



## Research article

# Evaluation of fungicides to manage rice false smut (*Ustilagoidea virens*) in the hills of Nepal

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## ABSTRACT

Rice False Smut (RFS) caused by *Ustilagoidea virens* is a major emerging disease of rice due to expanded area of hybrid rice cultivars, increasing use of nitrogenous fertilizers and change in climate. Due to the increasing incidences of this disease across the globe, there is a pressing need to develop techniques for false smut management. The application of fungicides with high efficiency, low toxicity, and low residue is currently the best option to control RFS. Therefore, current research was conducted to determine the effectiveness of fungicides to manage RFS. The experiments were conducted in a completely randomized block design with three replications of seven treatments at RFS-prone subtropical hills of Nepal in the main rice growing season, during 2020 and 2021. The fungicides include trifloxystrobin 25 % + tebuconazole 50 %, chlorothalonil 75 %, carbendazim 12 % + mancozeb 63 %, propiconazole 25 %, azoxystrobin 50 %, carbendazim 50 % and untreated control. Fungicides were applied as two foliar sprays, one at booting and the other at flowering. Fungicide spray significantly increased number of tillers per plant ( $P \leq 0.01$ ) and reduced the number of false smut-infected tillers per plant ( $P \leq 0.05$ ), false smut severity ( $P \leq 0.05$ ), and incidence ( $P \leq 0.05$ ). False smut incidence percentages were significantly reduced by all the fungicides except mancozeb + carbendazim compared to the non-treated control. The reduction in RFS incidence was 70 % in propiconazole, 71 % in trifloxystrobin + tebuconazole sprayed plots compared to the non-treated control plots. Thus, the application of suitable fungicide at the appropriate stage would give the satisfactory suppression of RFS in a farmers' field in Nepal.

## 1. Introduction

Rice false smut (RFS) caused by *Ustilagoidea virens* is becoming an intensively challenging threat for rice production due to the expanded area of hybrid rice cultivars, increasing use of nitrogenous fertilizers and change in climate [1]. Rice false smut declines the yield and quality of rice by replacing rice grains with false smut balls and produces a diversity of mycotoxins such as ustiloxins and ustilaginoidins. These toxins are considered to have a deleterious impact in human and animal health by inhibiting cell microtubule assembly and skeletal formation [2,3]. The yield losses caused by false smut ranged from 0.2 to 49 percent depending on rice varieties and disease severity [4–6].

False smut is previously recorded as a minor disease of rice in Nepal however recently it is established as a major production constraint. It was first recorded in 1964 [7] and is considered to be introduced with Taiwanese rice varieties in Nepal. Now the disease

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is distributed throughout the country from temperate hills to tropical plains.

*Ustilaginoidea virens* (Cook) is an ascomycete fungal pathogen that produces both sexual ascospores and asexual chlamydospores in its life cycle [3]. *Ustilaginoidea virens* replace rice grains by producing globose, velvety spore balls (1–5 cm), which burst out in between the glumes. The balls consist of three spore-producing layers surrounding a hard core of tightly woven mycelium. Premature spore balls are enclosed in a membrane and are orange in color. Rice ovary is mostly destroyed but style, stigma and anthers might remain intact and incorporated into the spore ball. Single to multiple, hard black, irregular sclerotia of 5–13 × 2–5 mm are developed in the center of spore balls.

Sclerotia are produced under low temperatures, particularly at the end of rice growing seasons (mostly in late autumn). Under favorable conditions such as appropriate lightness, temperature and wetness, sclerotia germinate to form stroma, asci and ascospores. Ascospores contribute to secondary infection. At the end of rice growing season, *U. virens* produces the thick-walled chlamydospores on the surface of the balls which serves as an overwintering structure for primary infection in the following season. Chlamydospores can survive up to four months and sclerotia can survive even longer in soil, therefore, the primary infection can be caused by both sclerotia and chlamydospores but sclerotia leads to produce ascospores which only cause to secondary infection [8].

Under conducive conditions, *U. virens* conidia germinate and produce a large number of secondary conidia which produce hypha in rice spikelets that extended to infect stigma through the gap between lemma and palea. This infection blocks pollination and mimics fertilization to hijack the nutrient supply through ovaries. The fungal hyphae cover the stamen, anthers, stigmas, and styles of the pistils, and begin to grow out of the spikelets. Finally, ball-like colonies formed around ovaries, but the ovaries remain alive. The development of false smut balls not only impacts the rice grain filling but also causes the sterility of the spikelets which resulted in significant grain yield loss [9].

The infection with *U. virens* is enhanced by high relative humidity (>90 %) [10], high precipitation [11], high cloudy hours [12], moderate temperatures (25–30 °C) [10,13] late planting or flowering [12,14] and increased fertilizer specially high rate of nitrogen [15,16] as well as high amount of nitrogen [17]. Additionally, extensive use of chemical fertilizers, excessive irrigation [18], and the growth of high-yielding rice varieties on hybrid rice [19,20] have all been identified as contributing causes to the growing severity of this disease.

With the increasing severities of RFS across the globe, there is a pressing need to develop strategies for efficient and economical management of false smut. Several endeavors have been done to manage the diseases through different strategies including improvement in cultural practices, resistance breeding, and chemical and biological control.

False smut disease was less prevalent in sensitive varieties when conservation tillage, continuous rice cropping, and moderate nitrogen fertility rates were used [21]. The most effective, cost-effective, and ecologically friendly method of disease management is thought to be genetic resistance. False smut incidence is greatly impacted by the rice development period, inoculation procedures, and environmental conditions, such as humidity and temperature [22] which make the evaluation of genotypes against false smut resistance challenging. Although certain RFS-resistant cultivars have been created for commercial production, fungicides are still the mainstay of RFS treatment. The most adopted approach for controlling RFS disease at the moment is the use fungicides with high efficacy, low toxicity, and low residue, although this is often not an environmentally acceptable method [23].

Therefore, the quest for appropriate fungicides for RFS suppression is one of the most prioritized works. This dictates the evaluation of available fungicides to make successful and reliable advice for the RFS management. Therefore, the main objective of this study was to evaluate the efficacy of available fungicides to manage RFS in hilly rice ecosystems.

## 2. Materials and methods

### 2.1. Field experiment and fungicide application

The field experiments were conducted in a Farmer's field at Dhading, Dhunibesi, Nepal during two consecutive rice-growing seasons in 2020 and 2021. The experimental site lies in the sub-tropical zone between 27.40 °N latitude and 85.24 °E longitude

**Table 1**

Fungicides and formulation used in 2020 and 2021 field experiments in hills of Nepal.

Treatment (common name and formulation <sup>x</sup> )	Active ingredient	FRAC Code	Trade name	Rate <sup>y</sup>	Company
Azoxystrobin 50 %	Azoxystrobin	11	Topcare	1g/lit	Ningbo Generic Chemical Co. Ltd
Carbendazim 12 % + mancozeb 63 %	Carbendazim + mancozeb	1+M3	Saaf	2g/lit	UPL Limited IGC, Samba (J and K)
Carbendazim 50 %	Carbendazim	1	Bavistin	2g/lit	Heranba Industries LTD, Gujrat, India
Chlorothalonil 75 %	Chlorothalonil	M05	Protector	1g/lit	Greenriver Industry Co., LTD. ShenZhen, China
Propiconazole 25 %	Propiconazole	3	Tilt	1ml/lit	Syngenta, Greensboro, North Carolina, USA)
Trifloxystrobin 25 % + tebuconazole 50 %	Trifloxystrobin + tebuconazole	11 + 3	Nativo 75 WG	0.5g/lit	Bayer Crop Science Ltd, India

Abbreviations: WG, wettable granule; WP, wettable powder.

<sup>x</sup>Percentage of active ingredient in commercial product.

<sup>y</sup>Spray volume was 400 L/ha for each fungicide.

and at an altitude of 1579 m above sea level.

Rice seedlings were grown in raised beds, where seeds were primed in water overnight and dried in shade to enhance the germination before broadcasting in seed beds. Seeds of var. US312 was broadcasted in finally prepared well tilled, raised (10–15 cm) nursery bed which is supplied with well-decomposed farmyard manure at 20 mt ha<sup>-1</sup> without any chemical fertilizers. The nursery bed was mulched with straw for a week to enhance germination. Light irrigation was provided every day to keep soil moist. Rice seedlings were transplanted 30 days after sowing. The mulching maintains the uniform moisture in the soil and enhances the germination. Light irrigation was given in the nursery bed before uprooting the seedlings.

The experiments were laid out in completely randomized block design (RCBD) with seven treatments of three replications. The treatments include six fungicides and one non-treated control (Table 1). The plot size was 4 m<sup>2</sup> (2 × 2 m), plot to plot distance was 75 cm and the blocks were separated by 100 cm.

Farmyard manure (FYM), 6 mt ha<sup>-1</sup> was incorporated in the field at the time of first plowing (one week prior to transplanting). Fertilizers were applied 100:30:30 kg NPK/ha through urea, diammonium phosphate and muriate of potash. One-third dose of nitrogen and full dose of phosphorus and potash were applied at puddling as a basal dose and the remaining dose of nitrogen was applied in two equal split doses, one at the active tillering stage (35 days after transplanting, DAT) and the other at panicle initiation stage (55 DAT).

Seedlings were transplanted at 20 cm row to row and 10 cm plant to plant distance with 2–3 seedlings per hill, individual plots received 200 hills. Rice field was kept flooded as much as possible with irrigation, two weedings at tillering, and the booting stage were done manually. Fungicides were sprayed two times at booting (80 DAT) and flowering (100 DAT) using a knapsack sprayer. The spray volume was 400 lit ha<sup>-1</sup> for all plots. Water was sprayed in untreated treatment.

Data including on the total number of tillers per plant, number of tillers with RFS, number of spikelets per panicle, number of infected spikelets per panicles were recorded at harvesting, and number of infected panicles m<sup>-2</sup>, number of infected spikelet panicle<sup>-1</sup>, number of infected panicles m<sup>-2</sup> was calculated.

For data recording, a quadrat made up of an iron frame of m<sup>2</sup> (1 m × 1 m) was randomly thrown in the middle of each plot and 10 plants were randomly selected from the quadrat. RFS severity was scored in randomly selected 10 plants using 0–9 scale, 0: no incidence, 1: less than 1 % infection in florets, 3: 1–5%, 5: 6–25 %, 7: 26–50 %, and 9: 51–100 % [24].

Furthermore, whole plot grain yield and thousand-grain weight (TGW) were also recorded. Harvesting was done at full maturity using sickles manually. Threshing was done manually, and after threshing and winnowing, the grains were dried under the sun for 5 days. Grain weight was taken when the moisture content was 14 %. A thousand grains were taken randomly from the total grains of each plot and weighed (g) at 14 % moisture content.

## 2.2. Benefit-cost analysis

The cost of each fungicide was acquired from the Mount Everest Agri-seed Centre in Kalanki, Kathmandu, while the cost of rice at the farm gate was acquired from nearby farmers. Total cost of cultivation for rice was calculated as described [25] for Nuwakot district (adjacent to study site). Afterwards, gross margin was determined using the following formula:

Then, gross margin was calculated using formula: gross margin = gross return – total variable cost (fungicide cost + total cultivation cost), where gross return = farm gate price of rice × total rice production. The following formula was used to calculate the benefit-cost ratio in the end:

$$B/C \text{ ratio} = \text{Gross Return} / \text{Total variable cost.}$$

## 2.3. Statistical analysis

Shapiro-Wilk, Bartlett, and histogram tests were used to determine whether the residuals' normality and the variance's homogeneity were present in the data. Prior to performing an ANOVA, data that didn't fit the normal distribution were square-root converted. The linear model function "lm" in RStudio (R-3.2.5) (R Core Team 2022) was used to analyze the data. Fisher's test of least significant difference (LSD), which was generated from the Agricolae package, was used if there was a significant difference among the treatment means [26].

## 3. Results

### 3.1. Weather variables

Daily total rainfall at the experimental site ranged from 0 to 58 mm, average minimum temperature 15 to 24 °C, maximum temperature 22 to 33 °C, and average relative humidity 57 to 98 % in 2020 crop season. Similarly, daily total rainfall ranged from 0 to 84.2 mm, the average minimum temperature 13 to 25 °C, average maximum temp 22 to 34 °C and relative humidity 52–81 % during the crop period in 2021 (Fig. 1 A and 1 B).

### 3.2. Analysis of variance

Effect of sources of variation, years, fungicides, and their interaction on the mean square for number of tillers per plant, numbers of false smut infected tillers per plant, number of infected spikelets per panicle, number of infected panicles m<sup>-2</sup>, false smut severity, false

smut incidence, thousand-grain weight, and yield of rice are presented for both years in [Table 2](#).

No significant effect of year on number of infected spikelets per panicle, number of infected panicles  $m^{-2}$ , false smut incidence and TGW was observed. However, the effect of year was significant for number of tillers per plants, numbers of false smut infected tiller per plant, false smut severity and yield ( $P < 0.05$ ). Fungicide spray significantly affected number of tillers per plant ( $P \leq 0.01$ ), numbers of false smut-infected tillers per plant ( $P \leq 0.05$ ), false smut severity ( $P \leq 0.05$ ), and incidence ( $P \leq 0.05$ ). Year and fungicide interactions were non-significant for all the parameters evaluated. Due to the non-significant interaction of year and fungicide treatment, the data were pooled, and ANOVA were used and presented.

### 3.3. Effect of fungicides on disease severity

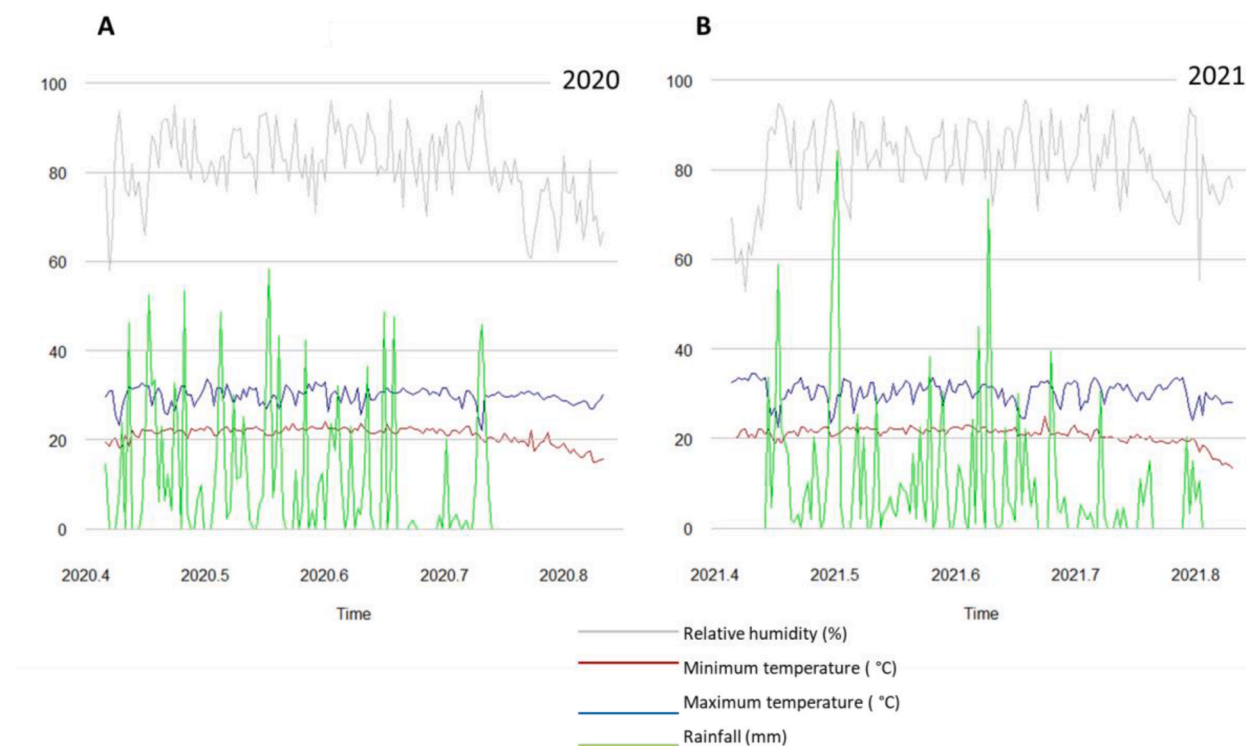
False smut severity was lower to moderate in both years of testing, with maximum 20.6 percent in non-treated control ([Fig. 2 Table 3](#)). Among the five fungicides, trifloxystrobin + tebuconazole, propiconazole and azoxystrobin consistently and significantly reduced the false smut severity as compared to control. The reduction was 55.6 percent in azoxystrobin, 66.1 percent in propiconazole and 60.5 percent in tebuconazole + trifloxystrobin. Carbendazim, mancozeb + carbendazim and chlorothalonil did not significantly reduce the false smut severity as compared to control.

False smut incidence percentages were moderate to high in both years of testing, with a maximum 42.8 percent in non-treated control ([Fig. 2](#)). False smut incidence percentages were significantly reduced by all the fungicides with the exception of mancozeb + carbendazim as compared to the non-treated control. The reduction in percentage incidence was 46.2 percent in carbendazim, 50.9 percent in chlorothalonil, 58.5 percent in azoxystrobin, 70.8 percent in propiconazole, 71.6 percent in trifloxystrobin + tebuconazole sprayed plots as compared to the non-treated control plots ([Table 3](#)).

### 3.4. Effect of fungicides on number of tillers and number of infected tillers per plant

Both number of tillers and number of infected tillers per plant were significantly affected by fungicide spray ([Fig. 3](#)). A significantly higher number of tillers per plant was observed in all fungicide-treated plots compared to non-treated control with the exception of carbendazim and mancozeb + carbendazim sprayed plots.

The maximum number of effective tillers was found on propiconazole 25 % (7.33) and trifloxystrobin 25 % + tebuconazole 50 % (7.33) which was significantly different from all other treatments during crop season 2020 ([Table 3](#)). In 2021 crop season there was no considerable difference among the treatments on number of total tillers. However, the maximum number of total tillers were found in chlorothalonil 75 % (10.33) followed by trifloxystrobin 25 % + tebuconazole 50 % (9.67), propiconazole (9.33) and azoxystrobin



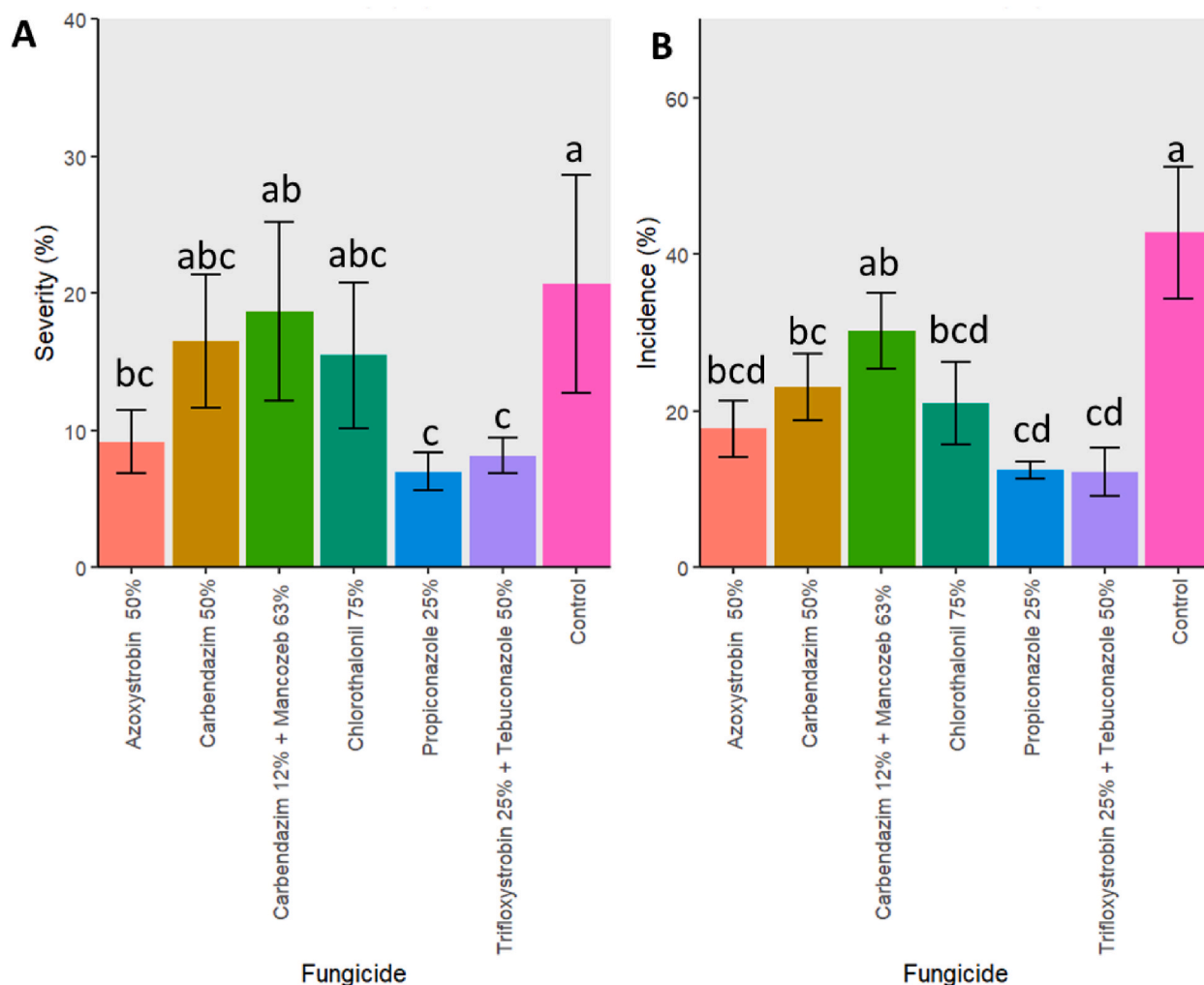
**Fig. 1.** Relative humidity, maximum and minimum temperatures, and rainfall during crop season of 2020 and 2021 at experimental site (A: 2020 and B: 2021).

**Table 2**

Effect of fungicide application on combined mean squares for number of tillers per plants number of infected tiller per plant, number of infected spikelets per panicle, number of infected panicles  $m^{-2}$ , severity (%), incidence (%), thousand-grain weight and rice yield in 2020 and 2021 in field experiment in the hills of Nepal.

Source	Degree of freedom	<sup>P</sup> Number of tillers per plant	<sup>Q</sup> Number of infected tiller per plant	<sup>T</sup> Infected spikelet per panicle	<sup>S</sup> Number of infected panicles $m^{-2}$	<sup>U</sup> Severity (%)	<sup>V</sup> Incidence (%)	<sup>W</sup> TGW	Yield
Year	1	0.96995**	0.43222*	1.05301	1.15825	0.3858*	0.032462	0.39829	3.6908*
Rep	2	0.05094	0.02916	0.38141	0.30111	0.00419	0.000779	0.01851	0.0046
Fungicide	6	0.27017**	0.25874*	0.61241	0.43981	0.031*	0.10519*	2.24952	0.6355
Year × Rep		0.01451	0.10714	0.04129	0.10714	0.02229	0.028075	0.9072	0.9531
Year × Fungicide		0.07639	0.1257	0.20057	0.06313	0.0158	0.026105	0.32372	0.2009
Residuals		0.0508	0.10147	0.53767	0.32086	0.01292	0.020046	0.93071	0.5458

Significance: \* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; ns: non-significant i.e.  $P > 0.05$ .



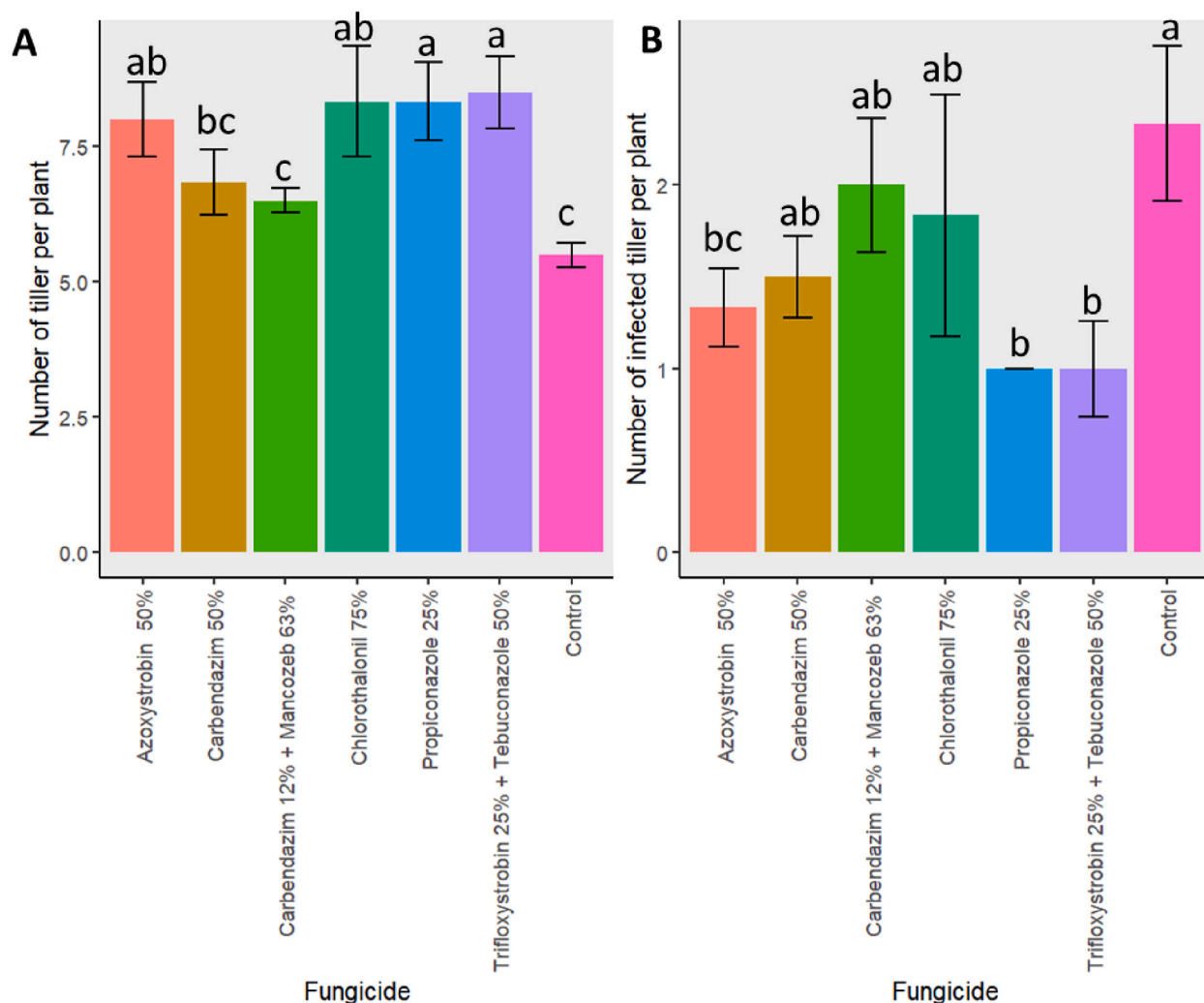
**Fig. 2.** False smut severity and incidence in rice affected by different fungicide treatments during crop season of 2020 and 2021 in hills of Nepal (A: Severity (%), B: Incidence (%)) Means followed by different letters are significantly different at P-value <0.05, according to Fisher’s LSD test after square root of arcsine transformation. Data presented are from two independent experiments each with three replications. Error bars indicate standard error.

**Table 3**

Effect of fungicide sprays on grain yield and total number of tillers per plant and thousand-grain weight of rice in field trails in Dhading district in hills of Nepal in 2020 and 2021.

Treatment	Yield (mt/ha)		Total number of tillers per plant				Thousand-grain weight (g)		
	2020	2021	2020		2021				
Azoxystrobin 50 %	4.87	3.97	7.00	(2.64)	ab	9.00	(2.99)	ab	19.5
Carbendazim 12 % + mancozeb 63 %	4.50	4.23	6.00	(2.45)	ab	7.00	(2.65)	bc	20.9
Carbendazim 50 %	4.27	4.10	6.67	(2.58)	ab	7.00	(2.63)	bc	21.0
Chlorothalonil 75 %	4.90	4.10	6.33	(2.50)	ab	10.33	(3.21)	a	20.1
Propiconazole 25 %	5.40	4.33	7.33	(2.70)	a	9.33	(3.05)	ab	21.3
Trifloxystrobin 25 % + tebuconazole 50 %	5.10	4.30	7.33	(2.70)	a	9.67	(3.11)	a	20.4
Control	4.03	3.73	5.33	(2.31)	b	5.67	(2.38)	c	19.6
P-value	0.2	1.0					0.007 **		0.4

Values in a column followed by different letters are significantly different at the indicated P-value according to Fisher’s LSD test after square root transformation; values inside the parenthesis indicate square root transformed values, values are means of two experiments, each with three replications.



**Fig. 3.** Number of rice tillers per plant and number of false smut infected tillers per plants in rice affected by different fungicide treatments during crop season of 2020 and 2021 in hills of Nepal (A: Number of rice tillers per plant, B: Number of false smut infected tillers per plants Means followed by different letters are significantly different at P-value <0.05, according to Fisher's LSD test after square root of arcsine transformation. Data presented are from two independent experiments each with three replications. Error bars indicate standard error.

(9.00) and by and the minimum in control (5.67) during 2021.

The percent increase in the number of tillers per plot was 54.6 percent in trifloxystrobin + tebuconazole, 51.5 percent in chlorothalonil, 51.5 percent in propiconazole and 45.5 percent in azoxystrobin sprayed plots compared to the non-treated control. Number of tillers per plant ranged from 6 (non-treated control) to 9 (trifloxystrobin + tebuconazole) (Table 3).

The number of infected tillers per plant was also significantly affected by fungicide treatment. The number of infected tillers per plant was significantly lower in propiconazole (1.0) and trifloxystrobin + tebuconazole (1.0) treated plots compared to the non-treated control (2.3). However other fungicide treatments did not change the number of infected tillers per plant compared to the non-treated control. The reduction percent in the number of infected tiller per plant was 42.7 percent in azoxystrobin, 57.0 percent in propiconazole, 57.0 percent in trifloxystrobin + tebuconazole sprayed plots compared to the non-treated control. The number of infected tillers per plant ranged from 1 (trifloxystrobin + tebuconazole) to 3 (non-treated control) (Supplementary Table 2).

### 3.5. Effect of fungicides on rice yield and test weight

Rice grain yield and test weight (1000 grain weight) did not differ significantly among the treatments in both years of testing (Table 3).



### 3.6. Financial analysis

The cost of azoxystrobin 50 % was higher followed by propiconazole 25 % (Table 4). Among the fungicides, carbendazim 12 % + mancozeb 63% required the least investment due to low cost of fungicides compared to other fungicides. In both years, economic return and B:C ratio was not significantly affected by fungicide application (Table 4).

## 4. Discussion

In recent years, RFS has become an emerging threat to rice cultivation. Strategies for effective and affordable disease management are urgently needed for the given emerging threats of RFS. The disease is highly influenced by weather conditions and thus chemical spray would give satisfactory control of disease. Chemical fungicides are currently a major approach to control RFS. Results from two years of studies in RFS-prone areas in the subtropical hills of Nepal indicate that the application of suitable fungicides would provide the effective suppression of the RFS.

The weather conditions were conducive for disease development in both years of testing. False smut incidences were moderate to high in both years of testing. The disease incidence and severity were higher in 2020 and lower in 2021 since weather conditions was favorable for disease development in 2020 where RH was ~90 percent and with high rainfall during the flowering period (month of September) in 2020 instead rainfall and RH were low during the flowering period of 2021.

Current study reported the fungicide sprays significantly influenced most of the disease parameters including number of tillers per plant, number of infected tillers per plant, RFS severity and incidence. All fungicide sprays consistently reduced the RFS incidence by more than 50 percent with the exception of plots treated with carbendazim and mancozeb + carbendazim as compared to untreated control. Azoxystrobin 60 % in propiconazole and 66 % in tebuconazole and trifloxystrobin sprays reduced the RFS severity consistently by more than 50 %.

The observation made by Raji et al. [27] lends more weight to our findings. In India, they discovered that trifloxystrobin + tebuconazole 75 WG when sprayed at booting or 50 % panicle emergence stage resulted in the lowest severity of RFS. By using EBI fungicides such as propiconazole, Chen et al. [28] observed that false smut and sheath blight on rice were suppressed in China. Bagga et al. [29] reported that Tilt 25 EC (propiconazole) effectively controlled the RFS incidence when these fungicides were applied at booting stage. Furthermore, false smut was suppressed significantly by the application of a combination of propiconazole + difenoconazole and *B. subtilis* with validamycin in China [13]. Anasari et al. [30] reported significant reduction of false smut infection as compared to non-treated control in Bangladesh when fungicides such as difeconazole + tebuconazole, validamycine, azoxystrobin + cyproconazole, copper + mancozeb, tebuconazole + trifloxystrobin and difeconazole + propiconazole applied twice at panicle initiation and flowering stages. Furthermore, Barnwal et al. [31] also reported the significant suppression of rice false smut by spraying propiconazole and hexaconazole in India. Tebuconazole, prochloraz, and propiconazole are among the fungicides, including demethylation inhibitors (DMI), that are frequently used in China to manage rice false smut [1,32,33].

It has been reported that chlamydospores are the primary source of inoculum to infect rice plants which can survive in soil. After germination of chlamydospores on coleoptile epidermal cells, reach to meristematic tissues intercellularly [34]. Infection levels slow down after the coleoptiles reach 10 mm in length. Hu et al. [35] and Hu et al. [23] reported injecting conidia and hyphae at the booting stage resulted in higher floret infection and the production of a larger number of smut balls. Therefore, application of fungicide at the booting stage is critical to control RFS. These might have resulted in the variation in panicle infection due to the application of different fungicides.

Numerous studies on rice have found that application of fungicides boosted grain output because the plants experienced lesser biotic stress during crucial growth stages [36–38]. However, our results indicate a reduction in RFS severity and incidence, but the fungicide application did not increase the rice yield compared to the non-treated control. Some of the earlier workers e.g. Ref. [29]; and [10] reported an increase in grain yield after the application of fungicide along with a reduction in RFS reduction.

Triazole fungicides, known as quinone outside inhibitors (QoI), prevent electron transport at the cytochrome *bc1* complex's (complex III) quinol-oxidizing site in the mitochondrial respiration chain [39–41]. Propiconazole and tebuconazole are triazole fungicides that inhibit ergosterol synthesis in true fungi. Ergosterol is a major sterol that is essential for the integrity of the membrane structure and function in true fungi. It is essential for membrane structure and function. Triazole fungicides inhibit sterol production and cell wall synthesis in most fungi. Trifloxystrobin is under the group of strobilurins (oximinoacetate) fungicides which inhibit the electron transport chain resulting the respiration in mitochondria by blocking the cytochrome *bc1* complex in true fungi. Chlorothalonil is under chloronitrile fungicides group which disrupts the enzyme synthesis in true fungi resulting inactivation amino acids, proteins and enzymes by combining with amino and thiol groups. Mancozeb is under alkylenebis (dithiocarbamate) group which disrupts the enzyme synthesis in true fungi by inactivating SH groups in amino acids, proteins and enzymes. Carbendazim is under benzimidazole (thiophanate-methyl) fungicides group which inhibits nucleic acid metabolism and protein synthesis which particularly inhibits DNA synthesis (nuclear division) [42].

Rice false smut (RFS) is emerging as one of the most economically important grain diseases of rice in Nepal and around the globe. The disease could be managed by spraying the combination of fungicides such as trifloxystrobin 25 % + tebuconazole 50 % (Nativo 75 WG) at the rate of 0.4 gm/lit and propiconazole at booting and flowering stage under field conditions. In order to reduce immediate economic damage caused by RFS, it is necessary to adopt appropriate management techniques to prevent the disease. These fungicides have a single site of the mode of action, therefore rotation of fungicide application and use in tank mix are always recommended. As the disease is highly influenced by nitrogen application [21], therefore balance use of fertilizers, and the application of suitable fungicides at booting and flowering stage is recommended to manage RFS in Nepal.



**Table 4**

Financial analysis of fungicide spray program based on field experiments conducted for the management of false smut in 2020 and 2021 crop seasons in hills of Nepal.

Treatment	Cost of fungicide (US\$/ha)		Total cost of cultivation (US\$)*		Revenue (US\$/ha)**		B:C ratio	
					2020	2021	2020	2022
Azoxystrobin 50 %	102.0	a	805.7	a	1014.4	832.4	1.27	1.03
Carbendazim 12 % + mancozeb 63 %	22.2	f	725.9	f	930.9	887.8	1.30	1.20
Carbendazim 50 %	46.9	d	750.6	d	889.8	860.5	1.40	1.17
Chlorothalonil 75 %	30.6	e	734.3	e	1020.4	858.8	1.20	1.17
Propiconazole 25 %	71.4	b	775.1	b	1127.9	905.6	1.47	1.17
Trifloxystrobin 25 % + tebuconazole 50 %	64.3	c	767.9	c	1058.5	896.9	1.37	1.17
Control	0.0	g	703.7	g	841.1	776.3	1.17	1.07
P-value	<2e-16 ***		<2e-16 ***	0.25	0.96	0.55	0.95	

Values in a column followed by different letters are significantly different at the indicated P-value according to Fisher's LSD test, values are means of two experiments, each with three replications. \*Total cost of cultivation of potato was estimated according to Ref. [25] including US\$ 20/ha for fungicide spray, cost of fungicide and other expenses. \*\*Revenue was calculated by multiplying total yield and local farm gate value at harvest (NRS 25,000/t). Exchange rate was calculated based on US\$ 1 equivalent to NRS 120.

### Data availability statement

Data will be made available on request.

### CRediT authorship contribution statement

**Pratiksha Sharma:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ram B. Khadka:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Investigation, Formal analysis, Data curation. **Suraj Baidya:** Writing – review & editing, Visualization, Validation, Supervision, Funding acquisition, Conceptualization.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2024.e34151>.

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