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Use of non-conventional chemicals as an alternative approach to protect chickpea (*Cicer arietinum*) from *Sclerotinia* stem rot

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Abstract

Four non-conventional chemicals, viz., zinc sulphate (ZS), oxalic acid (OA), sodium malonate (SM) and sodium selenite (SS), were applied as foliar sprays to chickpea (*Cicer arietinum*) and the plants were subsequently challenged against *Sclerotinia sclerotiorum*, the causal agent of stem rot in chickpea. All the chemicals reduced mortality of chickpea from *S. sclerotiorum* infection. Among them, ZS at 10^{-3} mmol gave the best result as only 13.6% mortality was recorded after 28 d compared to 100% in the control. High performance liquid chromatographic analysis of treated chickpea leaves revealed activation of shikimic acid as well as phenyl propanoid pathways and synthesis of several phenolic compounds increased specially after application of OA, ZS and SM. Individual treatment of the chemicals showed better results than their combinations as plant mortality was reduced and accumulation of phenolics increased in their individual treatments. A positive correlation was observed between induction of phenolic compounds and survival of the plants. *In vitro* assay of the four chemicals showed only SS to be antifungal. The protection of plants by ZS, OA and SM is possibly because of induction of resistance in the host against *S. sclerotiorum*.

Keywords: Non-conventional chemicals; Phenolics; Induced resistance; *Sclerotinia sclerotiorum*

1. Introduction

Sclerotinia sclerotiorum (Lib.) de Bary affects yield in several economically important crops by inciting serious stem or root rot diseases in more than 500 plant species comprising mostly dicotyledonous plants (Willetts and Wong, 1980). Various control measures are adopted to manage the pathogen in fields. Moore (1949) reported that flooding fields caused decay of sclerotia of *S. sclerotiorum*. Crop rotation was also found effective in managing *Sclerotinia* rot (Schwartz and Steadman, 1978). Wider spacing between rows, use of wire trellis to raise foliage from the ground, and pruning of branches were also found to reduce *Sclerotinia* stem rot in some crops. Although several synthetic fungicides are reported to be effective against the pathogen, search for alternative approaches to manage crops under field conditions are gaining importance due to the often-hazardous effects of synthetic fungicides. A number of *in vitro* studies were conducted in the past to assess the potential of non-conventional chemicals as an alternative means for managing various soil-borne diseases (Bag and Sinha, 1997; Bhattacharya and Roy, 1998; Sinha and Giri, 1979; Cartwright et al., 1977) and many of them are reported to induce resistance in the hosts against several pathogens (Sarkar and Sinha, 1988; Manibhushanrao et al., 1990).

Phenolic compounds are the natural constituents in all the plants investigated until now. Besides several other classes of compounds, antibiotic phenols have been implicated in plant defence mechanisms (Elgersma and Liem, 1989; Nicholson and Hammerschmidt, 1992). Among them, some occur constitutively and are thought to function as preformed inhibitors associated with non-host resistance (Millar and Higgins, 1970; Stoessl, 1983). Others are formed in response to the ingress of pathogens and their appearance is considered as part of an active defense response (Nicholson and Hammerschmidt, 1992). Recently, Sarma et al. (2002) showed successful reduction of collar rot of chickpea (*Cicer arietinum*) caused by a

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soil-borne pathogen, *Sclerotium rolfsii*, through enhanced synthesis of phenolic compounds in the host. Sarma and Singh (2003) also demonstrated the sensitivity of *S. rolfsii* towards higher concentrations of ferulic acid. Looking into the potential showed by some non-conventional chemicals in reducing plant diseases *in vitro*, the present study was conducted to evaluate (i) four non-conventional chemicals for their capacity to reduce chickpea stem rot caused by *S. sclerotiorum* and (ii) their mode of action.

2. Materials and methods

2.1. Pathogen

S. sclerotiorum was isolated by picking up individual sclerotia from infected chickpea plants. Such sclerotia were surface sterilized with 0.1% mercuric chloride for a few seconds followed by three washings in sterile distilled water (SDW). They were then singly placed on potato dextrose agar (PDA, peeled potato 250 g, dextrose 20 g, agar powder 15 g and distilled water 1 L) medium in Petri dishes and incubated at $25\pm2\,^{\circ}C$. The cultures were purified on PDA slants by selecting single sclerotia produced on such plates.

2.2. Mass culturing

Sclerotia of *S. sclerotiorum* were produced on bajra (*Pennisetum typhoides* Pers.) seed meal-sand medium (bajra seed 250 g, washed white sand 750 g, distilled water 250 ml) by transferring mycelial strands of *S. sclerotiorum* (5–6 d old) grown on PDA in Petri plates. Cultures consisting of mycelium and mature sclerotia were homogenized by stirring vigorously with a glass rod for 5–10 min in a glass beaker containing SDW. The water was decanted several times to remove fungal mycelium, bajra seeds and sand. Homogenate containing sclerotia either free or attached to bajra seeds was filtered through a cheese cloth and washed thoroughly with SDW thrice to remove traces of sand and bajra seeds. Sclerotia thus obtained were blotted dry between two pieces of sterilized filter papers and dried at room temperature ($25\pm2\,^{\circ}C$) for 2 d and finally stored at $4\,^{\circ}C$ until required.

2.3. Experimental set-up

Chickpea seeds (cv. Avrodhi) were sown in earthen pots (10 cm diametre) containing sterilized soil, at the rate of 5 seeds/pot in a glasshouse. Necessary care was taken to ensure uniform germination of the seeds. In the ambient glasshouse conditions, temperature ranged from 8 to $24\,^{\circ}C$, relative humidity 85–90%, and photoperiod of approximately 10 h light and 14 h darkness. Solutions of zinc sulphate (ZS) 10^{-3} and 10^{-5} mmol, oxalic acid (OA) 4 and 6 mmol, sodium malonate (SM) 10^{-4} and 10^{-5} mmol, sodium selenite (SS) 10^{-4} and 10^{-5} mmol, SM 10^{-5} mmol + ZS 10^{-3} mmol, SS 10^{-5} mmol + ZS 10^{-3} mmol, OA 4 mmol + ZS 10^{-3} mmol, OA 4 mmol + SS 10^{-5} mmol, OA 4 mmol + SM 10^{-5} mmol, SM 10^{-5} mmol + SS 10^{-5} mmol, ZS 10^{-3} mmol + OA 4 mmol + SM 10^{-5} mmol + SS 10^{-5} mmol were prepared by adding the required amount of chemicals to distilled water. A foliar spray was applied to 21-d-old chickpea plants at the susceptible stage (5 plants/pot) as a pre-inoculation treatment. Treatments were applied to a set of pots comprising 10 pots/treatment and plants sprayed with distilled water served as control. The whole set of treatments was divided into two halves and after 24 h, a single mature sclerotium of *S. sclerotiorum* was inoculated at the collar region of each plant in one-half of treatments comprising 5 pots/treatment. The other five pots of the same treatment were left uninoculated. The pots were arranged in a randomized complete block design where each block consisted of one pot of all the treatments and considered as single replication. Mortality of chickpea plants was recorded at 14, 21 and 28 d after inoculation with sclerotia and compared with uninoculated plants, which received the spray of the same chemicals. Plant mortality in each individual pot that received the same treatment and distributed in the five blocks was considered as one replication and the mortality percentage was calculated from the number of plants killed due to stem rot from the total number of plants germinated. The data were subjected to ANOVA for statistical significance at $P\leqslant0.05$. The whole experiment was repeated once and the data were pooled and averaged.

2.4. Extraction of phenolic acids from chickpea leaves

From the treatments mentioned above ten treatments were selected for extraction of phenolic acids. The treatments were as follows: T1 = Control; T2 = Sclerotia inoculated; T3 = ZS 10^{-3} mmol; T4 = ZS 10^{-3} mmol + Sclerotia, T5 = OA 4 mmol, T6 = OA 4 mmol + Sclerotia, T7 = SM 10^{-5} mmol, T8 = SM 10^{-5} mmol + Sclerotia, T9 = SS 10^{-5} mmol, T10 = SS 10^{-5} mmol + Sclerotia. Fresh leaves from the lower nodes of chickpea plants from each treatment were collected from randomly selected plants from the five pots at 24 h after the foliar spray and mixed together. Similarly, leaves were also harvested at 48, 72 and 96 h after treatment. Samples, each comprising of 2 g, were taken from each treatment and the leaves were macerated in a mortar with a pestle followed by suspension of the fine crushed samples in 10 ml of ethanol:water (80:20, v/v) mixture. Samples were collected in screw-capped tubes and the suspensions were subjected to ultra sonication (Branson Sonifier, USA) at 60% duty cycles for 15 min. The clear greenish supernatant in glass tubes was mixed with charcoal for removal of pigments. After 3 h of charcoal treatment, the clear colourless supernatant was filtered through Whatman No. 1 filter paper and collected in glass tubes. The whole extraction process was repeated twice and extracts from the same treatment were pooled. The supernatant was evaporated under vacuum (Buchi Ratavapor Re Type). Dried samples were re-suspended in 1.0 ml high performance liquid chromatography (HPLC) grade methanol by vortexing and stored at $4\,^{\circ}C$ for further