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Exemplification of Manganese with Salicylic Acid on Growth, Antioxidants and Productivity of Indian Mustard (*Brassica juncea* L.)

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ABSTRACT

Background: In India, mustard, an essential oilseed crop, encounters several challenges including diminished yield and environmental apprehensions. The diligent mix of micronutrients and plant growth regulators indicates the potential for enhancing mustard crop output while retaining environmental sustainability. Manganese and salicylic acid play a vital role in plant metabolism.

Methods: The trial was planned with ten treatments with three replications under randomized complete block design. The treatments consisted of control, varying concentrations of salicylic acid (75, 150, 300 ppm), varying concentrations of manganese (0.25, 0.5, 0.75 mM $MnSO_4$) and combinations of both.

Result: The experimental findings indicate the synergistic use of salicylic acid and manganese resulted in enhanced growth dynamics and improved yield potential compared to the separate treatments of manganese and salicylic acid. The exogenous application of 0.5 mM manganese with 150 ppm salicylic acid increased plant height by 10.83%, total dry matter by 49.6%, chlorophyll content by 51.61%, number of siliquae by 81.94%, test weight by 5.8%, grain yield by 41.26% and stover yield by 48.76% over the control. Growth and yield attributes positively correlated with grain and stover yield. Hence the application of both 0.5 mM Mn with 150 ppm SA is feasible for enhancement of Indian mustard productivity.

Key words: Chlorophyll content, Grain yield, Plant growth regulator, Test weight, Total dry matter.

INTRODUCTION

Oilseeds stand out as one of India's most important crops owing to their increased adaptability to a range of agroclimatic conditions. Mustard, a large oilseed crop, is widely produced on most continents. India is leading country in mustard productivity which accounts 9.2 M ha area, 11.7 MMT production with the average productivity 1270 kg ha-1 (USDA, 2024). Rapeseed-mustard ranks second in total oil productivity in India after groundnut. In Punjab mustard accounts 30.5 thousand hectares area, 46.5 thousand tons production and 1524 kg ha-1 productivity (INDIASTAT, 2020). Due to the importance of Mustard and rapeseed oil, it is cultivated in more than 80% of the land during *rabi* seasons of northern and eastern part of the India. The mustard seed has 51.6% fat, 23.11% protein, 9.34% fibre and 8.23% carbohydrate. The average Indian consumes about 14.3 kg of oil, compared to 23 kg globally because most of the land (90% -95%) relies on rain, 80% of which is arid and lack of irrigation systems. Insufficient rainfall, during before its maturity could impact both yield and oil content. Similarly, low productive soils having limited quantity of micronutrients which hamper mustard productivity. Furthermore, there are challenges related to various stress accord during crop growth under changing climate.

Micronutrients are vital for plants in trace amounts for healthy growth and development. These elements have a role in different physiological and metabolic processes. Plants[-1] development and production can all be significantly impacted by an excess or deficit of these elements (Srivastava and Dubey, 2011). Manganese is a micronutrient that is crucial to numerous plant physiological processes, including photosynthesis, enzymatic hormone activation and flavonoid production. Furthermore, manganese is a crucial co-factor for many antioxidants that support oxidative stress tolerance, including Mn-SOD and Mn-CAT. It is a vital mineral for plants when present in sufficient amounts. Understanding manganese's involvement in the growth and productivity of plants is crucial for plants to produce at their full potential and for food security. Similarly, plant defense mechanisms responses against various biotic and abiotic stresses are signaled by endogenous phytohormone development, which is vital for plants (Li *et al.,* 2022). Using its interactions with phytohormones and other signaling molecules, salicylic acid regulates several physiological and biochemical processes, reduces oxidetive damage, alters stomatal behavior, reduces metal absorption, scavenges reactive oxygen species (ROS) and controls nitrogen metabolism. The complex phenomena

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of salicylic acid and abiotic stress interaction in plants is contingent upon the kind of stress experienced by the plant, its speciesand its environmental conditions under changing climate. Adoption of new strategies to improve productivity under changing climate, it is necessary to study the importance of Mn with SA in mustard. There is enough information known on salicylic acid's response to various abiotic stimuli. However, little is known about how varying quantity of salicylic acid reacts with varying levels of Mn in *Brassica juncea*. Thus, the current work seeks to optimize the dose of Mn and SA and their effect on growthand productivity of mustard.

MATERIALS AND METHODS

The experimental study was carried out at Lovely Professional University's experimental field in Punjab, India with 31°15' 29″ North latitude and 75° 42' 28″ East longitudes and elevation of 249 m MSL. Ten treatments with three replications were allocated in randomized complete blocked design. They are- T_1: Control, T_2: 0.25 mM $MnSO_4$, T_3: 0.5 mM $MnSO_4$, T_4: 0.75 mM $MnSO_4$, T_5: 75 ppm SA (Salicylic Acid), T_6: 150 ppm SA, T_7 : 300 ppm SA, T_8 : 0.25 mM $MnSO_4$ + 300 ppm SA, T_9 : 0.5 mM $MnSO_4$ + 150 ppm SA, T_{10}: 0.75 mM $MnSO_4$ + 75 ppm SA. *Brassica juncea* var. GSC 7 (98% genetic purity) was used as test crop with spacing 30 cm × 10 cm during *rabi* season of 2022-2023. Urea, DAP and MOP were used to fulfil the recommended nutrient dose (100:30:15 kg NPK ha^{-1}). As per the treatment combinations, the foliar spray of Mn and SA were applied at 20, 40, 60 and 80 days after sowing.

From each net plot, five random plants were selected and tagged for various growth attributes estimation. The five plants were cut at above ground level and used for dry matter estimation. The plant shoots were sun-dried and then put in an oven at a temperature of 65°C until their weight reached a constant. The dry weight of the shoot was expressed in grams per plant. The crop growth rate was measured with 20 days intervals.

Antioxidants: Chlorophyll ab content (mg g^{-1} FW)

The Acetone method was used for chlorophyll ab measurement. Third leaf from the top of the plant was selected and then grind using an 80% acetone solution. The required quantity was gatheredand the measurement of absorbance was conducted at wavelengths of 645 nm and 663 nm (Arnon, 1949). The following formula was used to do additional computations.

Chl. ab (mg/g FW) =

$$[(8.05 \times A663 + 20.29 \times A645) \times (V/W) \times (1/1000)]$$

Where,

V= Total volume of the extract.

W= Weight of the tissue used for pigment assays.

Statistical analysis

An analysis of variance (ANOVA) was performed to ascertain the effects of varying levels of manganese and salicylic acid on the growth and productivity of mustard. We used the RStudio-based statistical data analysis. The least significant difference (LSD) calculations were used with Duncan's multiple range test (DMRT) to identify the significant differences between the different treatments (Gomez and Gomez, 1984). To determine the relationship between the different characteristics, Pearson's correlation was calculated and presented.

RESULTS AND DISCUSSION

Plant height

Significant maximum plant height was found in 0.5 mM $MnSO_4$ + 150 ppm SA (221.65 cm), which was at par with T_8 and T_{10} (215.81 cm to 217.99 cm) at crop maturity. Significantly lower plant height was found at control. The combined application of Mn and SA produced 7.91 % to 10.83 % higher plant height over the control (Table 1). Manganese significantly affects cell elongation and cell division and increases the photosynthetic rate resulting in higher plant height (Salomon and Keren, 2011). Salicylic acid plays a crucial role in the expansion and division of cells (Guin *et al.,* 2024). It also increases the uptake of nitrogen, phosphorus and potassium which affects the plant height positively (Koo *et al.,* 2020). So, the combined effect of manganese and salicylic acid (T_9) significantly affects the plant height as compared to the control which was found in this experiment.

Total dry matter

The significantly maximum total dry matter accumulation was found in the combined application of Mn with SA (T_9) with 66.5 g $plant^{-1}$, which was statistically similar with T_8 and T_{10} (60.52 g $plant^{-1}$ to 63.4 g $plant^{-1}$). The lowest total dry matter accumulation was found in control. The application of T_9, T_8 and T_{10} produced 36.14% to 49.6% higher total dry matter over the control (Table 1). $MnSO_4$ and salicylic acid both increased the dry matter accumulation through increasing photosynthetic rate. But manganese promotes the accumulation of dry matter not as much as when we apply salicylic acid. The results of our study show that the application of 0.5 mM manganese with 150 ppm salicylic acid resulted in increased total dry matter of the plant. The same results were noticed by Fariduddin *et al.* (2003) in *Brassica juncea*.

Crop growth rate

Foliar application of Mn with SA (T_9) with 21.93 g m^{-2} day^{-1} was show maximum crop growth rate at 80 DAS to 100 DAS. However, T_7, T_8 and T_{10} (17.03 g m^{-2} day^{-1} to 20.67 g m^{-2} day^{-1}) were statistically at par with T_9. Similarly, the combined application of 0.75 mM $MnSO_4$ + 75 ppm SA (T_{10}) was at par with the rest of the sole application of Mn and SA except T_2 and control. The lowest crop growth rate was found in the control. Applying Mn and SA (T_7 to T_{10}) produced 157.24 % to 231.3 % higher crop growth rate over the control (Fig 1). As the dry matter accumulation is increased in the plant, the crop growth rate is also affected positively by the combined foliar spray of manganese with salicylic acid.

Chlorophyll ab content

Varying levels of $MnSO_4$ and Salicylic acid significantly affected the mustard chlorophyll content during 2022-23. Application of Mn with SA (T_9) produced maximum chlorophyll ab content at 40 DAS (1.23 mg g^{-1}), followed by T_8 and T_{10}. Application of $MnSO_4$ with 0.25 mM to 0.75 mM produced more chlorophyll content (0.85 mg g^{-1} to 0.9 mg g^{-1}) over the control (0.81 mg g^{-1}) but lower than the foliar spray of SA with 75 ppm to 300 ppm (0.9 mg g^{-1} to 1.11 mg g^{-1}). The application of T9 had 51.61% higher chlorophyll content over the control (Table 1). Manganese is one of the important micronutrients involved in photosynthesis. It increased the production of chlorophyll content by activating various enzymes that are involved in the biosynthesis pathways of chlorophyll (Marschner *et al.,* 2003 and Korav *et al.,* 2020). Higher concentrations of salicylic acid applied in *Brassica juncea* lead to increased chlorophyll content (Sharma *et al.,* 2017).

Number of siliquae

The maximum number of siliquae was found in the application of both Mn and SA (T_9) (420.99 plant^{-1}), which was statistically at par with T_8 and T_{10} (360.68 plant^{-1} to 370.07 plant^{-1}). Application of Mn with 0.50 to 0.75 mM $MnSO_4$ and 75 ppm SA (259.08 plant^{-1} to 274.28 plant^{-1}) was found statistically at par with each other but lower than T_6 and T_7 (308.98 plant^{-1} to 355.95 plant^{-1}) which are statistically at par. The lowest number of siliquae was found in the control (231.39 plant^{-1}). Combined application of Mn and SA (T_9, T_8 and T_{10}) produced 55.88% to 81.94% more number of siliquae per plant over control (Table 1). Manganese is involved in carbohydrate metabolism and photosynthesis. Efficient photosynthetic rate increased the production of seed, which directly affects the number of siliquae (Bankoti *et al.,* 2021). When salicylic acid (SA) is applied as a foliar spray, it improves yield quantities in *Brassica juncea*. Specifically, it increases the quantity of siliquae (Sharma *et al.,*

Table 1: Effect of foliar spray of manganese and salicylic acid on plant height (cm), total dry matter (g plant^{-1}), chlorophyll ab (mg g^{-1}), number of siliquae (no. plant^{-1}) and test weight (g) of Indian mustard during 2022-2023.

Treatments	Growth attributes			Yield attributes	
	Plant height	Total dry matter	Chlorophyll ab	No. of siliquae	Test weight
T_1: Control	199.99^{d}	44.45^{f}	0.81^{h}	231.39^{e}	3.91^{d}
T_2: 0.25 mM $MnSO_4$	206.98^{c}	48.84^{ef}	0.85^{g}	259.08^{de}	3.95^{d}
T_3: 0.5 mM $MnSO_4$	208.32^{c}	51.13^{de}	0.90^{f}	263.69^{de}	3.97^{cd}
T_4: 0.75 mM $MnSO_4$	210.82^{bc}	52.11^{de}	0.90^{f}	274.28^{de}	4.03^{bc}
T_5: 75 ppm SA (Salicylic Acid)	212.98^{bc}	53.20^{de}	0.90^{f}	293.88^{cde}	4.04^{b}
T_6: 150 ppm SA	213.66^{bc}	56.06^{cd}	1.00^{e}	308.98^{bcd}	4.05^{b}
T_7: 300 ppm SA	213.82^{bc}	59.00^{bc}	1.11^{d}	355.95^{bc}	4.06^{b}
T_8: 0.25 mM $MnSO_4$ + 300 ppm SA	217.99^{ab}	63.40^{ab}	1.19^{b}	370.07^{ab}	4.08^{ab}
T_9: 0.5 mM $MnSO_4$ + 150 ppm SA	221.65^{a}	66.50^{a}	1.23^{a}	420.99^{a}	4.14^{a}
T_{10}: 0.75 mM $MnSO_4$ + 75 ppm SA	215.81^{ab}	60.52^{bc}	1.14^{c}	360.68^{ab}	4.07^{ab}
C.D. (p=0.05)	6.69	5.29	0.01	59.56	0.06

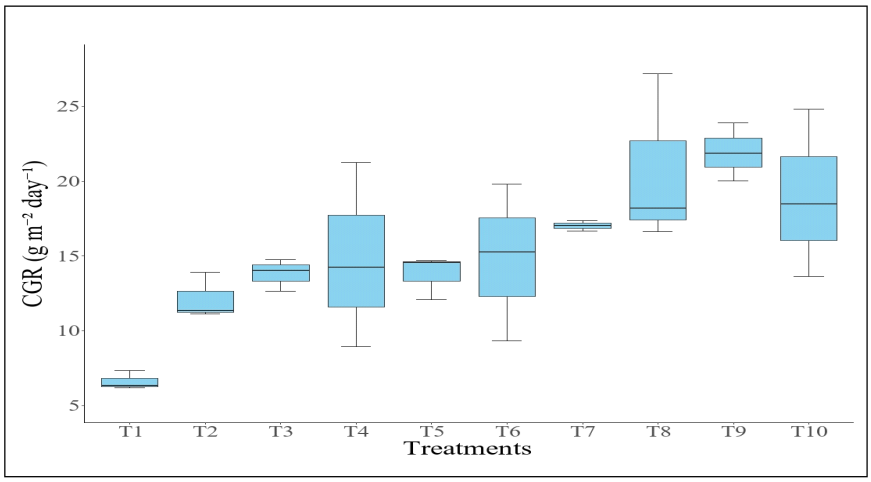

Fig 1: Effect of foliar spray of Manganese and Salicylic acid on crop growth rate (g m^{-2} day^{-1}) of Indian mustard during 2022-2023.

2013). In our results, the same trend was found with the application of 0.5 mM manganese with 150 ppm salicylic acid.

Test weight

Foliar application of Mn and SA (T_9) was superior (4.14 g) over the rest of the treatment in thousand seed weight. However, T_8 and T_{10} (4.07 g to 4.08 g) were statistically at par with T_9. Similarly, the combined application of 0.25 mM $MnSO_4$ + 300 ppm SA (T_8) was at par with the rest of the alone application of Mn and SA except T_3, T_2 and control. The lowest thousand seed weight was found in the control. Applying Mn and SA (T_9, T_8 and T_{10}) produced 4.18% to 5.8 % higher thousand seed weight over the control (Table 1). Research has found that manganese significantly increased the different yield attributes. So, we can say that the test weight is also synergistically affected by the application of $MnSO_4$. Sharma *et al.* (2013) found that the test weight of *Brassica juncea* acts synergistically with the foliar application of salicylic acid. Our experiment also found the same combined effect of manganese and salicylic acid on the test weight of mustard.

Grain yield

Varying levels of $MnSO_4$ and Salicylic acid significantly affected the grain yield of mustard during 2022-23. Application of both Mn and SA (T_9) produced maximum grain yield (2602.03 kg ha^{-1}), which was statistically at par with T_8 and T_{10} (2402.17 kg ha^{-1} to 2520.7 kg ha^{-1}). Application of $MnSO_4$ with 0.25 mM to 0.75 mM produced less grain yield (1914.53 kg ha^{-1} to 1988.2 kg ha^{-1}) than the application of SA with 75 ppm to 300 ppm (2111.07 kg ha^{-1} to 2201.73 kg ha^{-1}) but more than the control (1842.03 kg ha^{-1}). The application of T_8 to T_{10} had 30.41 % to 41.26 % higher grain yield over control (Fig 2). The exogenous foliar spray of MnSO4 directly affects the metabolism of carbohydrates and the rate of photosynthesis which increases the grain yield of *Brassica juncea*. Same way the salicylic acid also improves the grain

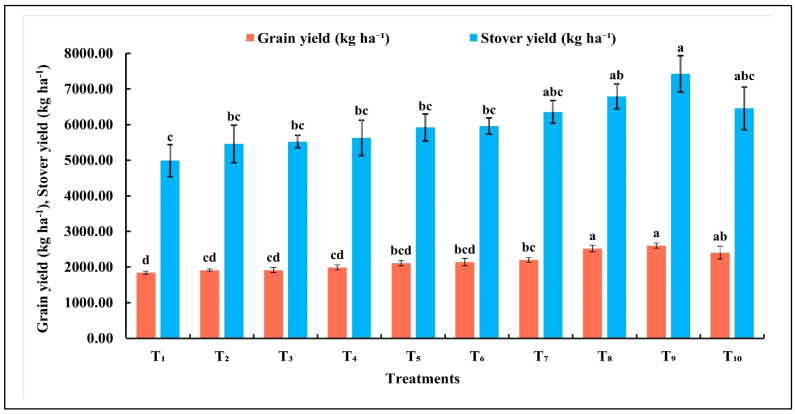

Fig 2: Effect of foliar spray of Manganese and Salicylic acid on grain yield (kg ha^{-1}) and stover yield (kg ha^{-1}) of Indian mustard during 2022-2023.

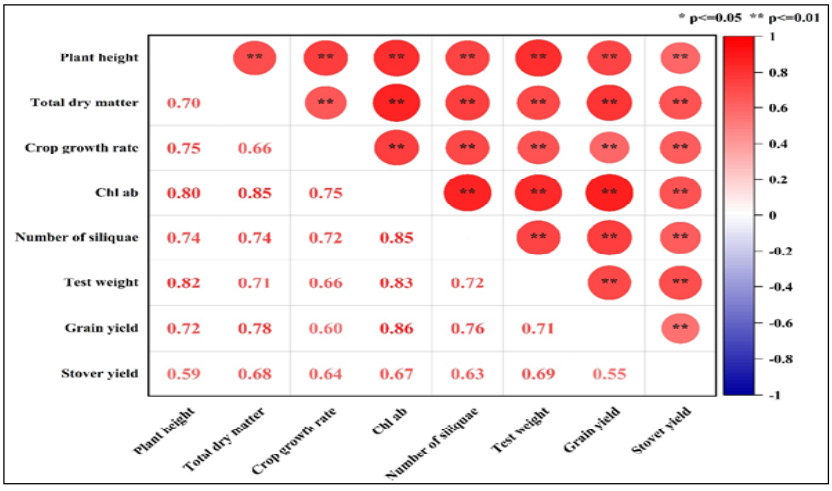

Fig 3: Relationship between crop growth, chlorophyll and yield attributes with grain yield of Indian mustard under exogenous spray of manganese and salicylic acid during 2022-2023.

yield by increasing the rate of photosynthates (Sharma *et al.,* 2013 and Tanin *et al.,* 2023). The same results were noticed in the combined application of manganese and salicylic acid (T_9).

Stover yield

The maximum straw yield was found in the application of Mn with SA (T_9) (7423.27 kg ha^{-1}), which was statistically at par with T_7 - T_{10} (6356.54 kg ha^{-1} to 6789.97 kg ha^{-1}). Application of Mn with 0.25 to 0.75 mM MnSO4 and 75 to 150 ppm SA (5456.6 kg ha^{-1} to 5955.94 kg ha-1) was found statistically at par with each other. The lowest stover yield was found in the control (4989.94 kg ha^{-1}). Combined application of Mn and SA (T_7 - T_{10}) produced 27.39% to 48.76 % more stover yield over control (Fig 2). The stover yield also increased in the same way that total dry matter increased. Manganese and salicylic acid synergistically affect different factors that interact with photosynthesis and increase the dry matter accumulation as well as the stover yield of *Brassica juncea*. The results of our experiment found that exogenous application of 0.5 mM manganese with 150 ppm salicylic acid increased the stover yield.

Correlation studies among growth and productivity of mustard influenced by $MnSO_4$ and salicylic acid

Correlation analysis produces a full view of parameters that work synergistically under the foliar application of MnSO4 and SA. At the end of the experiment, a strong positive correlation was noticed among the different growth parameters and productivity (Fig 3). Various growth attributes like plant height (r= 0.72), total dry matter (r= 0.78), crop growth rate (r= 0.60) and chlorophyll ab content (r= 0.86) are positively correlated with Grain yield. In addition, different yield attributes like the number of siliquae (r= 0.76)and test weight (r= 0.71) were showed a positive correlation with grain yield.

CONCLUSION

The foliar spray of salicylic acid (SA) and manganese (Mn) had a beneficial impact on the growth and yield attributes of Indian mustard. Also, it improves the photosynthesis by enhancing chlorophyll development. The of application of 0.5 mM Manganese with 150 ppm Salicylic acid enhanced grain and stover yield by 41% and 48%, respectively over control. Hence these results are environmentally feasible to use manganese and salicylic acid for enhancing the mustard productivity.

Conflict of interest

All authors declare that they have no conflicts of interest.

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