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Role of Nutrients and Lower Leaf Removal Against Alternaria Blight in Indian Mustard (*Brassica juncea* L.)

¹P.D. Meena, ¹Asha Rani, ²M.C. Meena, ¹Pankaj Sharma, ¹Basant Kandpal and ¹Dhiraj Singh

¹ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur (Raj), 321303, India

²Division of Soil Science, ICAR-Indian Agricultural Research Institute, New Delhi, 110012, India

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Corresponding Author:

P.D. Meena,

ICAR-Directorate of Rapeseed-Mustard
Research, Bharatpur (Raj), 321303,
India

ABSTRACT

Alternaria Blight (AB) caused by *Alternaria brassicae* and *A. brassicicola* is a major constraint in rapeseed-mustard production in India. An experiment was conducted during 2009-10 and 2010-11 to study the effect of nutrients and lower leaf removal in Indian mustard (*Brassica juncea* L.) on AB disease. Results indicated that maximum AB reduction (26%) was observed by soil application of Potash at 40 kg ha⁻¹+Zinc sulphate at 25 kg ha⁻¹+Copper sulphate at 40 kg ha⁻¹+Sulphur at 10 kg ha⁻¹+Ridomil-MZ 72 as foliar spray at 0.25% was better when applied as foliar spray (21%). However, three lower leaves removal at 40 Days After Sowing (DAS) with Foliar Spray (FS) of Ridomil MZ- 72 at 0.25 also reduced the disease (19%) as similar with leaf removal (18.9%) alone in combination with no treatment. Soil application of K+ZnSO₄+CuSO₄+S+leaf removal+FS of mancozeb+carbendazim and lower leaf removal at 40 DAS+FS of ridomil altogether reduced the maximum disease severity (37.9%). A linear equation was found between Zn, Cu, S and K with R² = 0.55, 0.38, 0.15 and 0.59, respectively, during 2009-10 and R² = 0.52, 0.30, 0.27 and 0.51, respectively during 2010-11 showed decrease in AB severity with increase of nutrient content in leaves. Data revealed that leaf removal at 40 DAS vis-a-vis use of nutrients along with fungicidal foliar spray reduced the AB of Indian mustard. Because the use of nutrients take part in induced tolerance and lower leaf removal keep away from the reach of inoculum to the plants, both plays a key role to minimize disease severity in Indian mustard resulted higher seed yield and there adoption could be economic and eco-friendly to the farmers.

Key words: Alternaria blight, Indian mustard, lower leaf removal, nutrient application

INTRODUCTION

Alternaria blight caused by *Alternaria brassicae* (Berk) Sacc. and *A. brassicicola* (Schwein.) Wiltsh. remains a serious disease of Indian mustard (*Brassica juncea* (L.) Czern and Coss.) causes yield losses upto 35% (Kolte and Awasthi, 1987). The pathogen affects the crop at every growth stages of plant. Initially, symptoms appear as brown to black pin points which later enlarge to develop into prominent, round, concentric rings of different sizes. Lower leaves are affected first and consequent to their defoliation middle and upper

leaves are also affected. Finally, disease reached on silique and stem which may coalesce to form elongated lesions leading to complete blackening of silique or weakening of stem may result a drastic reduction in yield and oil content of the seeds (Meena *et al.*, 2010). Management of this destructive pathogen is very difficult and it is still a challenge for the Plant Pathologists. The role of nutrients as a source of fungitoxic chemicals and their importance has been reported in controlling different plant pathogens.

Elemental sulphur (S⁰) is the oldest known fungicide and newest phytoalexin in plant-pathogen interactions which was

reported to produce as a component of active defense to fungal pathogens (Williams and Cooper, 2004). Maximum amounts of S^o was reported in *B. oleracea* (0.37 µg g⁻¹) and *Arabidopsis thaliana* (6.3 µg g⁻¹) showed the possible fungitoxic source (Williams and Cooper, 2004). The mixture of copper sulphate and zinc has been showed antifungal activity under laboratory experiments against many fungi including *A. brassicae* (Williams and Cooper, 2004). Soil application of sulphur was found to increase resistance against different fungal pathogens in many crops (Klikocka *et al.*, 2005). Its low toxicity to plants and beneficial insects has made sulphur attractive as a chemical control agent (Emmett, 2003). The plants require S for the synthesis of glucosinolates reported to be part of the plant's defense mechanism against fungi. In addition, glucosinolates may play a role as S storage source which can be used in the event of S starvation (Schnug and Haneklaus, 1993).

Hossain and Mian (2005), integrated the different management practices including soil treatment with sulphur-zinc-magnesium-molybdenum-boron (S-Zn-Mg-Mo-B) at the rate of 30-5-1-1-1 kg ha⁻¹ in addition to the recommended dose of nitrogen-phosphorus-potassium (N-P-K) and spraying of fungicide iprodione (0.2%) recorded the best AB reduction (93.2%) and increased a high cabbage seed yield in comparison to normal management practices. The decrease in *Alternaria* blight severity due to K application of 40 kg ha⁻¹ gave consistently (68%) seed yield over control (Sharma and Kolte, 1994; Godika *et al.*, 2001). However, limited efforts by Dubuis *et al.* (2005) have been made in use of nutrients but no reports found of lower leaf removal for effective management of AB of Indian mustard. Therefore, present study was undertaken to examine the potential of nutrients applied as soil application at the time of sowing and foliar spray at critical stages of 45 to 75 days after sowing (Meena *et al.*, 2004) and lower leaf removal for substituting the recommended chemical fungicides against the AB of Indian mustard in the present study.

MATERIALS AND METHODS

Field experiment was conducted at Experimental Farm, ICAR-Directorate of Rapeseed-Mustard Research (ICAR), Bharatpur, India during 2009-10 and 2010-11 to study the role of nutrients and lower leaf removal in effective management of AB of Indian mustard. Ten treatments in three replications were taken including (1) Three lower leaf removal at 40 Days after Sowing (DAS), (2) Three lower leaf removal at 40 DAS with Foliar Spray (FS) of ridomil at 0.25%, (3) Soil Application (SA) of potash (K) at 40 kg ha⁻¹, (4) SA of ZnSO₄ at 25 kg ha⁻¹+FS of ZnSO₄ at 0.2%, (5) SA of CuSO₄ at 40 kg ha⁻¹ and FS of CuSO₄ at 500 ppm, (6) SA of sulphur (S) at 10 kg ha⁻¹+FS of S (0.2%), (7) SA of K+ ZnSO₄+CuSO₄+S, (8) FS of ridomil at 0.25%, (9) FS of mancozeb+carbendazim at 0.1%, (10) SA of K+ZnSO₄+CuSO₄+S+leaf removal+FS of ridomil, (11) SA of K+ZnSO₄+CuSO₄+S+leaf removal+FS of mancozeb+carbendazim at 0.1% and (12) control as water spray. Indian mustard cultivar Varuna was sown on

25 October during both the years in 5×3 m plots size with row to row and plant to plant spacing of 30×10 cm, respectively in Randomized Block Design (RBD) using three replications. Treatments were applied as soil application and foliar spray at 45 and 60 days after sowing. Leaf samples were collected at 44 and 59 days after sowing vis-a-vis before foliar spray of treatments and at 75 days after sowing vis-a-vis after treatment spray for micro-nutrient analysis. The AB severity was assessed as percentage of leaf area covered by selecting five leaves per plant on ten plants from each plot on a random basis in each replication. Disease severity was recorded using Conn *et al.* (1990) scale. The yield of net area of each plot without border rows was obtained. The obtained yield of each genotype was calculated in kg ha⁻¹.

Extraction: Extraction was done particular pre-weighted powdered samples were wet ashed using 50 mL (1:1) of a concentrated HNO₃/HClO₄ acid mixture, few drops of Hydrofluoric Acid (HF) was added and the mixtures were then heated to a clear solution and continued to complete dryness. The cooled residue was dissolved in 5 mL 2N HCl and heated again until complete digestion. After cooling the clear solution was transferred into 50 mL volumetric flask and completed to the mark using bi-distilled water (Mohamed *et al.*, 2003).

Analysis: Certified atomic absorption spectroscopic standards of BDH (UK) for Zn and Cu were used and analyzed using Perkin-Elmer (Model HGA-3110) atomic absorption spectrophotometer equipped with a Perkin-Elmer HGA-600 carbon furnace and auto sampler As-60. Analysis of S was done by using spectrophotometer and K was estimated by using flame photometer. Three replicates from each sample were analyzed.

Statistical analysis: The significance of difference between nutrient contents in the Indian mustard variety compared to control at different application stages of leaves was analyzed by using one-way analysis of variance (ANOVA) using Indostat software. Means were then separated using the Least Significant Difference (LSD) test at p≤0.05. The F-values were calculated and compared with the table value at p≤0.05. Also the difference between soil and foliar application of micronutrients in leaves at different stage was assessed.

RESULTS AND DISCUSSION

Data revealed that all the treatments were found effective in reducing the AB severity on the leaves as well as silique over control (Table 1). Sulphur and zinc, significantly reduced AB on leaves of Indian mustard while copper also reduced the disease to some extent. Maximum AB reduction (26%) was observed with combination of soil application of potash at 40 kg ha⁻¹+zinc sulphate at 25 kg ha⁻¹+copper sulphate at 40 kg ha⁻¹+sulphur 10 kg ha⁻¹ and foliar spray of ridomil MZ 72 at 0.25% followed by their foliar spray (21%). However, the lower three leaves removal at 40 DAS with foliar spray of ridomil MZ-72 at 0.25% reduced the disease (19%) followed by only leaf removal (18.9%).

Data revealed that all the treatments were significantly reduced the AB severity in comparisons to check. The percentage reduction of AB disease severity significantly superior with 33% reduction by removal of leaves alone over check and subsequently significant increased the seed yield. We conclude the results that farmers can use both nutrients as well as leaves removal simultaneously or alone because both are significantly different. Leaf removal may be environmental friendly technology additionally providing nutritive fodder for animals could be substitute of fungicidal spray.

The above results underline the importance of mineral nutrition as a component of disease management practices (Hossain and Mian, 2005). Dubuis *et al.* (2005) studied that the sulphur deficiency of oilseed rape negatively affects disease resistance caused by the reduction of sulphur dependent phytoanticipins. However, sulphur applied in soil was found to increase resistance against a variety of fungal pathogens on different crops (Klikocka *et al.*, 2005). The possible role of elements in defence and the form, location and levels *in planta* are considered. Sulphur is one of many S-containing defence-related compounds and it is ironic that sulphur deficiency has recently become a widespread nutrient disorder in crops (Singh, 2001). Singh *et al.* (2005) reported

that more than 50% (10 m ha) of the agricultural soils in India is zinc-deficient. Role of calcium in improving tolerance in plants to diseases have been indicated earlier (Reddy, 2001). The problem is being addressed by application of sulphur, borax and zinc. However, this seems to be the possible first report of use of such nutrients including sulphur, zinc, etc. for effective management of AB of mustard. Our results from the two-year study comprehensively proved that nutrients viz., calcium sulphate, borax and sulphur which significantly reduced the disease severity could be used to supplement the lower leaf removal and foliar spray as an effective substitutes of mancozeb for better control of disease in Indian mustard.

Pathogens such as some *Fusarium* spp. and *Alternaria* spp. that are mild in normal conditions of plant growth and exist mainly as saprophytes or endophytes, may cause severe disease under conditions of nutrient stress or aluminium toxicity (Desaeger *et al.*, 2004). Nutrient content is seems to be responsible for enhance the tolerance level of leaves. Our results demonstrated a linear equation between Zn, Cu, S and K with $R^2 = 0.55, 0.38, 0.15$ and 0.59 , respectively during 2009-10 and similarly, $R^2 = 0.52, 0.30, 0.27$ and 0.51 , respectively during 2010-11 showed decrease in AB severity with increase of nutrient content in leaves (Fig. 1). Our

Table 1: Reduction percentage of Alternaria blight severity in Indian mustard by different treatments

Treatments	AB reduction over control (%)		Seed yield (kg ha ⁻¹)	1000-seed wt (g)	Oil content (%)
	2009-10	2010-11			
Lower leaf removal at 40 DAS	19.1	33.1	2461	5.44	39.1
Lower leaf removal at 40 DAS+FS of Metalaxyl 64% +mancozeb 8% at 0.25%	18.9	37.9	2906	5.16	39.3
SA of K at 40 kg ha ⁻¹	9.7	26.5	2483	5.23	38.7
SA of Zn SO ₄ at 25 kg ha ⁻¹ + FS of Zn SO ₄ at 0.2%	4.4	19.8	2850	5.28	39.5
SA of Cu SO ₄ at 40 kg ha ⁻¹ and FS of Cu SO ₄ at 500 ppm	11.9	23.9	2928	5.16	39.5
SA of S at 10 kg ha ⁻¹ +FS of S	11.9	25.9	2811	5.18	39.3
SA of K+ ZnSO ₄ +Cu SO ₄ + S	9.7	32.6	2833	5.13	39.1
FS of Metalaxyl 64% +mancozeb 8% at 0.25%	21.3	33.7	2556	5.13	39.3
FS of Mancozeb+Carbendazim at 0.1%	-	33.7	2933	5.17	39.3
SA of K+Zn SO ₄ +Cu SO ₄ +S+Leaf removal+FS of Metalaxyl 64% +mancozeb 8%	25.7	37.9	2947	5.39	39.5
SA of K+Zn SO ₄ + Cu SO ₄ +S+ Leaf removal+FS of Mancozeb+Carbendazim at 0.1%	-	39.3	2978	5.49	39.5
Control (Water spray)	-	-	2022	4.81	38.7
CD (p>0.05)	3.9	3.8	394	0.32	0.69

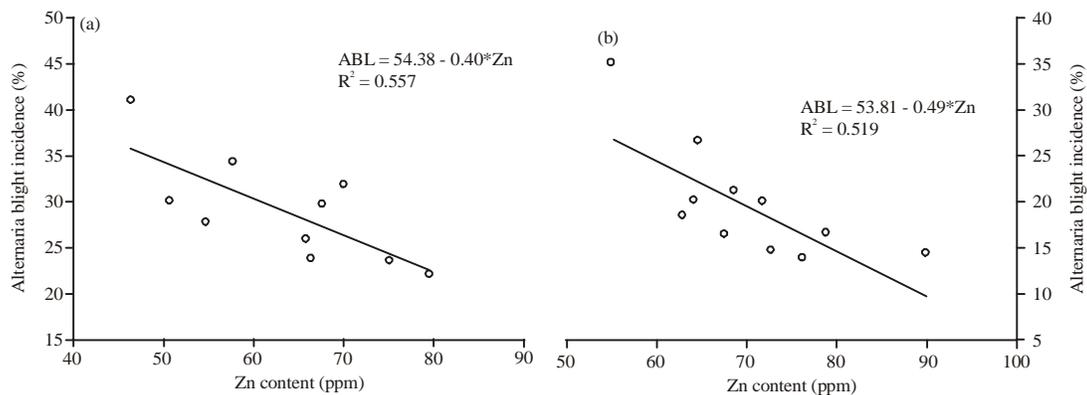


Fig. 1(a-h): Continue

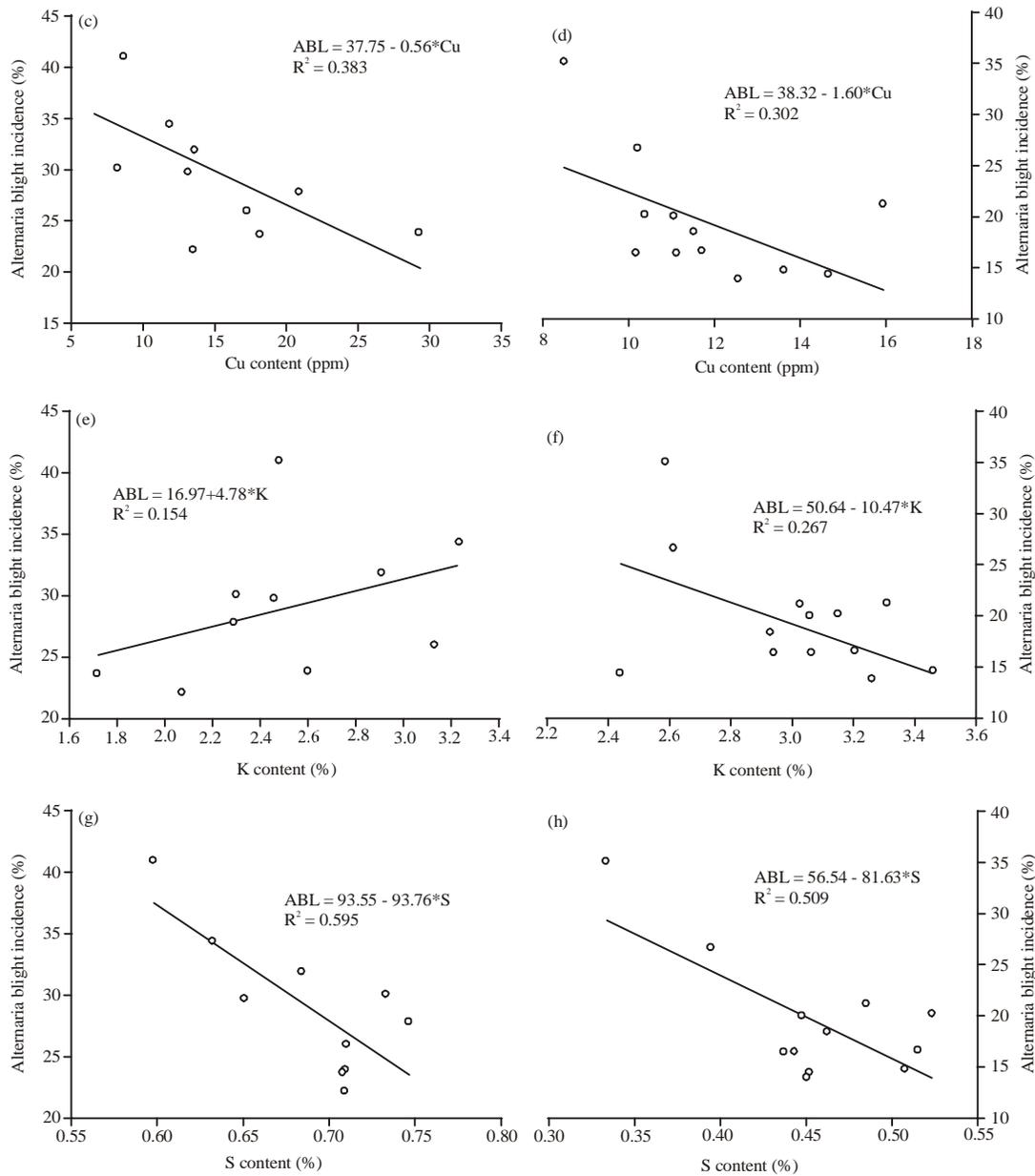


Fig. 1(a-h): Effect of Zn, Cu, K and S content in Indian mustard leaves on percentage of Alternaria blight severity (a, b) Zn content, (c, d) Cu content, (e, f) K content and (g, h) S content in leaves (2009-10 and 2010-11)

results supported with the findings of earlier studies. The mechanism of resistance in some disease-resistant genotypes might be related to a greater efficiency in K uptake (Prabhu *et al.*, 2007). Similar results were observed by Haneklaus *et al.* (2007) with the application of S to deficient soil reduced leaf spot, caused by *Pyrenopeziza* in oilseed rape and stem canker caused by *Rhizoctonia solani* in potato. In case of Zn, low levels in soils and leaf tissues were associated with a high incidence of Phytophthora pod rot (or black pod) of cocoa in Papua New Guinea (Nelson *et al.*, 2011). Copper has direct toxic effects on pathogens as well. Consequently, the use of nutrient which take part in induced

tolerance and lower leaf removal may keep away from the reach of inoculum to the plants, could together perform to minimize AB disease severity in Indian mustard resulted higher seed yield and there adoption could be economically beneficial to the farmers.

CONCLUSION

In conclusion, we have demonstrated that deficiency of nutrients including K, S, Zn and Cu increases the AB susceptibility in *B. juncea* plants because when a plant becomes infected by Alternaria blight, its natural defenses

might be triggered. The infection causes increased production of *Alternaria* inhibiting compounds, both at the site of infection and in other parts of the plant. The production and transport of these compounds is controlled in large part by the nutrition of the plant. Therefore, shortages of key nutrients such as K, Cu, Zn and B diminish the quantity of the plants expected antifungal compounds at the site of infection. The results of the disease severity were observed from leaves of plants without application and after application of nutrients. Even if these effects should appear only minor on an individual plant basis, the effect on a population level could be much more dramatic and has the potential to lead to an increased use of nutrients instead of fungicides. Correspondingly, the use of nutrients facilitate induced tolerance at the same time as lower leaf removal keep away from the reach of inoculum to the plants. Their role to minimize disease severity in Indian mustard resulted higher seed yield and there adoption could be economically and environmental friendly to the mustard farmers.

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