphate	40.41 (39.47)	31.24 (33.98)	45.56 (42.40)	4160.0
T3 – SiO_2	46.99 (43.27)	36.17 (36.97)	54.44 (47.55)	3856.0
T4 – Control (Un-inoculated)	5.59 (13.63)	2.52 (9.11)	11.11 (19.47)	4560.0
T5 - Control (Inoculated)	52.30 (46.31)	40.96 (39.79)	55.56 (48.19)	3480.0
S Em±	0.5655	0.4882	1.3452	38.331
CD (5%)	1.7048	1.4717	4.0550	117.052

*Figures in parenthesis are arc sine transformed value

During kharif 2017, the data presented in table 3 showed that the copper sulphate treatment is significantly reduced the sheath blight disease incidence (39.59%) is followed by zinc sulphate (41.50%) and SiO_2 (42.55%) over the incoulated control treatment (51.60%). Minimum HRLH% (Highest Relative Lesion Height percent) of sheath blight disease was observed in copper sulphate (30.42%) and was on par with zinc sulphate (31.13% HRLH) followed by SiO_2 (32.70% HRLH) over the inoculated control (40.16% HRLH). The copper sulphate significantly reduced the sheath blight disease (42.23% PDI) followed by zinc sulphate (50.0% PDI) and silica (53.34% PDI) over the inoculated control with 55.56% PDI. The significantly increased grain yield was recorded in copper sulphate (4326.25 kg/ha) and was on par with zinc sulphate (4264.0 kg/ha) and SiO_2 (4161.20 kg/ha) over the inoculated control. The minimum grain yield (3568.0 kg/ha) was observed in inoculated control treatment.

Table 3: Effect of micronutrients on sheath blight disease development under pot condition (Year-2017)

Treatments	Disease incidence (%)	HRLH (%) (Highest Relative lesion height (%)	PDI (%)	Grain yield (Kg/ha.)
T1 - Copper sulphate	39.59 (38.98)	30.42 (33.47)	42.23 (40.49)	4326.25
T2 - Zinc sulphate	41.50 (40.10)	31.13 (33.91)	50.00 (44.98)	4264.0
T3 – SiO_2	42.55 (40.71)	32.70 (34.83)	53.34 (46.91)	4161.2
T4 – Control(Un-inoculated)	7.04 (15.33)	3.64 (11.0)	11.11 (19.47)	4441.25
T-5 - Control (Inoculated)	51.60 (45.91)	40.16 (39.32)	55.56 (48.19)	3568.0
S.Em±	0.5137	0.4028	1.4779	61.535
CD (5%)	1.5486	1.2142	4.4550	185.487

*Figures in parenthesis are arc sine transformed value

The pooled data of year 2016 and 2017 presented in the table 4 and fig. 3. revealed that the treatment copper sulphate significantly reduced the sheath blight disease incidence (38.34%) followed by zinc sulphate (40.96%) and SiO_2 (44.77%) over the incoulated control treatment (51.95%). The HRLH % (Highest Relative Lesion Height percent) of sheath blight disease ranged from 28.93 to 34.44 per cent and found significant difference among the micronutrients. Minimum HRLH% (Highest Relative Lesion Height percent) of sheath blight disease was again interpreteted in copper sulphate (28.93%) treatment is followed by zinc sulphate (31.19%) and SiO_2 (34.44%) treatments over the inoculated control (40.56%). The copper sulphate significantly reduced the sheath blight disease (with 38.34% PDI) followed by zinc sulphate (47.78% PDI) and silica (53.89% PDI) over the inoculated control (with 55.56% PDI). The pooled study also showed that the treatment with copper sulphate significantly enhanced the yield of paddy (4397.93 kg/ha) and recorded superior over all treatments was followed by zinc sulphate (4212.00kg/ha) and SiO_2 (4008.60 kg/ha) over the yield of inoculated control treatment (3524.00 kg/ha). This study showed the potential contribution of copper, zinc and silicon to reducing the sheath blight severity and increasing rice yield as compared with the control treatment. Silicon fertilization could be incorporated as a sustainable and environmentally friendly practice for management of rice sheath blight disease.

Similar results in the agreement with Khaing *et al.*, 2014 [12], which examined that the use of micronutrients reduced the incidence and severity of sheath blight disease regardless of the variety. Fertilization with Si was significantly more effective than treatment with Cu and Zn in both varieties in order to minimize the loss of yield. These results are consistent with other results (Rodrigues and Datnoff 2005 and Wang Meiqin, 2005) [6, 26] on disease reduction. Increased resistance to diseases due to the use of Si can be associated with an accumulation of Si in epidermal cells of leaves, which acts as a mechanical barrier against fungal attack (Cai *et al*., 2008) [4]. The results of several workers were in agreement with the present result because they also reported the effectiveness of various micronutrients against R. solani (Kannaiyan and Prasad, 1979; Phillip and Joshi, 1980; Bhattacharya and Roy, 2001; Lakpale, 1992[14]; Walia *et al.* 1992 and Ganguli and Sinha, 2003) [11, 6, 3, 14, 25, 7]. In pots trial inoculated with R. solani, initial symptoms developed on 15 to 20% of the stems within 7 to 14 days of inoculation, and these infection levels were within sheath blight treatment thresholds for the varieties in the study. In the inoculated / untreated (control), severe sheath blight disease occurred, ranging from 49 to 53% infected stems with 38 to 43% of the lesion length to the height of the rice plant. During the vegetation period, mild sheath blight was observed in the non-inoculated test pots, but did not reach the treatment threshold.

Table 4: Effect of nutrients on sheath blight disease development under pot condition (Pooled data of year 2016 and 2017)

Treatments	Disease incidence (%)	HRLH (%) (Highest Relative lesion height %)	PDI (%)	Grain yield (Kg/ha.)
T1 - Copper sulphate	38.34 (38.24)	28.93 (32.53)	38.34 (38.24)	4397.93
T2 - Zinc sulphate	40.96 (39.79)	31.19 (33.95)	47.78 (43.72)	4212.00
T3 – SiO_2	44.77 (42.0)	34.44 (35.91)	53.89 (47.23)	4008.60
T4 – Control(Un-inoculated)	6.32 (14.56)	3.08 (10.10)	11.11 (19.47)	4500.63

T-5 - Control (Inoculated)	51.95 (46.12)	40.56 (39.56)	55.56 (48.19)	3524.00
S.Em±	0.4191	0.3119	1.0046	30.542
CD (5%)	1.2634	0.9403	3.0282	92.066

*Figures in parenthesis are arc sine transformed value

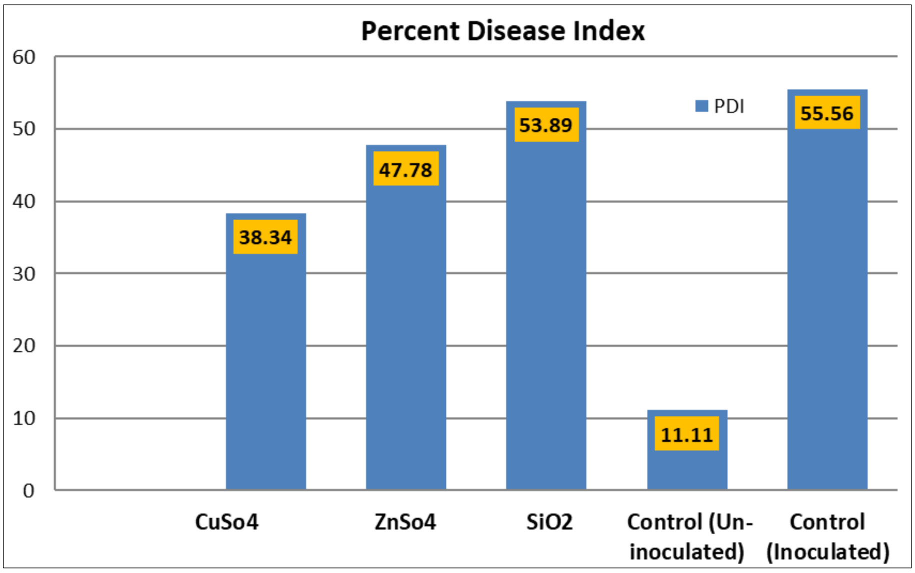


Fig 3: Effect of nutrients on sheath blight disease development under Pot condition (Pooled data of kharif 2016 and kharif 2017)

The study on *in vitro* effects of micronutrients on mycelial growth of R. solani showed that the copper sulphate and zinc sulphate at 500 ppm (100%) and SiO_2 (18.61%) decrease the mycelial growth of R. solani over control treatment. These micronutrients were also applied to the rice under pot conditions by mixing in soil prior to the transplanting against sheath blight and results revealed that among all micronutrients, the treatment of copper sulphate significantly reduced the sheath blight incidence was recorded to lowest lesion length along with lowest PDI (38.34%) as compared to the control treatment (55.56% PDI). This treatment also enhanced the yield of paddy significantly (4397.93 kg/ha) over all treatment as compared to untreated (3524.00 kg/ha) treatment. With the reconfirmation and the refinement in present experiment findings about management of sheath blight of rice by using micronutrients under the study may be helpful for development of IDM module for management of sheath blight of rice in Chhattisgarh region.

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