Fungitoxic effect of inorganic salts for the management of seed borne *Macrophomina phaseolina* and *Fusarium* sp. causing charcoal rot and wilt disease in blackgram

N. Indra*, A.S. Kauvyashree, D. Swetha, M. Asmina and Shalini

Seed Centre, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.

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**ABSTRACT**

The laboratory experiments were carried out to study the effect of different salts *viz.*, potassium chloride (KCl), potassium phosphate dibasic (K\(_2\)HPO\(_4\)), sodium carbonate (Na\(_2\)CO\(_3\)), sodium bicarbonate (NaHCO\(_3\)) and ammonium molybdate (NH\(_4\))\(_2\)MoO\(_4\)) on seed borne *M. phaseolina* and *Fusarium* sp. as an alternative to synthetic fungicides for the control of charcoal rot and wilt diseases in blackgram. The evaluation of different salts was performed in vitro using various concentrations *viz.*, 0.5, 1.0, 2.0, 4.0 and 8.0 per cent (w/v). Among the salts tested against *M. phaseolina*, sodium carbonate, sodium bicarbonate and ammonium molybdate at 0.5, 2.0 and 4.0 per cent respectively inhibited the fungal growth. Among the salts tested against *Fusarium* sp. sodium carbonate (Na\(_2\)CO\(_3\)) at 4.0 per cent concentration recorded complete inhibition of the mycelial growth compared to the other salts. Also seed priming of these salts significantly reduced the seed borne infection due to *M. phaseolina* and *Fusarium* sp. under standard blotter test. The salts like potassium chloride and potassium phosphate dibasic (K\(_2\)HPO\(_4\)) at all concentrations did not inhibit *M. phaseolina* and *Fusarium* sp. which recorded 100 per cent mycelial growth as that of control.

**Key words:** Ammonium molybdate, Blackgram, *Fusarium*, *Macrophomina phaseolina*.

**INTRODUCTION**

Black gram (*Vigna mungo*) L. Hepper belonging to the Family fabaceae is an important pulse crop in India. It plays an important role in human diet as a rich source of protein. India is one of the principle pulse growing countries in the world with an area of about 23.9 million hectare and with an average production of 15.8 MT per year. About 88% of proteins consumed in India are of vegetable origin and pulses contribute about 17-43% of vegetable proteins and also as good forage and grain concentrates in cattle food. Despite growing pulses in a large area, the production and average yield/ha is very low of 656 kg/ha (Annual report, 2017-18, Ministry of Agriculture).

The pulse seeds in general are heavily infected by many different seed borne fungi and the associated fungal flora causes deterioration of seed and seed contents. *Macrophomina phaseolina* is a fungal pathogen inciting a stem canker disease in blackgram that is often referred as charcoal rot. The pathogen is a seed borne and seed to seedling transmission has been documented in infected seeds (Pun *et al.*, 1998). *Macrophomina* infection causes both pre and post emergence plant mortality. The fungicidal method of seed treatments are the most commonly practiced method to protect the seeds from seed borne fungi. On the other hand, the use of fungicides has risen concern due to the impact on environment, human and other living organisms.

*Corresponding author’s e-mail: nindra73@yahoo.com*

The use of natural compounds such as organic and inorganic salts is one of the best alternative methods for controlling the disease. Organic and inorganic salts have low mammalian toxicity and are widely used in the food industry as preservatives, pH regulators, antimicrobial agents (Oliver *et al.*, 1998). Non conventional chemicals provide nutrients to the host plant which affect the relationship between crop and pathogen in many ways. Some chemicals were reported as resistance inducers against plant diseases. Induced resistance by chemicals is a promising approach to prevent diseases caused by soil borne pathogens (Okubara and Paulitz, 2005). Treating seeds with resistance inducers may represent a novel approach to control diseases caused by soil borne pathogens.

**MATERIALS AND METHODS**

**Isolation and identification of black gram seed borne pathogens:** The blackgram seeds (cv. CO6) were collected from the Department of Pulses, Tamil Nadu Agricultural University, Coimbatore and preserved and used for the studies. The seed borne pathogens (*Macrophomina phaseolina, Fusarium* sp) from blackgram seeds were isolated on PDA medium by agar plate method in seed health laboratory, Seed Centre, Tamil Nadu Agricultural University, Coimbatore.

**Agar plate method:** Ten ml of sterilized PDA medium of pH 5.6 was poured in pre sterilized borosil glass Petri plate of 9 cm diameter. The Petri plate was allowed to cool at...
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room temperature. Then 25 seeds of test samples were placed at equidistance under aseptic condition. The plates were then incubated at room temperature for 5 days. The seeds were then examined under microscope for the preliminary determination of seed mycoflora. The Macrophomina phaseolina and Fusarium infection found on each and every seed were detected and identified. The identification of the isolated fungi was verified according to their morphological features using the description of Barnett and Hunter (1998). The fungi were isolated, purified using the hyphal tip method (Hildebrand, 1938). The pure cultures were maintained on PDA slants kept at 5°C for further studies.

**Evaluation of various salts on the mycelial growth of Macrophomina phaseolina and Fusarium sp.:** The effect of various salts on the mycelial growth of Macrophomina phaseolina and Fusarium sp. was assayed according to Meacteau et al. (2002). The salts with different concentrations viz., 0.5, 1.0, 2.0, 4.0, 8.0 per cent w/v were added to autoclaved and cooled PDA medium at 50°C. Ten ml of the medium was dispensed aseptically into 9mm diameter Petri plates. The plates without the salts were used as control. The mycelial disc of 5mm diameter from 7 day old fungal cultures of M. phaseolina and Fusarium sp. were placed in the centre of the plates. The plates were then sealed with parafilm and incubated at 24±1°C for 5 days. The mycelial growth was measured at two perpendicular colony diameters until the growth in the control plates reached the edge of the plates. The mycelial growth values were converted into inhibition per cent of mycelial growth inhibition (MGI) in relation to control using the formula,

\[
\text{MGI (\%)} = \frac{[\text{dc}-\text{dt}]}{\text{dc}} \times 100
\]

Where dc and dt represents mycelial growth diameters in control and salt amended Petri plates respectively. Each treatment was replicated three times.

**Effect of salts on the seed borne diseases:** The blackgram seeds were soaked in the salts (w/v) viz., sodium carbonate (0.5%), sodium bicarbonate (2.0%) and ammonium molybdate (4%) for 4 hours. The seeds were drained, shade dried and brought to the original moisture condition. The seeds were stored in autoclaved polythene bags and used for further studies. The seed borne infection of Macrophomina phaseolina and Fusarium sp. were detected by moist blotter plate method as recommended by ISTA (1996), Neergaard (1971) and Agarwal (1981). Four hundred seeds were tested with 100 seeds per replication.

**Moist blotter plate method:** A pair of blotter papers and a filter paper of 8.5 cm diameter were jointly soaked in sterile distilled water and placed in pre sterilized plastic Petri plates of 9 cm diameter. Twenty five seeds were placed at equal distance aseptically on the moist blotter paper with 16 seeds on the outer circle, 8 seeds in the inner circle and 1 seed in the middle. The plates were then incubated for 7 days at 22± 2°C under 12 h of alternating cycles of light and darkness. After incubation, the M. phaseolina and Fusarium sp. infection on each seed was examined under stereomicroscope for the seed infection. The per cent disease incidence was worked out by using the formula

\[
\text{PDI} = \frac{\text{NO. of seeds infected}}{\text{Total No. of seeds}} \times 100
\]

**RESULTS AND DISCUSSION**

**Isolation and identification of seed borne pathogens:** The experiment was conducted to isolate and identify the seed borne pathogens Macrophomina phaseolina and Fusarium sp. from blackgram seeds. The pathogens were isolated, identified as per the keys suggested by Neergaard and Saad, 1962. The pure culture was maintained in the agar slants for further studies.

**Evaluation of salts on the mycelial growth of Macrophomina phaseolina and Fusarium sp.:** The results (Table 1) revealed that among the various salts tested with different concentrations viz., 0.5, 1.0, 2.0, 4.0 and 8.0 per cent under invitro conditions, the linear growth of Macrophomina phaseolina was completely inhibited by sodium carbonate at 0.5 per cent followed by sodium bicarbonate and ammonium molybdate at 2.0 and 4.0 per cent respectively. The per cent inhibition of the mycelial growth over control was recorded maximum (100 %) in the above treatments (Fig 1). The complete inhibition of the linear growth of the mycelium of Fusarium sp. was recorded in sodium carbonate at 4.0 per cent (Table 2) and the per

| Table 1: Evaluation of various salts on the linear growth of mycelium of Macrophomina Phaseolina. |
|---------------------------------------------------------------|
| **Concentration (%)**                                      |
| **Mycelial growth (cm)**                                   |
| **Salts**                                                   | 0.0 | 0.5 | 1.0 | 2.0 | 4.0 | 8.0 |
| Potassium chloride                                         | 9.0 | 9.0 | 9.0 | 9.0 | 9.0 | 9.0 |
| Potassium phosphate dibasic                                 | 9.0 | 9.0 | 9.0 | 9.0 | 9.0 | 9.0 |
| Ammonium molybdate                                         | 9.0 | 4.6 | 3.1 | 2.0 | 0.0 | 0.0 |
| Sodium bicarbonate                                         | 9.0 | 7.3 | 1.9 | 0.0 | 0.0 | 0.0 |
| Sodium carbonate                                           | 9.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| SEd                                                        | -   | 0.46| 0.22| 0.13|-   |-   |
| CD (0.05)                                                  | -   | 1.04| 0.50| 0.29|-   |-   |
Table 2: Evaluation of various salts on the linear growth of mycelium of Fusarium sp.

| Salts              | Concentration (%) | Concentration (cm) |
|--------------------|-------------------|--------------------|
|                    | 0.0               | 0.5                | 1.0 | 2.0 | 4.0 | 8.0 |
| Potassium chloride | 9.0               | 9.0                | 9.0 | 9.0 | 9.0 | 9.0 |
| Potassium phosphate dibasic | 9.0 | 9.0 | 9.0 | 9.0 | 9.0 | 9.0 |
| Ammonium molybdate | 9.0               | 4.3                | 4.4 | 5.0 | 4.2 | 3.5 |
| Sodium bicarbonate | 9.0               | 7.4                | 4.3 | 3.2 | 3.2 | 2.0 |
| Sodium carbonate   | 9.0               | 4.5                | 3.3 | 1.5 | 0.0 | 0.0 |
| SED                | -                 | 0.47               | 0.25 | 0.38 | 0.24 | 0.04 |
| CD (0.05)          | -                 | 1.05               | 0.56 | 0.84 | 0.55 | 0.10 |

Fig 1: Effect of inorganic salts on the per cent inhibition of M. phaseolina.

Fig 2: Effect of inorganic salts on the per cent inhibition of Fusarium sp.

Per cent inhibition was also recorded maximum (100 per cent) (Fig 2). Potassium chloride and potassium phosphate dibasic had no significant effect in inhibiting the seed borne pathogens Macrophomina phaseolina and Fusarium sp. The results are in line with the reports of Turkkan (2013) that potassium sulphate and sodium carbonate strongly reduced the mycelial growth of Fusarium oxysporum f. sp. cepae whereas potassium phosphate dibasic had greatest stimulatory effect. High salt concentration lowers the production of mycelial, conidial formation and sporulation of fungi through the effects of negative osmotic potential as well as toxic and nutritional effects. Jones et al. (2011) reported that the conidial germination and the mycelial growth of Verticillium species were declined under reduced osmotic potential. Similar findings were also made by Gouderzi et al. (2008) on the sporulation of Pythium oligandrum and Macrophomina phaseolina. Nunes et al. (2001) reported that ammonium molybdate inhibited the spore germination of Penicillium expansum and Botrytis cinerea in vitro. Bicarbonates of potassium, sodium and sodium metabisulphite showed highest antifungal activity against Fusarium oxysporum, Alternaria alternata and Botrytis cinerea (Muharrem and Ismail, 2014; Masoud zaber, 2014). Jabnoun-Khiareddine et al. (2016) reported that potassium sorbate at concentrations above 0.25% completely inhibited the tomato fungal pathogens.
Effect of salts on the seed infection of *Macrophomina phaseolina* and *Fusarium* sp.: The results (Fig 3) revealed that the blackgram seeds treated with the salts *viz.*, sodium carbonate, sodium bicarbonate and ammonium molybdate at 0.5, 2.0 and 4.0 per cent respectively recorded complete reduction of the *Macrophomina* infection on the blackgram seeds which causes post emergence rotting of the seedlings. Similarly, the seeds treated with sodium carbonate (4%) recorded nil incidence of *Fusarium* infection on seeds in blotter method compared to control (Fig 4). The results are in line with the reports of Pham *et al.* (2001) that rice seeds treated with natri-tetra borate reduced the disease incidence from 19-27 % in greenhouse and about 7% neck blast incidence under field condition. The treatment of basil seeds with chemical inducer $K_2HPO_4$ resulted in the reduction of disease severity of *Botrytis fabae* (Mirostera *et al*., 2002).

It is possible that the chemical agents elicit the release of a signal triggers the plants general response, the signal may also affect the expression of the defense genes which make the plant more responsive after subsequent infection (Kuc, 1990). El-Mougy and Abdel Kader (2009) reported that sodium bicarbonate or calcium chloride significantly reduced the early blight incidence and severity of tomato plants in pot experiments under artificial infestation with pathogenic fungus. Kostandi and Soliman (1998) stated that the effect of saline irrigation containing NaCl or Na$_2$SO$_4$ reduced the smut occurrence by 22.7% & 10.8% respectively. The salinity resulted in soil suppressiveness to *F. oxysporum* a fungal agent of vascular wilt disease through reduced germination of conidia and mycelial growth (Amir *et al.*, 1996).

**CONCLUSION**

In this study, it was observed that inorganic salts tested possess variable antifungal activity invitro depending on fungal pathogens and concentrations used. Based on these findings, it could be concluded that the salts like sodium carbonate, sodium bicarbonate and ammonium molybdate at respective doses when applied as seed priming completely suppressed the *Macrophomina phaseolina* and *Fusarium* sp. infection on black gram seeds and enhance plant growth. However, this is only a preliminary study and the efficacy of these salts needs to be investigated in natural environmental conditions. The findings of the present investigation will provide a non toxic and environmentally safe option for alternative control of phytopathogenic fungi.

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