



Short communication

Control of chickpea blight disease caused by *Didymella rabiei* by mixing resistance inducer and contact fungicideKamal Dev Sharma^{a,*}, Vinod Sharma^b, Ravinder Singh^b, Harsh Nayyar^c^a Department of Agricultural Biotechnology, CSK Himachal Pradesh Agricultural University, Palampur-176062, HP, India^b Krishi Vigyan Kendra, CSK Himachal Pradesh Agricultural University, Berthun, HP, India^c Department of Botany, Punjab University, Chandigarh-160014, India

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ABSTRACT

Elicitors of systemic acquired resistance are well known to reduce severity of several plant pathogenic diseases caused by fungi, bacteria and viruses. Their field applications for management of plant diseases are, however, limited because of yield penalties. Our studies on affect of Benzo (1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH), an elicitor of systemic acquired resistance, on chickpea blight caused by a fungal pathogen *Didymella rabiei* showed that multiple foliar applications of the chemical were effective in management of the disease under economic threshold levels. Multiple applications, however, affected chickpea grain yield adversely. The BTH induced yield penalties could be prevented by foliar spray schedule comprised of BTH and a contact fungicide mancozeb. One spray of BTH (50 ppm) followed by another of mancozeb (0.2%) was less effective (8.3% severity) than three sprays of BTH (4.2% severity) in blight control, however, this treatment enhanced grain yield significantly (1.241 t ha⁻¹) over three sprays of BTH (0.922 t ha⁻¹).

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1. Introduction

Chickpea (*Cicer arietinum* L.) is the third most important cool season grain legume in the world. Its seed are important source of proteins to human and animals. Among several biotic and abiotic stresses, the blight caused by *Didymella rabiei* (Kovachevski) v. Arx, (anamorph *Ascochyta rabiei* (Pass.) Lab.) is one of the major diseases of chickpea in cool and humid climates of the world (Nene and Reddy, 1987; Khan et al., 1999; Chongo et al., 2003). The disease under favorable climatic conditions can cause 100% yield losses and plants are susceptible to infection at any stage of crop growth (Reddy and Singh, 1990). Though conidia of *D. rabiei* penetrate the host directly through the cuticle after formation of appressorium-like infection structures, the mechanical forces are not considered to facilitate host penetration, rather hydrolytic enzymes produced by the fungus were suspected to aid penetration (Kohler et al., 1995).

Contact or systemic fungicides such as thiabendazole, maneb, mancozeb, zineb and chlorothalonil as seed dressers or foliar sprays are being used for the management of the disease (MacLeod and Galloway, 2002; Gaur, 2003; Chongo et al., 2003) as a part of integrated blight management strategy that includes host

resistance and cultural practices (Gan et al., 2006). A new category of chemicals i.e. 'inducers of systemic acquired resistance (SAR)' are not lethal to pathogens but potentiate plants' innate defenses against subsequent biotic challenges. SAR functions through accumulation of pathogenesis-related proteins and salicylic acid throughout the plant (Dong, 2001). One such inducer of SAR, Benzo (1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH, common names: Acibenzolar-S-methyl, benzothiadiazole) (Görlach et al., 1996) has been demonstrated to manage several plant diseases caused by fungi, bacteria and viruses e.g. barley powdery mildew, bean rust, tomato bacterial canker, tomato spotted wilt virus and chrysanthemum yellows phytoplasma (Görlach et al., 1996; Csinos et al., 2001; Baysal et al., 2003; Faoro et al., 2008; Abo-Elyousr et al., 2009; D'Amelio et al., 2010; Maffi et al., 2011). Resistance inducing chemicals have not been evaluated for blight management in chickpea so far. Chemical induced resistance may prove more useful for disease management compared to traditional fungicides especially when chickpea plants are infected simultaneously with many pathogens. BTH is also a factor for priming (Goellner and Conrath, 2008). Priming is a unique physiological situation in which plants after treatment with various chemicals or biotic stresses are able to 'recall' the previous chemical treatment or infection. As a consequence of priming, the BTH treated plants respond more effectively and quickly to biotic or abiotic stresses. BTH is, however, suspected to have yield penalties (Heil et al., 2000;

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Walters and Heil, 2007). The physiological cost of resistance induction in host has been debated a lot, limited experiments have, however, been conducted on affect of BTH on diseases and yield of leguminous crops (Dann et al., 1998; Maffi et al., 2011).

In the present study, we report the effect of BTH on chickpea blight management and grain yield, and demonstrate that a spray of BTH followed by another of contact fungicide mancozeb (broad spectrum, non-systemic with protective action, acts by disrupting lipid metabolism) was more effective in grain yield enhancement in chickpea than BTH alone.

2. Material and methods

For all field and greenhouse trials, a popular variety of chickpea in northern India, Him Chana 2, was used. A single spore isolate of *D. rabiei* (ARL1) was used for all greenhouse experiments. The pathogen was multiplied on V8 agar medium (200 ml V8 juice, 3 g CaCO₃, 15 g agar per liter, Himedia, India) at 22 ± 1 °C. The field experiments were conducted at CSK HP Agricultural University, Research Substation Berthin, HP, India (altitude 664 m amsl). Average temperature and humidity during the crop growing season (November–April) at this location were 15.6 °C and 70.2%, respectively during 2007–08 and 17.0 °C and 73.8%, respectively during 2008–09. The disease in this area appears during the month of March when temperatures are conducive for infection and subsequent disease development. The disease progresses till the end of April when temperature further rises and plants starts maturing. Average temperature and humidity during March and April in 2007–08 were 21.4 °C and 64.7%, respectively whereas during 2008–09 these were 20.9 °C and 63.5%, respectively.

The greenhouse experiments were conducted at CSK HP Agricultural University, Palampur using three to four leaf stage seedlings grown in 4" diameter pots (three seedlings per pot). The experiment was conducted at a temperature of 22 ± 1 °C and light/dark period of 12 h/12 h. The seedlings were inoculated with *D. rabiei* isolate ARL1 (1 × 10⁵ spores/ml) using a mini-dome technique described by Chen et al. (2005). To standardize appropriate dose of a commercial formulation of BTH i.e. Bion (Sandoz, 50%WP BTH), six concentrations of Bion (50, 100, 200, 300, 400 and 500) were used as foliar sprays (instrument used: atomizer, 10 ml solution per pot having three plants) on young seedlings 24 h prior to inoculation. One hundred ppm Bion (50 ppm BTH) was found to provide optimum reduction in blight severity (data not shown). In subsequent trials, 100 ppm of Bion was used. To standardize the time required for effective SAR induction, plants were sprayed with Bion as per the treatments in Fig. 1. When the plants were to be inoculated simultaneously, these were first sprayed followed by inoculation after 1 h. The disease data for greenhouse

experiments were recorded using 9-class scale of disease severity (Chen et al., 2004). The greenhouse experiments were conducted in completely randomized design with six replications in each treatment. There were three plants per pot with four pots per replication.

Field experiments were conducted during 2007–08 and 2008–09 in a randomized block design with three blocks and seven treatments during both the years (see Table 1 for treatments used). Between the plots (3 m × 1.8 m) about 75 cm unplanted space was kept to avoid spray drift. To each of the plots (7.2 m²), 1.5 kg blighted stubbles from last year crop were added to ensure sufficient primary inoculum. The chemicals used were Bion (100 ppm i.e. BTH 50 ppm) and mancozeb (0.2%). For treatment of seed with BTH, seed were soaked in 100 ppm Bion for 24 h prior to sowing. The crop was sprayed (3.5 L liquid per plot) with knapsack sprayer [Napsak (SRP50), American Spring and Pressing Works Pvt Ltd, New Delhi, India]. The sprayer was of 16 l capacity with hollow cone nozzle and a pressure of 40 psi. First spray during both the years was done at the beginning of March when initial symptoms of blight appeared on the crop. The interval between two sprays was 15 days. The crop was provided intermittent misting five times a day (7 am–7 pm) from March onward till the completion of flowering to facilitate disease development at times of no rainfall. The duration of mist at each time was 15 min and repeat interval between two mists was 3 h. Data on blight severity (%) on leaves were recorded when flowering was complete and plants were at pod filling stage. The dry grain yield from individual plots was also recorded.

For statistical analysis of blight data, transformed values were used. For greenhouse experiments where blight was scored using a 9-class scale, data were transformed as square root of log of blight score plus one. For field experiments the blight severity was scored in percent and the data were transformed as square root of value of blight severity plus one. For field trials, the blight and yield data were analyzed separately for both the years. The data were analyzed statistically to calculate the variance (ANOVA) using the computer programme, Windostat 8.0 (www.windostat.org). Treatment means were compared using Fisher's protected least significant difference (LSD) test at $p \leq 0.05$.

3. Results and discussion

3.1. Estimation of time required for induction of SAR by BTH

Fifty ppm of BTH (100 ppm Bion) applied 72 to 24 h prior to inoculations gave effective control of the disease whereas post-inoculation applications (24 and 48 h) were less effective (Fig. 1). It appeared that BTH induced SAR within 24 h after application as

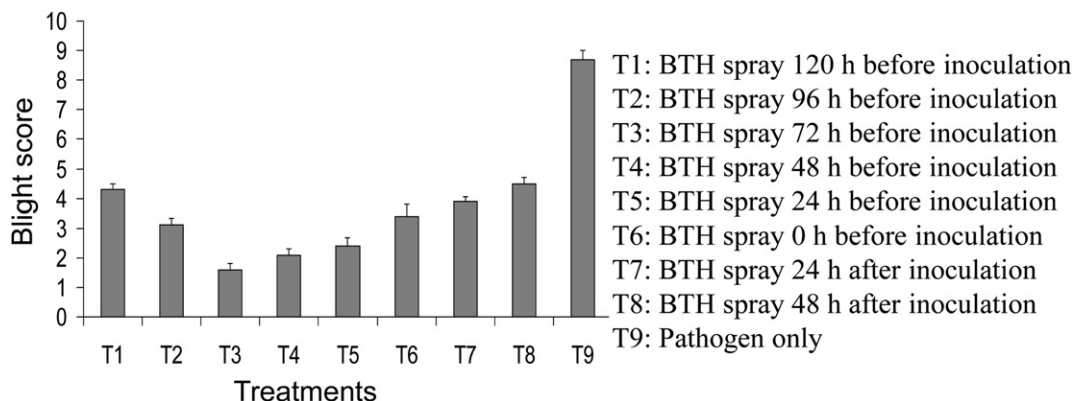


Fig. 1. Effect of pre- and post-inoculation applications of Benzothiadiazole commercial preparation Bion (100 ppm) in suppression of ascochyta blight of chickpea. When the crop was sprayed with water and BTH alone (data not shown in the fig), no blight was observed. [Blight score as per Chen et al. (2004), LSD ($p \leq 0.05\%$): 0.19, CV (%): 0.98].

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