ABSTRACT

Cumin botanically known as *Cuminum cyminum* L. belonging to Apiaceae family is an important seeds spice crop at world level and, India ranks first in terms of the acreage, production, consumption and export. Cumin is grown as a rabi crop in India. Within India, Gujarat is the largest producer of cumin seeds. But, several biotic and abiotic factors affect the cumin production. The major crop damage is caused by fungal diseases. The foremost fungal pathogens affecting cumin yield and growth are *Fusarium oxysporum* f. sp. *cumini* and *Alternaria burnsii* that causes wilt and blight diseases respectively. Significant drop in cumin yield is due to these diseases only. Several researches used several chemical, physical and biological methods to control the occurrence and spread of these diseases but failed in controlling the damage caused due to these pathogenic fungi. These controlling methods have their own limitations in terms of feasibility, cost, hazards and effectiveness. Apparently, there was a need for an efficient control measure that complies with the recent crop production trends of the environmentally sustainable agriculture. Thoughtful application of the plant’s own defense mechanism induced by biological elicitors commonly known as systemic acquired resistance (SAR) is such approach that meets all the above said needs hence, it was applied to control fungal diseases of cumin in the present study. Gujarat cumin-2 (GC-2) and Gujarat cumin-4 (GC-4) susceptible and resistant variety of cumin seeds respectively were used throughout the study. To induce SAR in cumin plants fungal culture filtrates (FCFs) prepared from *F. oxysporum* f. sp. *cumini*, *A. burnsii* and *Trichoderma harzianum*; cell free culture filtrate (CCF) prepared from *Bacillus subtilis*, MIX FCF which is a combination of FCF prepared from *F. oxysporum* f. sp. *cumini* and *A. burnsii*; commercially available biocontrol agents i.e. headline and monitor were used as bioelicitors. To prepare FCFs from pathogenic fungi *F. oxysporum* f. sp. *cumini* and *A. burnsii* were collected which were confirmed after checking their morphological characters. Concentration and treatment time was standardized by estimating the change in two important defense related enzymes i.e. phenylalanine ammonia lyase (PAL) and peroxidase (POX) after the treatment with varied concentration of selected elicitors. It was concluded that 10% *Fusarium oxysporum* f. sp. *cumin* (FOFCF) and 5% *Alternaria burnsii* (ABFCF), MIX FCF, 20% *T. harzianum* FCF, 5% *B. subtilis* CCF were most effective in inducing PAL and POX activities at 3 hrs, 1 hr, 30 min, 24 hrs, 1 hour respectively.
These standardized concentrations and time were used for the treatment throughout the study. Pathogenicity test of the selected pathogen, purification and determination of active compound of the pathogenic elicitors i.e. FOFCF and ABFCF, seed germination experiment were done. The selected fungi were found pathogenic as they showed identical symptoms of disease upon incubation with fresh cumin leaves. Purification of fungal elicitors revealed the presence of glycoprotein as an active constituent which trigger SAR related pathways via signaling. Treatment with selected pathogenic fungal elicitors was effective in germinating seeds faster than untreated or control seeds. Moreover, less mortality was seen in treated seeds compared to control. It was concluded from these experiments that FOFCF and ABFCF were the best agents which has supported faster germination with lowest mortality compared to other agents. Further, plants grown in infested soil in pots after seed treatment with selected elicitors, experimental plots study (seed and foliar spray treatment) and farmer’s field study (foliar spray treatment) were checked for elicitation of defense related enzymes such as phenylalanine ammonia lyase (PAL), peroxidase (POX), β-1,3 glucanase, polyphenol oxidase (PPO), catalase, nitrate reductase (NR), nitrite reductase (NiR), total protein and total phenol concentration which are base parameters to know the induction of SAR. Along with this, protein profiling by SDS-PAGE and phenol profiling by HPTLC, isozyme study was performed. Essential oil extraction, determination of cuminaldehyde concentration, physiological parameters such as height of the plants, number of umbels, number of branches per plant, number of seeds per plant and weight of 100 seeds were measured from the samples collected after field experiments along with estimations of defense related enzymes and protein profiling. Experimental design, selection of elicitors was done one by one based upon the results obtained and experiments were performed gradually. Highest fold induced enzyme activities and biochemical parameters were found in all treated plants compared to control plants in all experiments. More induced protein bands were seen in SDS-PAGE analysis and isozyme study from the samples treated with elicitors throughout the study. More additionally induced bands of phenols were recorded and some were identified. Salicylic acid, vanillic acid and trans-chlorogenic acid were found to be induced during phenol profiling in pathogenic fungal elicitor treated plants compared to control plants. Depending on the results obtained in the lab and experimental plot experiments, farmer’s field experiment were conducted to know the exact effect on efficacy of selected elicitors in combating the
disease at large scale. In field experiments, SAR was induced in the cumin plants treated with pathogenic elicitors. Highest activity of all defense related enzymes were recorded from the samples collected after the treatment with selected bioelicitors. More amount of essential oil was recovered from the seeds harvested from elicitors treated cumin plants grown at farmer’s field. The seeds recovered from treated plants also produced higher cuminaldehyde than seeds harvested from control plants. All physiological parameters were measured high compared to control plants in the study. More vigour, less disease incidence and higher yield was fetched from the elicitor treated plants compared to control. All theses enzyme activities and parameters were found highest in pathogenic FCFs treated plants compared to other selected elicitors and control as well. Two spray foliar application was finalized after conducting all experiments in which 1st spray using 10% FOFCF after 25 days of sowing and second spray after 55 days of sowing or just prior to flowering and recommended as one of the agronomic practices. Induction of defense related enzymes and pathogenesis related proteins (PR proteins) as well as all other biochemical parameters strictly confirms the induction of SAR in cumin plants that protects the cumin plants from wilt and blight diseases and also promotes better growth and development of the plant. The quality seeds obtained after treatment with elicitors appeared to be remunerative to farmers and also for the export.