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DEVELOPMENT OF A Na-ALGINATE-BASED BIOFORMULATION AND ITS USE IN THE MANAGEMENT OF CHARCOAL ROT OF SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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Abstract

The treatment of sunflower seeds with Na-alginate in combination with Ca-carbonate and carboxymethyl cellulose (CMC) showed maximum increase in plant length and weight followed by seed coated with Na-alginate in combination with Ca-carbonate and gum arabic. Maximum control of *Macrophomina phaseolina* infection was recorded when seeds were coated with Na-alginate in combination with Ca-carbonate and gum arabic followed by Na-alginate in combination with Ca-carbonate and CMC. In another experiment when sunflower seeds were coated with Na-alginate at 1, 2 and 4% w/w with or without *Trichoderma viride*, *T. resei*, significant increase in germination was recorded when seeds were coated with *Trichoderma* species using Na alginate at different concentrations as a sticker. Plant length and weight was significantly higher in treated seeds as compared to non treated control. Seeds coated with *T. viride* using 2% Na-alginate as a sticker showed maximum increase in plant length and weight followed by seed coated with *T. viride* in combination with 1% Na-alginate. Maximum vigor index were observed in sunflower seeds treated with *T. viridi* using 2% Na-alginate.

Introduction

Macrophomina phaseolina (Tassi) Goid., the charcoal rot fungus known to attack more than 500 species of plants (Sinclair, 1982, Shahzad *et al.*, 1988) is one of the most serious pathogens affecting sunflower plants in Pakistan (Mirza & Beg, 1982; Ghaffar, 1988). Sunflower plants infected by *M. phaseolina* showed a reduction in crude oil, ash content and weight of 100 seeds (Conte *et al.*, 1998) and protein content of seed besides producing considerable changes in the fatty acid composition of oil (Pustovoit & Bordin, 1983). Continuous use of fungicides may develop fungicide resistance to pathogen, cause environmental contamination and produce mutagenic affect in human, plants and animal (Rajavel, 2000).

In the previous studies several microbial antagonists and biocontrol agents have shown promising results in the control of soil-borne pathogens (Ghaffar, 1978, 1988, 1992). *Trichoderma* has gained considerable success (Denis & Webster, 1971). *T. harzianum* protects the root system against *F. solani*, *R. solani* and *M. phaseolina* infection on a number of crops (Malik & Dawar, 2003). Application of microbial antagonists on soil provide the antagonists a direct entry into the infection site (Windels *et al.*, 1985). Corncobs and pyrophyllite considerably enhanced the biocontrol activity (Fravel *et al.*, 1995). Mycophilic fungus *Trichoderma* is among the most frequently used antagonists (Estrella & Chet, 1998). Treatment of seeds not only promote establishment of seedling, also increase yield and educe quality of losses caused by plant pathogens. The characteristics of fungi to control fungal diseases made them an important part of disease protection. Seed treatment effectively control the pathogens survive on seed surface and inside seed. There are two type of seed treatment i.e., contact and systemic. Contact helps to control pathogens present on seed surface and systemic control seed borne pathogen present inside seed (Martha *et al.*, 2003). Formulation of biocontrol agents may greatly affect the success of biocontrol (Fravel & Lewis, 1992). Formulation can also influence the length of time, the biocontrol agent can be stored and survival and proliferation of the biocontrol agent in soil (Papavizas *et al*., 1987). Alginate prill have been used to deliver several biocontrol agents (Fravel *et al.*, 1986). Sodium alginate is commonly used in many food products (Connick, 1979) any residue in plant or soil should not be toxic.

Present work was designed to study the development of a Na-alginate based bioformulation of *Trichoderma* species in the management of charcoal rot of sunflower.

Materials and Methods

Soil was collected from experimental plots of Department of Botany, University of Karachi. The soil was sandy loam (Sand, Silt, Clay, 70, 19, 11, 1), pH ranged from 7.5-8.1 with moisture holding capacity (MHC) of 40% (Keen & Raczkowski, 1922), total nitrogen 0.077-0.099% (Mackenzie & Wallace, 1954), total organic matter 4.17-4.59%. The soil had a natural infestation of sclerotia of *Macrophomina phaseolina* (4-6 sclerotia/g soil) (Sheikh & Ghaffar, 1975). In an experiment, soil was placed in plastic pot of 8 cm diam. Seeds of sunflower var. Hysun-39 were surface sterilized by 5 % of household bleach $(NaOCl)_2$ for 5 minutes, washed three times in sterile distilled water, dry in a laminar airflow for 2 hr prior to coating. After sterilization, sunflower seeds were coated with Na-alginate, calcium carbonate separately and in combination with carboxymethyl cellulose (CMC), or starch or sabudana or gum arabic. Non treated seeds served as control and there were three replicates of each treatment. Pots were kept in a randomized fashion at the screen-house of Botany Department. After 30 days of seedling emergence plant growth parameters in terms of plant length, plant weight, vigour index and root incidence with *M. phaseolina* was recorded.

In another experiment, seeds of sunflower after surface sterilization with $(NaOCl)_2$ coated with Na-alginate @ (1, 2 & 4% w/w) alone or in combination with *T. viride*, and *T. resei* separately and 5 seeds were sown in each pot containing 300 g soil. The pots without treated seeds served as control and there were three replicates of each treatment. Pots were kept in a randomized fashion at the screen-house of Botany Department, University of Karachi. After 30 days of seedling emergence plant growth parameters in terms of plant length, plant weight, vigour index and root incidence with *M. phaseolina* was recorded. Data were analyzed using one way analysis of variance (ANOVA). Standard error was calculated for each treatment (Sokal & Rohlf, 1995).

Results and Discussion

An experiment was designed to find out the effectiveness of sodium alginate based formulation for growth and control of charcoal rot fungus *M. phaseolina* on sunflower. Seeds of sunflower were coated with Na-alginate alone, sodium alginate + calcium carbonate, sodium alginate + CMC+ $CaCO_3$, sodium alginate + starch + $CaCO_3$ and sodium alginate + sabudana + $CaCO_3$ and sodium alginate + gum arabic + $CaCO_3$. Results obtained showed that seeds of sunflower coated with sodium alginate based formulation did not show any adverse effect on germination. Similarly, Barrett (1978), Connick (1979, 1982), Goodwin & Somerville (1974) prepared chemical herbicides formulation with Na alginate and certain cations such as Ca^{++} in the form of gel. As sodium alginate used in many food products and any residue in plant or soil should not be toxic. Present result showed that seed coated with sodium alginate + $CaCO_3$ + CMC gave maximum increase in plant length and weight ($p<0.001$) followed by seed coated with sodium alginate + gum arabic + $CaCO_3$ (Fig. 1). Maximum vigor index was observed when seeds were coated with sodium alginate + $CaCO_3$ +CMC followed by seed coated with sodium alginate + $CaCO_3$ whereas maximum reduction ($p<0.001$) in *M. phaseolina* was recorded when seeds of sunflower were coated with gum arabic + sodium alginate followed by sodium Alginate + CMC + $CaCO_3$ (Fig. 1).

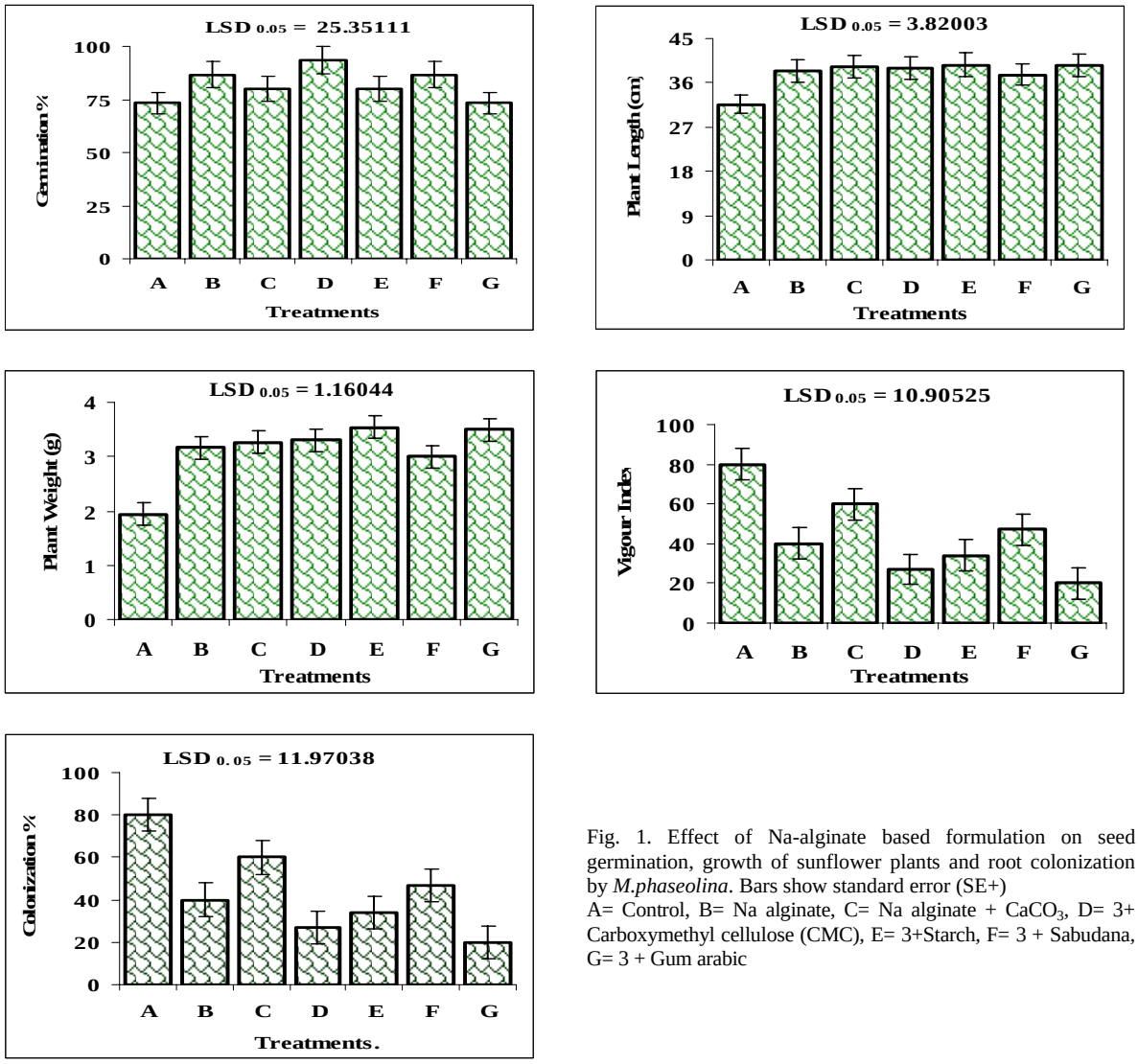

Fig. 1. Effect of Na-alginate based formulation on seed germination, growth of sunflower plants and root colonization by *M.phaseolina*. Bars show standard error (SE+)
A= Control, B= Na alginate, C= Na alginate + $CaCO_3$, D= 3+ Carboxymethyl cellulose (CMC), E= 3+Starch, F= 3 + Sabudana, G= 3 + Gum arabic

In the other experiment sunflower seeds were coated with *T. viride* using sodium alginate @ 1, 2 and 4% w/w as a sticker (containing 36 x 10^4, 31 x 10^4, 34 x 10^4 conidia/seed respectively), *T. resei* (containing 6 x 10^4, 4 x 10^4 and 9 x 10^4 conidia/seed respectively). Significant ($p<0.001$) increase in germination was recorded when seeds were coated with *Trichoderma* species (Fig. 2). Certain strains of *Trichoderma* are known to stimulate plant growth (Chang, 1986). 1 and 2% sodium alginate showed maximum increase in germination followed by seed coated with 4% sodium alginate alone and with *Trichoderma* species (*T. resei*, *T. viride*). A wide range of bioactive material can be incorporated in sodium alginate using an aqueous system in an ambient temperature suggested that this method would enable us to produce pelletized formulation of mycoherbicides (Connick, 1979). Presently it was observed that plant length ($p<0.001$) and weight ($p<0.0001$) were significantly higher in treated seeds as compared to non treated seeds (Fig. 2). Seeds coated with *T. viride* using 2% sodium alginate as a sticker showed maximum increase in plant length and weight followed by seed coated with *T. viride* using sodium alginate as a sticker (Fig. 2). Kucuk & Kivanc (2005) prepared a formulation of *T. harzianum* and observed the effect of formulation on its conidia. Similarly Papavizas *et al.* (1987) reported a successful formulation of mycelium of *Talaromyces flavus* in alginate pellete. Maximum suppression in colonization of roots of *M. phaseolina* was recorded when seeds were coated with *T. resei* using 1% sodium alginate as a sticker (Fig. 2). Similarly Fravel *et al*., (1986) found that *T. viride* decreased the incidence of root rot in barley plant by 65% and an increase in number of productive stem/sq mm. Kolombet *et al*., (2001) recorded that Chinese cabbage grown on soil containing conidia of *T. viride* had considerably greater weight of shoot and root. Present results showed that maximum vigor index was observed in seed treated with *T. viride* using 2% sodium alginate as a sticker (Fig. 2).

Sodium alginate which is relatively cheap and harmless to the environment can be used as formulation and delivery of a biocontrol fungus with sodium alginate provide better results which would be useful for obtaining the better yield of crops.

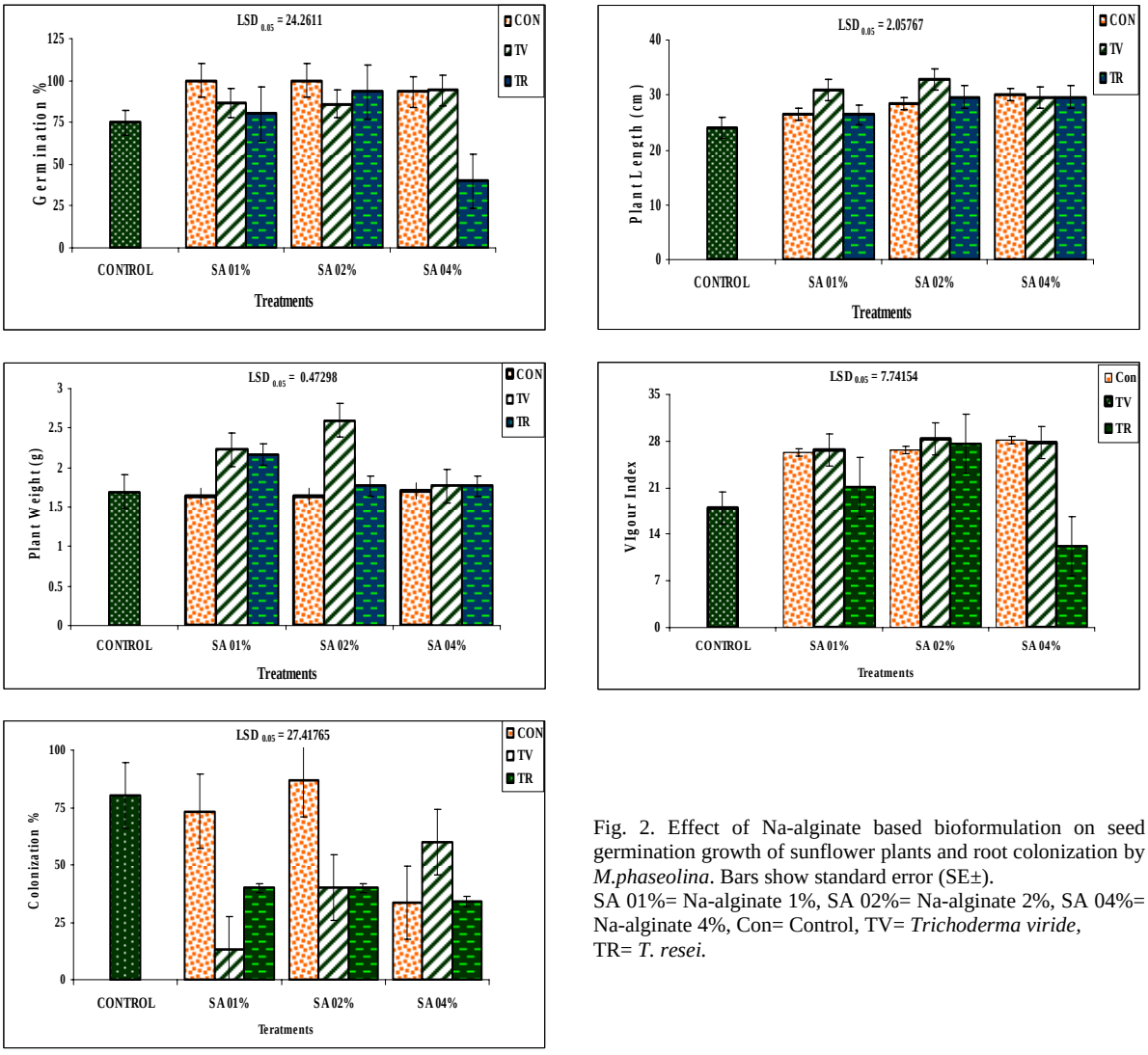

Fig. 2. Effect of Na-alginate based bioformulation on seed germination growth of sunflower plants and root colonization by *M.phaseolina*. Bars show standard error (SE±).
SA 01%= Na-alginate 1%, SA 02%= Na-alginate 2%, SA 04%= Na-alginate 4%, Con= Control, TV= *Trichoderma viride*, TR= *T. resei.*

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