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ACTIVATION OF DEFENSE SYSTEM OF EUCALYPTUS PLANTS TOWARDS BACTERIAL BLIGHT DISEASE BY USING PLANT ACTIVATORS AND NUTRIENTS

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Abstract

Eucalyptus camaldulensis belongs to family myrtaceae and native to Australia, is a tree with such diverse uses that it can be called a multipurpose tree. Eucalyptus is generally used for the reclamation of problematic soils in various parts of subcontinent. The versatile tree is highly vulnerable to several diseases, with the most serious threat being bacterial blight triggered by Xanthomonas axonopodis pv. Eucalypti. Six plant activators i.e. salicylic acid ($C_7H_6O_3$), benzoic acid ($C_7H_6O_2$), citric acid ($C_6H_8O_7$), dipotassium phosphate (K_2HPO_4), monopotassium phosphate (KH_2PO_4), 2,4 D Isonicotinic acid and nutritional mixture namely Compound (NPK) and Nutritop (Fe, Zn, Cu, B, Mn) were evaluated in field under RCBD and greenhouse under CRD in the Research area, Department of Forestry and Range Management, University of Agriculture Faisalabad. Out of these six plant defense regulators salicylic acid showed significant results and expressed minimum disease incidence (14.167%), as compared to others under greenhouse and (16.185%) field conditions. In nutritional management, mixture of nutrients viz boron (B), magasium (Mg), zinc (Zn), iron (Fe), calcium (Ca) Nutritop and Compound (NPK) were used alone and in combination. Minimum disease incidence (12.57%) was recorded in greenhouse and field (16.88%) when applied in combination (Compound+Nutriotop) as compared to control. The current relationship concluded that salicylic acid among six plant activators and combination of Compound+Nutriotop is a fruitful tool for the integrated management of the disease.

Keywords: Disease Incidence (%), Nutrients, Plant Activators, Bacterial Blight Management, *Eucalyptus Camaldulensis.*

INTRODUCTION

Eucalyptus camaldulensis is the most frequently occurring specie. Naturally, this tree occupies a riparian habitat along watercourses, rivers and water channels in Australia. However, owing to extensive root system this tree has tremendous capacity to acclimatize to temperate and sub-tropical regions where rain is more or less abundant. Moreover, it is the most cultivated tree in the world due to its rapid growth (Elgat et al., 2020; Angane

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et al., 2022). In Pakistan *E. camaldulensis* is commonly known as Sufeda. However, it is most commonly known as River Red Gum (Sabo et al., 2019; Singh et al., 2022).

E. camaldulensis first came to Pakistan in the mid of 20^{th} century, during various afforestation schemes and plans, on millions of hectares all across the country (Zahid, et al. 2010).

Eucalyptus is attacked by a variety of diseases, which are responsible for huge economic losses. Stem canker, pink disease, Crysoporthe stem canker, Amphilogia stem canker, Cytospora stem canker, Lasiodiplodia stem canker and Macrovalsaria stem canker, Coniella and Guignardia leaf spot and bacterial blight are the major diseases of eucalyptus. Among all diseases, bacterial blight, originate by *X. axonopodis* pv *eucalyptorum* (*Xae)*, is a potential threat to Eucalyptus and is responsible for huge economic losses. Water-soaked lesions, with central perforations on the leaves along with interveinal necrosis and defoliation are characteristics symptoms of this disease (Mulyaningsih *et al*., 2011**).** Various management strategies are in practice for the management of plant diseases which includes plant defense activators, nutrients and chemical (Amini and Sindovich, 2010).

In agriculture and forestry, little attention has been received for the management of plant disease by the application of nutrients. The physiological functions of nutrients are commonly well understood but lack of awareness about the connection between plant-pathogens and nutrients (Zekri and Obreza, 2015).

In plants, nutrients play vital role in minimizing disease level and ultimately increase plant growth and resistance (Ahmad et al., 2016). Occurrence of pathogen interrupt the physiological processes of plants by retarding nutrient uptake which results in nutrient deficiencies (Ryan et al., 2011). So in current study effects of macro and micro nutrients alone and in combinations were evaluated towards bacterial blight of eucalyptus.

Another management strategy is to boost up resistance in eucalyptus through application of plant defense activator like salicylic acid, jasmonic acid and citric acid etc (Francis *et al.,* 2009). Plant defense activators provide signals which activate the defense system of plants after the application of salicylic acid that activate the genes involved in defense mechanism of plants (Vidyashekaran et al., 2004).

These plant defense activators enhanced the resistance of plant against pathogen through signal transduction molecules like flavonoids, alkaloids and various phenolic compounds (Freeman and Beatie, 2008).

The defense system induced by these activators provide long term immunity against wide range of pathogens (Erb et al*.,* 2012). Plant activators do not possess antibiotic activity. Moreover, their contrary effects on human health and environment are negligible. So, due to their effectiveness, in the current study, plant defense activator and nutrients were evaluated against bacterial blight of eucalyptus.

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MATERIAL AND METHODS

Isolation, Purification, Identification and preservation of Pathogen.

Leaves of *E. camaldulensis* showing typical symptoms of bacterial leaf blight were collected in brown paper bag (10" x 12") and brought to Phytobacteriology laboratory for the isolation of the pathogen associated with disease (Ruangpan and Tendencia, 2004). Nutrient agar media was prepared for the isolation of bacterial pathogen. For the preparation of one liter NA (Nutrient Agar) media, 29g of synthetic NA was added into 1000 mL of water in a flask (1000mL), and mix them thoroughly and autoclaved (RTA85, Robus United Kingdom) it at 121^{o}C temperature and 15 Psi pressure for 15minutes. After this, autoclave was turned off and when media temperature reduced to 60^{o}C, nilstat (Antifungal) was added to prevent fungal contamination. Infected leave samples along with some healthy portion were cut into small pieces (2-3mm). These samples were washed with tap water to remove dust.

These leaves surface were sterilized with 1% (NaOCl) solution for 30 sec followed by three consecutive washings with sterilized distilled water for the removal of toxicity of sodium hypochlorite. After that, these leaves were dried on a sterilized tissue paper/ blotted paper. After this, media was poured into Petri plates (90mm) in laminar air flow chamber (RTVL-1312, Robus United Kingdom) (Hemraj *et al*., 2013).

After the solidification of media, samples were placed in Petri plates and these plates were wrapped, labeled and placed in an incubator (D-180) for 24 hours at 25-28^{o}C (Riaz *et al.,* 2008). Yellow ooze (a form of bacterial growth) was developed under the diseased samples after 24-36 hours. For the purification of bacteria, culture was transferred to new plates by using streaking method (Ranjan *et al.,* 2021). After that, Petri plates were wrapped and incubated at 25 ^{o}C.

Pathogenicity Test

Koch's postulates were followed for the conformation of the pathogen associated with bacterial blight disease of Eucalyptus (Juhasz et al., 2013).

For the pathogenicity test, *E. camaldulensis* plants were grown in pots (18 cm diam.) containing field soil (sandy clay) previously sterilized with a 1% solution of formalin (Goswami et al., 2010). All plants were irrigated and covered with a polythene sheet before inoculation. These covered plants were placed in a sunny environment because maximum stomata were opened under this condition. Inoculation was done early in the morning (when a maximum number of stomata were opened) under the greenhouse using the syringe method (tuberculin of 1 cm3).

Suspension of the bacteria (about two µL) was injected into the plant leaves (three areas of leaf on each side including mid vein) (Francis et al., 2009). When symptoms developed, the pathogen was isolated from the infected leaves sample, and it was compared with the parental pathogen based on morphological (size, shape, and sporulation) and taxonomical (growth pattern and color) characteristics (Amaral et al., 2014).

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Management of bacterial leaf blight disease through nutrients application under greenhouse and field conditions

One-year old Eucalyptus plants were grown in the pots (32×18cm) and arranged them under (CRD) on wooden table in greenhouse and inoculated them with bacterial suspension (1×10^6 CFU/mL of H_2O) Using Hydroponic syringe method (Model Number: 23G×1”). A mixture of macro (NPK) and micro (B, Zn, Fe, Mg, Cu) nutrients with trade name compounds, Nutritop and their combination (Compound and Nutritop) were applied by using simple hand sprayer early in the morning (when maximum stomata were opened). After one week of interval data regarding disease incidence was recorded for three times.

One year old *E. camaldulensis* plants were transplanted in the experiment area of Department of Forestry and Range Management under RCBD design. A mixture of compound Nutritop alone and in combination was evaluated against the bacterial leaf blight disease. Before the application of nutrients, inoculum was applied on plants through foliar application @1×10^6 CFU/mL. After one week interval data regarding disease incidence was recorded and calculated by using following formula: (Lee *et al*., 2000; Ashfaq, 2014).

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Number of total plants}} \times 100$$

Management of bacterial leaf blight disease of *Eucalyptus camaldulensis* through the application of plant defense activators under green house and field conditions

Plant defense activators like KH_2PO_4, K_2HPO_4, Citric acid, Benzoic acid, salicylic acid and 2,4- D Isonicotinic acid were evaluated under greenhouse conditions. For this purpose, One-year old plant of *E. camaldulensis* were grown in pots (32×18 cm) and placed in the greenhouse by using Completely Randomized Design (CRD). For this purpose, inoculum was applied through syringe method early in the morning (when maximum no of stomata were opened). After that, three concentration of plant defense activator (0.25, 0.75 and 1%) were prepared. Then, these prepared concentrations of plant defense activator were applied on eucalyptus plants. Data regarding disease incidence were recorded after first, second and third sprays with 10, 20 and 30 days interval of spray.

Further in field condition effective plant defense activator alone and in combination were evaluated under field conditions under randomized complete block design (RCBD). For this purpose, inoculum was applied through spray method early in the morning. Disease incidence was recorded on new flushes of leaves after ten days interval. In control, each plant was treated with sterilized water after the inoculation (Huang and Hsu, 2003). Data regarding disease incidence (%) was recorded 10, 20 and 30 days interval and calculated by using following formula;

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Number of total plants}} \times 100$$

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RESULTS

Assessment of plant activator towards bacterial leaf blight of eucalyptus under greenhouse conditions

Among treatments salicylic acid indicated minimum disease incidence (14.167%) followed by citric acid (17.185%), K_2HPO_4 (18.685%), benzoic acid (20.167%), KH_2PO_4 (21.648%), 2,4 D Isonicotinic acid (24.185%) as compared to control (60%) (Table 1, Fig. 1). Interaction between T×C expressed that maximum disease incidence was recorded at 0.25 % concentration of salicylic acid as compared to 0.50 percent and 0.75% , K_2HPO_4, benzoic acid, KH_2PO_4 and 2,4 D Isonicotinic acid disease incidence when applied @ 0.25, 0.50 and 0.75% concentrations respectively (Fig. 2). The interaction of treatment and days revealed that all treatment such as salicylic acid, citric acid , K_2HPO_4, benzoic acid, KH_2PO_4, 2, 4- D Isonicotinic acid expressed minimum disease incidence as compared to control at day 30 followed by day 20 and day 10 respectively (Fig. 3).

Table 1: Effect of plant activators on the development of bacterial leaf blight of eucalyptus under greenhouse conditions

Sr.#	Treatments	Chemical Formula	Disease incidence (%)
1	Salicylic acid	$C_7H_6O_3$	14.167g
2	Citric acid	$C_6H_8O_7$	17.185f
3	K_2HPO_4	K_2HPO_4	18.685e
4	Benzoic acid	$C_7H_6O_2$	20.167d
5	KH_2PO_4	KH_2PO_4	21.648c
6	2,4 -D Isonicotinic acid	$C_6H_5NO_2$	24.185b
7	Control	H_2O	60.000a
	LSD	**0.4640**	

Mean values in a column sharing similar letters do not differ significantly as determined by LSD test (P≤.0.05) 2,4-D. Isonicotinic acid =2,4-Dichloro Isonicotinic acid

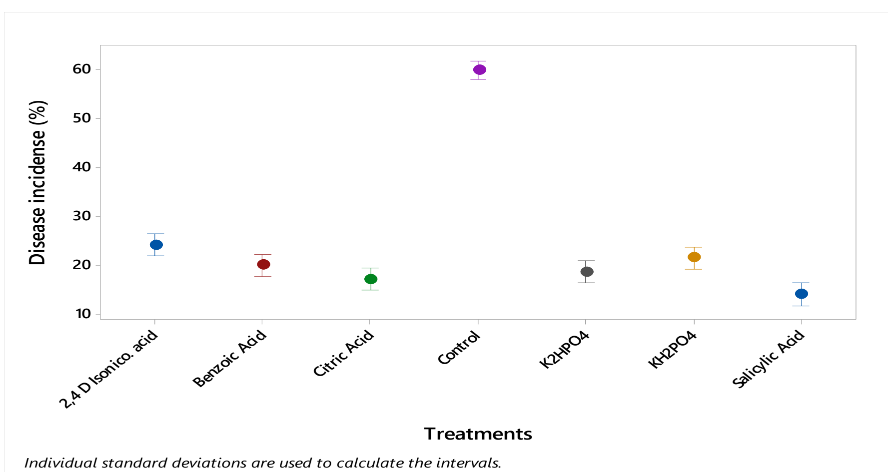

Fig 1: Effect of plant activators on the development of bacterial leaf blight of eucalyptus under greenhouse conditions

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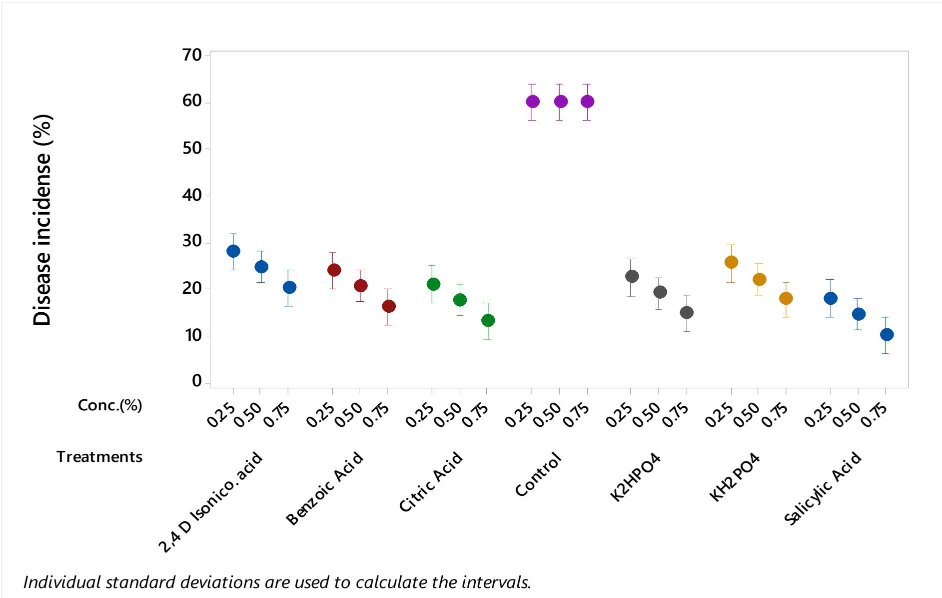

Fig 2: Effect of plant activators and their concentrations on the development of bacterial leaf blight of eucalyptus under greenhouse conditions

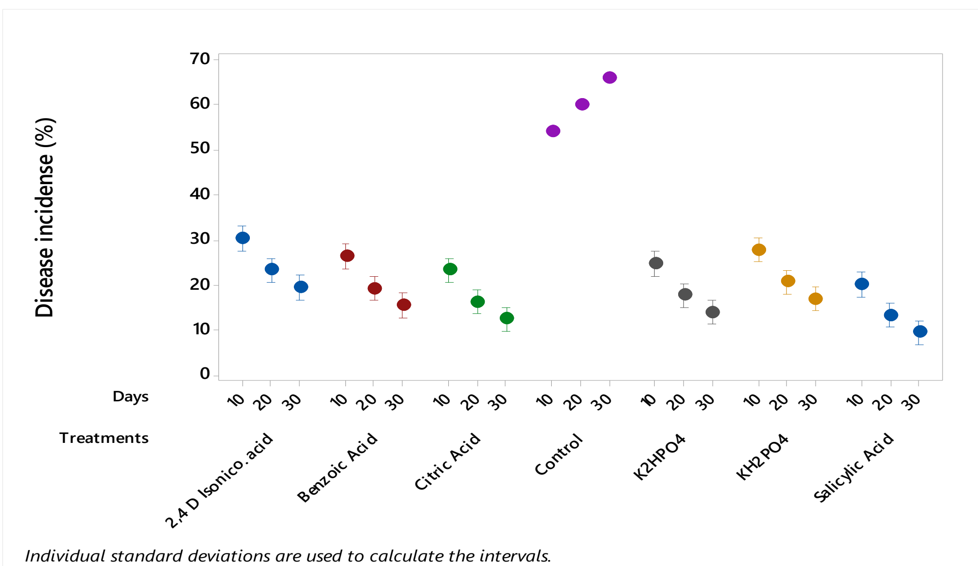

Fig 3: Evaluation of interaction between treatments and days (T×D) on the development of bacterial leaf blight of eucalyptus under greenhouse conditions

Evaluation of plant activator towards bacterial leaf blight of eucalyptus under field conditions

Among treatments salicylic acid expressed minimum disease incidence (16.185%) as compared to citric acid (20.185%), K_2HPO_4 (20.685%), benzoic acid (23.185%), KH_2PO_4 (24.648%), 2,4-D Isonicotinic acid (27.259%) and control (70%) (Table 2, Fig. 4). The

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interaction of treatment and concentration factors showed that salicylic acid exhibited maximum disease incidence at 0.25% concentration while, same treatment showed maximum disease incidence at 0.50 and 0.75% concentration respectively, where citric acid expressed, K_2HPO_4, benzoic acid, KH_2PO_4 and 2,4-D Isonicotinic acid expressed maximum disease incidence at 0.25, 0.50 and 0.75% concentrations respectively (Fig. 5). The interaction of treatment and days showed that all treatment viz. salicylic acid citric acid K_2HPO_4 benzoic acid , KH_2PO_4 , 2,4-D Isonicotinic acid and control recorded minimum disease incidence as compared to control at day 30 followed by day 20 respectively (fig. 6).

Table 2: Effect of plant activators on the development of bacterial leaf blight of eucalyptus under field conditions

Sr.#	Treatments	Chemical Formula	Disease incidence (%)
1	Salicylic acid	$C_7H_6O_3$	16.185
2	Citric acid	$C_6H_8O_7$	20.185
3	Di potassium hydrogen phosphate	K_2HPO_4	20.685
4	Benzoic acid	$C_7H_6O_2$	23.185
5	Potassium di hydrogen phosphate	KH_2PO_4	24.648
6	2,4-D Isonicotinic acid	$C_6H_5NO_2$	27.259
7	Control	H_2O	70.00
	LSD	**0.4822**	

Mean values in a column sharing similar letters do not differ significantly as determined by LSD test (P≤.0.05) 2,4-D Isonicotinic acid = 2,4-Dichloro Isonicotinic acid

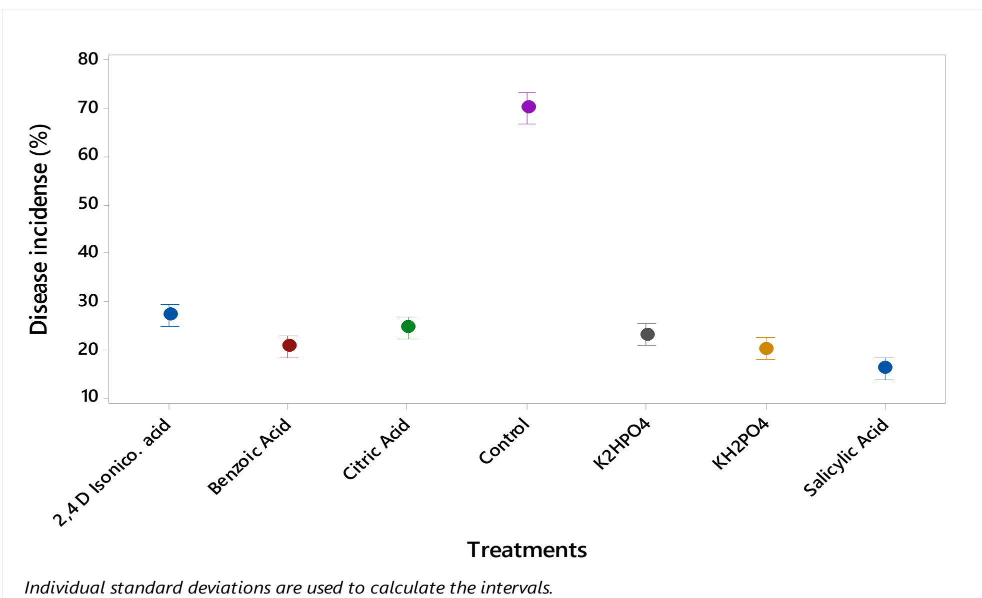

Fig 4: Effect of plant activators on the development of bacterial leaf blight of eucalyptus under field conditions

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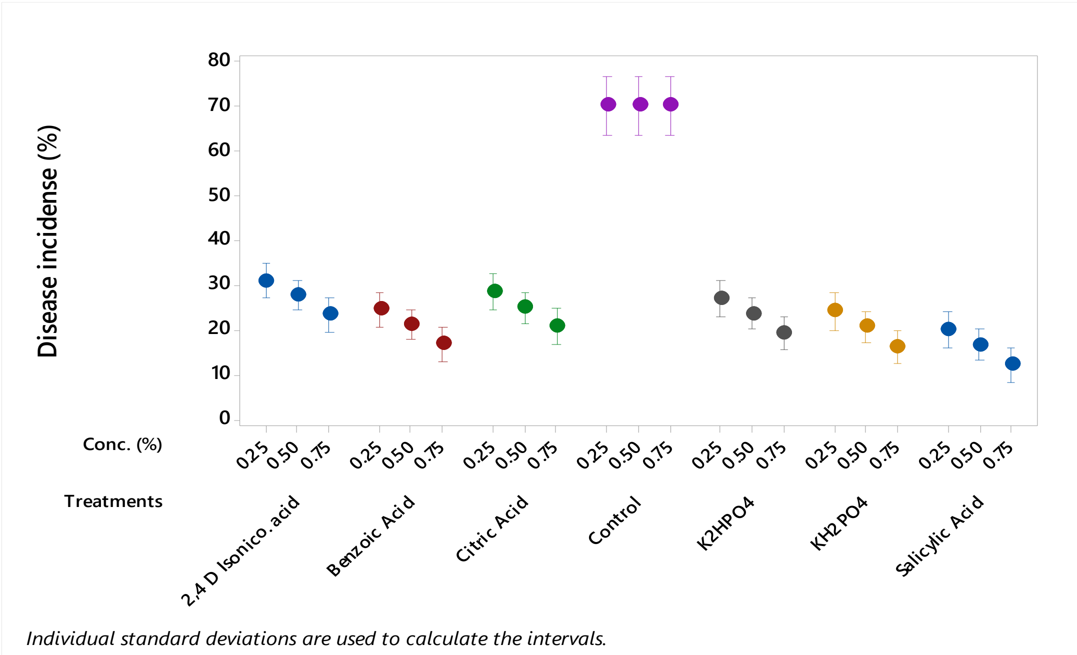

Fig 5: Effect of interaction between treatment and concentration on the development of eucalyptus under field conditions

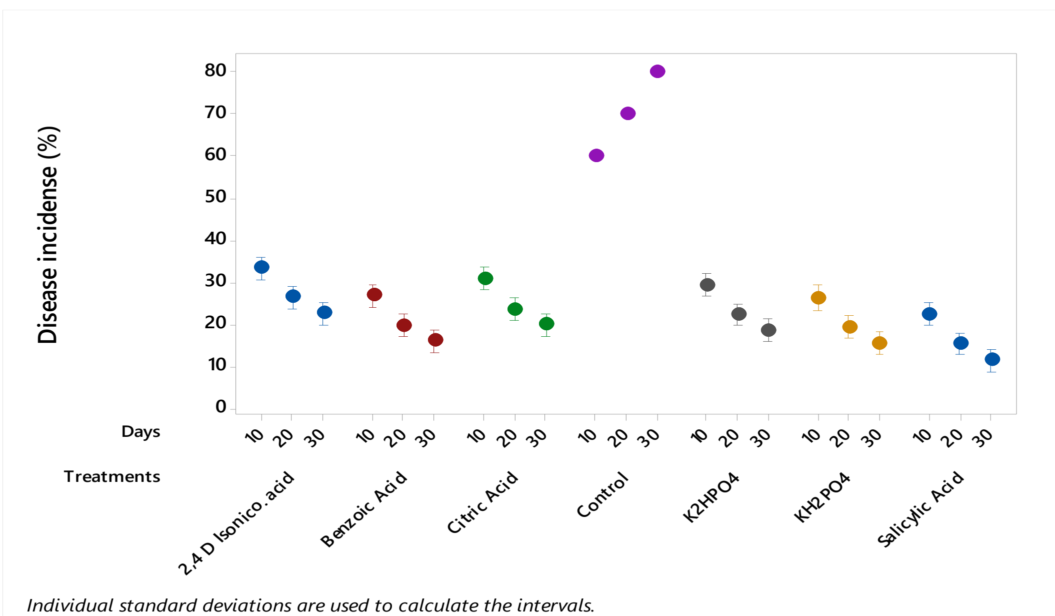

Fig 6: Effect of interaction between treatments and days (T×D) on the development of bacterial leaf blight of eucalyptus under field conditions

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Evaluation of macro and micro nutrients against bacterial leaf blight of eucalyptus under greenhouse conditions

Compound+Nutritop (12.574%) exhibited minimum disease incidence followed by Compound (21.870%) and Nutritop (27.278%) as compare to control (57.315%) (Table 3, Fig7). The interaction of treatment and concentration exhibited that minimum disease incidence (8.611%) was observed at C3 of Compound+Nutritop as compared to C2 and C1 while, Compound , Nutritop 31.444, 27.222, and 23.167%, and Control 57.444, 57.222, and 57.278% disease incidence at C1, C2, and C3 concentrations respectively (Table 27, Fig. 20). The treatment and days interaction showed that all treatments including Compound+Nutritop (9.722%), Compound (19.278%), and Nutritop (23.944%) exhibited minimum diseases incidence as compare to control (60.667%) at day 30 followed by day 20 and day 10 (15.278, 24.611, 30.667, and 54%) respectively (fig.9)

Table 3: Evaluation of macro and micro nutrients against bacterial leaf blight of eucalyptus under greenhouse conditions

Sr.#	Treatments	Chemical formula	Disease incidence (%)
1	Compound + Nutritop	NPK + Fe, Cu, Zn, Mg,Bo	12.574d
2	Compound	NPK	21.870c
3	Nutritop	Fe, Cu, Zn, Mg, Bo	27.278b
4	Control	H_2O	57.315a
	LSD	**0.3783**	

Mean values in a column sharing similar letters do not differ significantly as determined by LSD test (P≤.0.05)

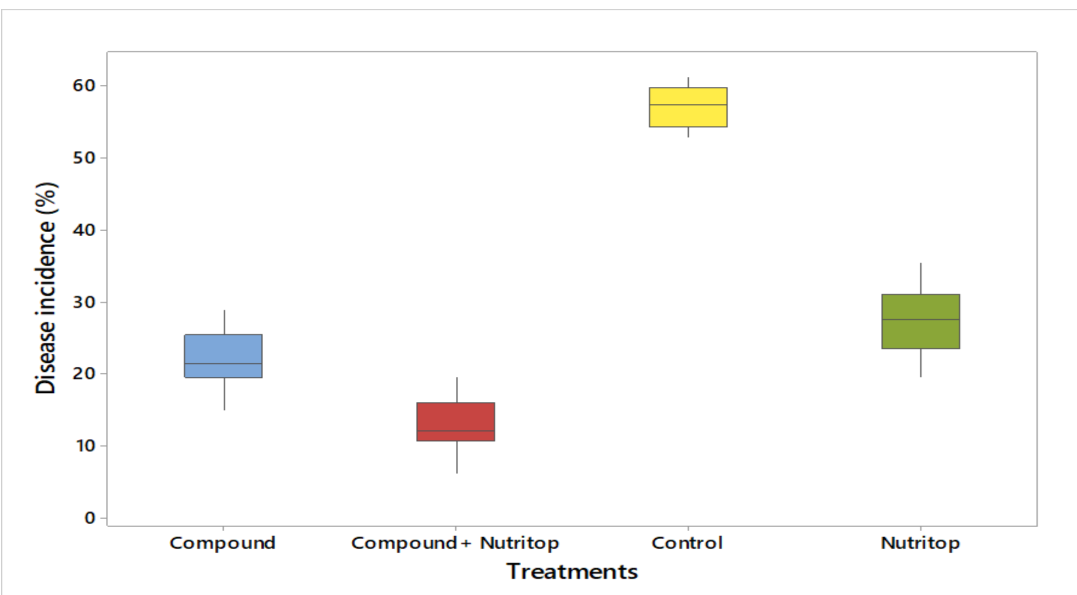

Fig 7: Evaluation of macro and micronutrients against bacterial leaf blight of eucalyptus under greenhouse conditions

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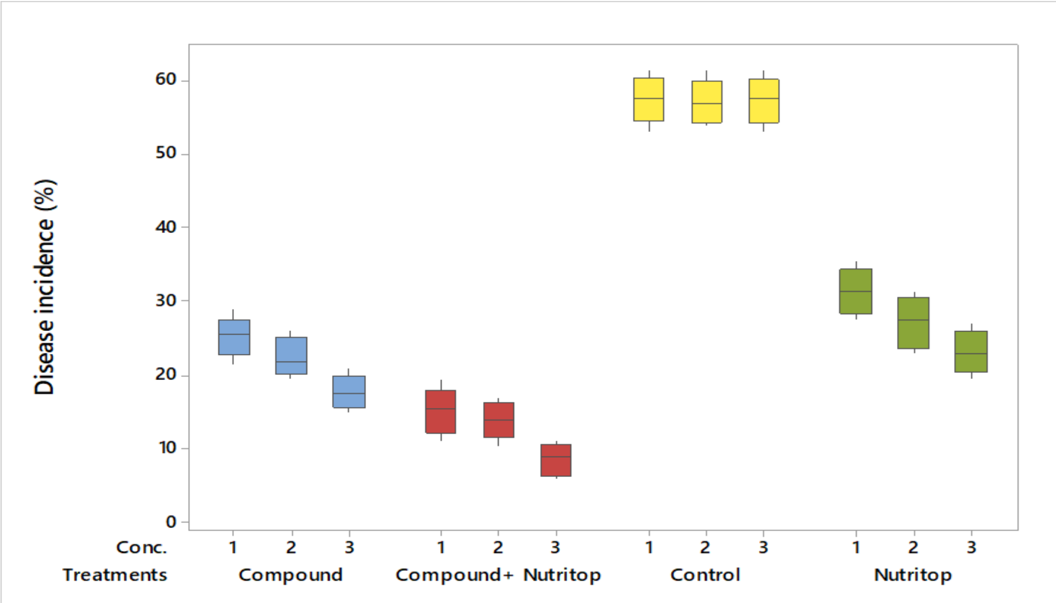

Fig 8: Evaluation of macro and micro nutrients against bacterial leaf blight of eucalyptus under greenhouse conditions

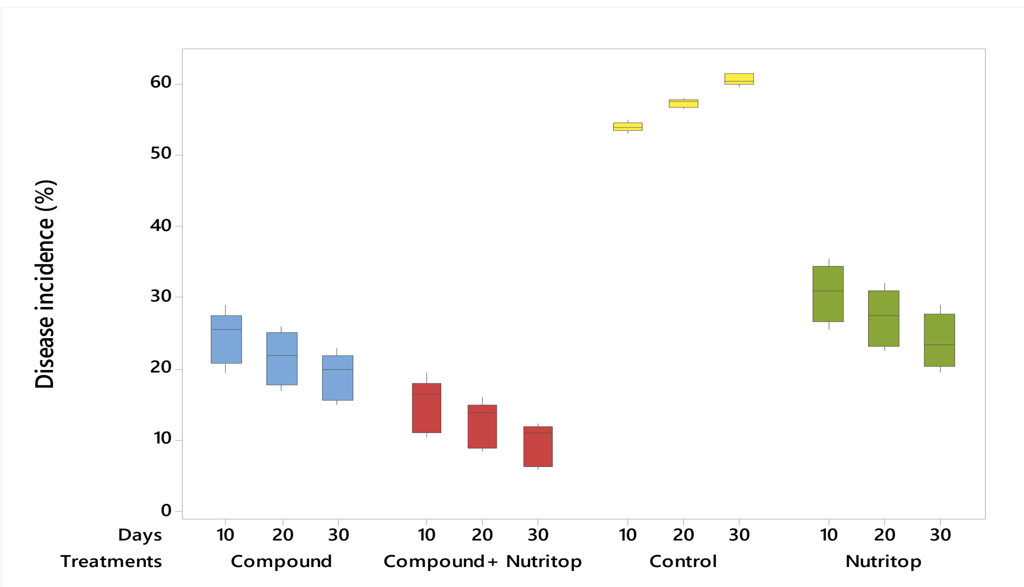

Fig 9: Impact of treatments × days on the development of bacterial leaf blight of eucalyptus under greenhouse conditions

Estimation of macro and micro nutrients against bacterial leaf blight of eucalyptus under field conditions

Compound+Nutritop (16.889%) exhibited minimum disease incidence followed by Compound (25.963%) and Nutritop (32.278%) as compared to control (57.944%) (Table

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3, Fig 10). The interaction of treatment and concentration exhibited minimum disease incidence at C3 of Compound+Nutritop as compared to C2 and C1, while Compound and Nutritop alone exhibited maximum disease incidence at C1, C2, and C3 concentrations respectively (Fig. 11). The treatment and days interaction showed that all treatments including Compound+Nutritop Compound and Nutritop exhibited minimum diseases incidence as compare to control at day 30 followed by day 20 and day 10 respectively (Fig 12).

Table 4: Evaluation of nutrients against bacterial leaf blight of Eucalyptus under field conditions

Sr.#	Treatment	Chemical formula	Disease incidence (%)
1	Compound + Nutritop	NPK + Fe, Cu, Zn, Mg, Bo	16.889d
2	Compound	NPK	25.963c
3	Nutritop	Fe, Cu, Zn, Mg, Bo	32.278b
4	Control	H_2O	57.944a
	LSD	**0.459**	

Mean values in a column sharing similar letters do not differ significantly as determined by LSD test (P≤.0.05)

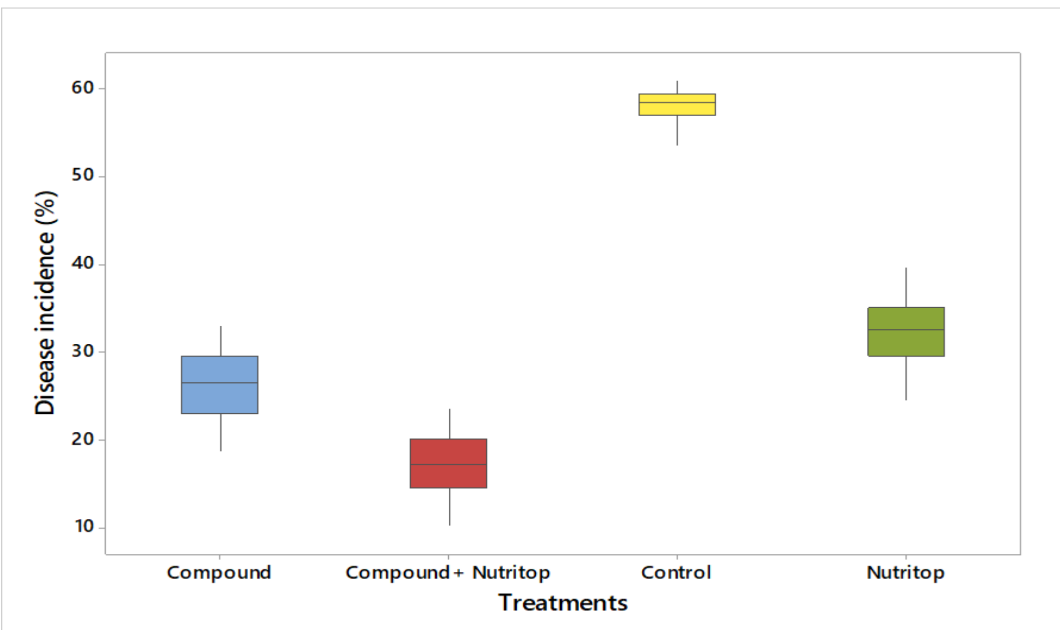

Fig 10: Evaluation of nutrients against bacterial leaf blight of eucalyptus under field conditions

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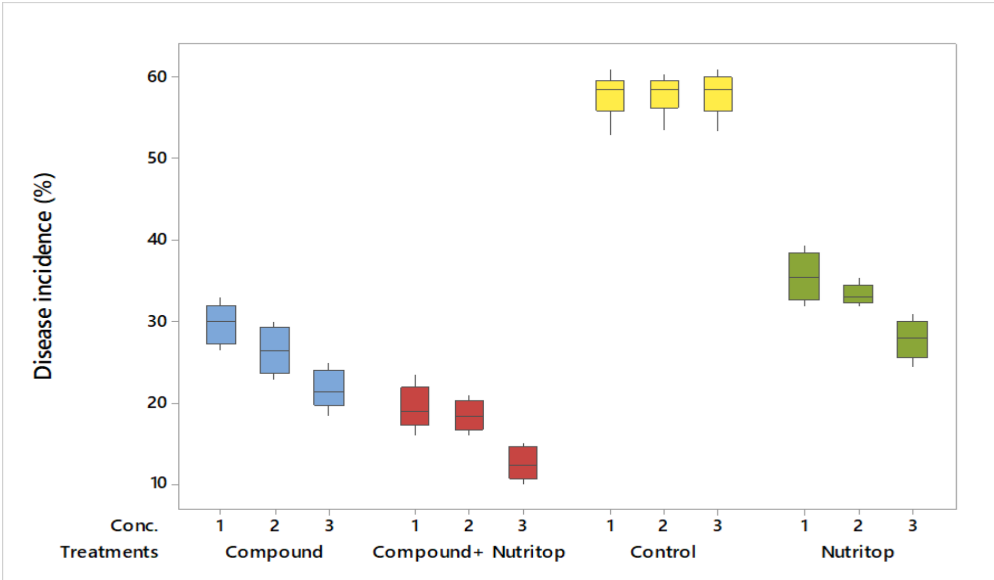

Fig 11: Evaluation of T ×C on the development of bacterial leaf blight of eucalyptus under field conditions

	C_1	C_2	C_3
*Compound	@ (4000	5000	6000 mL/ha)
*Nutritop	@ (1000	1200	1400 mL/ ha)
*Compound + Nutirtop	@ (3000	4000	5000 mL/ha)

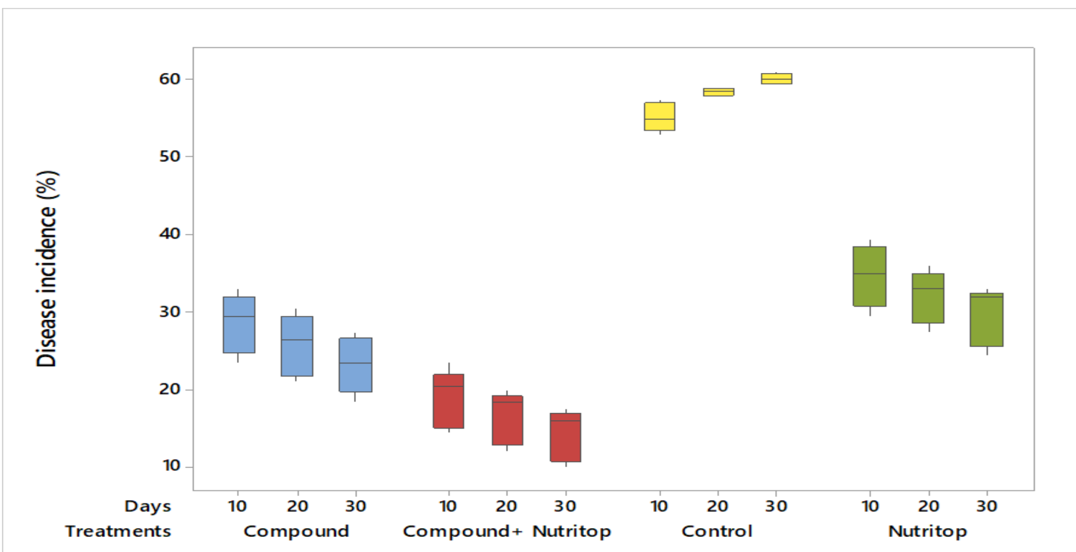

Fig 12: Evaluation of T × D on the development of bacterial leaf blight of eucalyptus under field conditions

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DISCUSSION

Eucalyptus's bacterial blight is one of the most distressing leaf disease, mostly this disease attacked plants in nursery and in fields. In nurseries, the emergence of leaf lesions and subsequent defoliation renders plants unsuitable for transplantation to fields. In the current study, six plant defense activators were tested towards bacterial leaf blight of eucalyptus under greenhouse and field conditions. Among six plant defense activators, salicylic acid expressed minimum incidence of bacterial leaf blight as compared to other activators and control. Results of the present study are in agreement with the findings of Hanieh et al. (2013) and sarwar *et al.* ((2005) who evaluated different plant defense activators towards bacterial disease and conclude that salicylic acid expressed promising results towards bacterial diseases due to enhancement of phyto-hormones like indole acetic acid which is responsible to cause resistance in plants towards diseases (Hayat *et al.,* 2005). Similarly activity of resistant genes towards diseases also increases in the presence of sufficient quantity of salicylic acid (Howard *et al.,* 2000; Khan *et al.,* 2012). Due to activation of plant defense system, there is increase production of reactive oxygen species, defense related protein, phytoalexins takes place in the host plant due to application of salicylic acid, which expressed resistance towards bacterial leaf blight of eucalyptus (Hayat and Ahmad, 2007; Vinod and Sabah, 2018). Similar results were also reported by Wang and Liu, (2012) who also reported decrease in incidence of bacterial diseases due to application of salicylic acid.

The current study appraised the effectiveness of nutrients (Compound+Nutriotop) in combination and alone applied on diseased plant. It was observed that minimum disease incidence was recorded when compound and Nutritop applied in combination form. Minerals play vital role in plant disease management as these have key role in the physiology, resistance mechanism and biochemical reaction taking place in the plants. Nitrogen is the main part of the protein and play crucial role in the growth of the plant. Application of nitrogen in plants, activate the plant defense genes that boost up plant defense system (showalter, 1993). Phosphorous is known as energy unit of plant that provide systematic resistance against pathogens by regulating the various metabolic process in plants (Huber *et al*., 2012). Potassium play key role in the growth and developmental phases of the plants and play important role in various metabolic reactions occurring in the plants (Rengel and damon, 2008; white, 2013). Calcium is another important minerals present in the plants. It is the main component of middle lamella which strengthen the cell wall and inhibits the degradation of cell wall from enzyme secreted by plant pathogens (Nadeem *et al.,* 2018). Zinc is an important part of enzymatic systems that is necessary for overcome oxidative stress (Zekri and obreza, 2016). Iron is important for the growth and development of plants as it is involved in production of chlorophyll and activate defense genes that stimulate plant defense system Copper is an important micronutrients for plants that is involved in seed production, chlorophyll synthesis and provide resistance to plants (Noules *et al*., 2018).

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When pathogen attacks on the host plant, it changes its physiological functions like nutrients uptake and utilization along with water translocation in different parts of the plant (Khan *et al.,* 2012). Because nutrients play a prime role in the growth and development of the host plant by activating its metabolic processes and cellular functions which are necessary to cause resistance towards invading pathogen and process of disease development (Saikia *et al.,* 2009) (Ahmad et al., 2013). Sufficient application of NPK and Zn, B, Fe, Ca, Mg, Cu and Fe cause reduction in the disease incidence due an important part of co-enzymes which activate different metabolic processes to resist towards invading pathogen (Suharja and Sutarno,2009). As pathogen attack reduce the availability of these nutrient that make the host plant vulnerable towards bacterial pathogens. So, in current study macro and micro nutrients namely compound and nutritop were evaluated towards bacterial blight of eucalyptus. It was observed that there is reduction in the intensity of bacterial leaf blight when compound and nutritop were used in combination. Outcomes of the present study are in agreement with the findings of Kusakabe *et al.,* 2006.

CONCLUSION

The present study offers valuable insights into the management of bacterial leaf blight using nutrients and plant activators. Salicylic acid demonstrated the most effective result among all tested plant defense activators, surpassing citric acid, K_2HPO_4, benzoic acid, KH_2PO_4 , 2,4-D Isonicotinic acid both under greenhouse and field conditions. Similarly, another managing strategy was used. Likewise the combined application of nutrients in the form of Compound+Nutriotop demonstrated minimal disease incidence in both greenhouse and field conditions, in comparison to the application of Nutriotop and compound alone.

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