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Research Article

SEED PRIMING EFFECT OF *ASPERGILLUS* SP., (NBP-53) ON PLANT GROWTH AND PROTECTION AGAINST ANTHRACNOSE DISEASE OF CHILLI

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A B S T R A C T

The efficacy of rhizospheric fungus *Aspergillus* sp., (NBP-53) was investigated for plant growth promotion and also tested for its effect on inducing resistance against anthracnose disease of chilli. The result indicated that seed treatment with isolated NBP-53 PGPF showed enhanced seed germination (66%) and seedling vigor (522.97) in comparison to that of control. The treatment also enhanced mean shoot and root length, fresh shoot and root weight of chilli plants under greenhouse conditions. In addition, it also offers considerable protection (58%) against anthracnose disease-causing pathogen *Colletotrichum capsici*. However, the maximum effect of enhanced seedling growth and protection was observed in positive control (Dithane M45) under both *in vitro* and greenhouse conditions. Therefore the study validated the use of beneficial microbes like PGPFs as a bio control agents in sustainable management of the plant diseases.

INTRODUCTION

Agriculture forms the basic need for food and a source of income in a populated country like India. Sustainable agriculture is gaining importance in today's scenario when compared to conventional agriculture as it fulfils the demands of forthcoming agricultural needs and played a vital role in the development of farmers, protects the environment by adopting eco-friendly methods. Chilli is a cash crop cultivated all over the world including India and is incorporated into the daily diet. Like humans, plants are also get infected by pathogens which in turn reduces the plant immune system. Anthracnose/ fruit rot/ die back disease is one of the major disease suffered by the chilli plant caused by *Colletotrichum capsici* (Berke *et al*., 2005) and the pathogen is so virulent that it can survive in extreme environmental conditions as conidial spores which in turn decrease the fruit marketability causing severe yield losses to poor farmers as well as affects the economic value of the country. The disease has greater side effects on seedling growth and vigor (Asalmol *et al*., 2001). Currently, the disease is managed by spraying fungicides. Dithane M-45 is effective fungicide used against fruit rot of chillies including bean anthracnose caused by *Colletotrichum* spp., downy mildew and anthracnose of cucurbits, citrus greasy spot caused by *Cercospora* spp., maize leaf blight and blights caused by *Alternaria*, *Phytophthora* and *Cercospora* on crops (Agropedia, 2012). As of we know, these fungicides have their own limitations, hazardous effects on the environment and human health.

An alternative to fungicides, biological control using beneficial micro-organisms is a promising option for the management of plant diseases and have been discovered as disease resistance inducers which play a key role in the induction of localized or systemic resistance in susceptible plants (Thakur and Sohal, 2013). Fungi which are associated with the roots of the plants were termed as rhizospheric fungi. Not all rhizospheric fungi promote growth as some may be pathogenic and some may be beneficial to the host plants. Some of these beneficial rhizospheric fungi are gaining attention for their improvement in plant growth and resistance against disease-causing microorganisms' called as plant growth promoting fungi (PGPFs). Utilization of these beneficial PGPFs could be a good approach for improving the health of the host plant (Hartman *et al*., 2009). The active nature of these PGPFs in the rhizosphere makes a possible venture for disease management strategies. PGPFs act as 'elicitor molecules' by sending signals to plant to trigger defense response upon pathogen attack (Dana *et al*., 2001) which eventually activates early signaling events of signal transduction cascade and there by inducing resistance (Nurnberger and Brunner, 2002). PGPFs are so quick in colonizing roots that it doesn't allow the growth of other microbes present in the soil and act as lymphocytes in plants. They compete for food; space and micro nutrients with other soil microbes which ultimately lead to the death of the soil microbes thereby resulting in enhancement of growth and resistance in the host plant (Bakker *et al*., 2007). Thus, the present study was undertaken to evaluate the effect of NBP-53 PGPF isolate in growth improvement and offering protection against chilli anthracnose disease under *in vitro* and green house conditions.

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MATERIALS AND METHODS

Source of pathogen, PGPF, seed samples and Dithane M-45

Colletotrichum capsici was isolated from the infected chilli plant and pure cultures were maintained in agar slants and Potato dextrose agar (PDA) plates. *Aspergillus* sp., (NBP-53) was isolated from the rhizospheric soil samples using serial dilution technique. Susceptible G-4 chilli seed samples were purchased from different agro chemical farms. Dithane M-45 (a positive control) was purchased from Himedia laboratory, Mumbai, India.

Preparation of inducers

PGPF isolate (NBP-53) was mass multiplied in PDA plates and incubate at room temperature for seven days. The conidial suspension obtained was adjusted to 1 x 10^8 CFU mL^{-1} using Haemocytometer. Dithane M-45 (Positive control) was prepared by weighing 0.1 g and make up the final volume to 100 mL with SDW (Buts *et al*., 2014).

Pathogen inoculum preparation

C. capsici was cultured in PDA plates and after complete growth, the conidial suspension was prepared by scraping the *C. capsici* PDA plates and final concentration of the suspension was adjusted to 4.5x10^5 conidia/ mL with sterile distilled water (SDW) using Haemocytometer (Sharma *et al*., 2005).

In vitro effect of NBP-13 isolate on seed germination and seedling vigor

G-4 chilli seeds were superficially sterilized with 0.2% sodium hypochlorite solution for 1-2 mins, washed with SDW to eliminate the traces of sterilant. After superficial sterilization, the seeds were treated by soaking them in PGPF inoculum prepared as above. All the sets (NBP-53, Dithane M-45 and control) were kept in rotary shaker for 6 h. After seed treatment, as per the ISTA rules of 2005 i.e. 100 seeds in replicates of four were placed in between paper towels at equal distance, rolled, covered with rubber bands on either side and kept for fourteen days. At fourteen day, seed germination per cent and seedling vigor was calculated using the standard formula of Abdul Baki and Anderson, 1973.

$$\% \text{ germination} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds plated}} \times 100$$

Effect of NBP-13 isolate on growth and protection under greenhouse conditions

Chilli seeds treated (NBP-53 and Dithane M-45) as above were sown in pots (10 seeds per pot) containing sand, soil and manure in 3: 1: 1 ratio and were allowed to grow for 30 days. Water was added in alternative days in order to maintain moisture content. A control set was separately maintained. On the 30th day, Mean Shoot Length, Mean Root Length and Fresh weight of shoot and root were recorded in all the plants (Agarwal, 1994). The same 30 day old chilli plants were sprayed with inoculum of *C. capsici* mentioned above, covered with polythene bags to avoid any cross contamination and plants were observed daily for anthracnose disease symptoms. Per cent protection was assessed after 30 days of challenge inoculation. The experiment was repeated four times.

$$\% \text{ protection} = \frac{C-T}{C}$$

C- % anthracnose disease symptoms in control plants
T- % anthracnose disease symptoms in treated plants

Statistical analysis

All the experiments of the study (*in vitro* seed germination and seedling vigor, vegetative growth and protection) were conducted in four replicates and individual experimental data was subjected to analysis of variance (ANOVA) using SPSS Inc. 11.5. The significance of the treatment in the test experiments were determined by the magnitude of the F value ($p \leq 0.05$) and means of all the experiments were separated by Tukeys'-b Post HSD test.

RESULTS

Effect of NBP-53 isolate on seed germination and seedling vigor in chilli under in vitro conditions

Seeds soaked for 6 h with NBP-53 isolate enhanced seed germination of 66% and vigor of 522.97 whereas non-treated control showed per cent germination of 62 and vigor of 327.07. A positive control (Dithane M-45) fungicide was also tested at 0.1% concentration which showed maximum seed germination of 75% and 889.15 of vigour under *in vitro* conditions (Table 1).

Effect of NBP-53 isolate on growth of chilli plants under greenhouse conditions

The seed treatment with NBP-53 soaked for 6 h was also tested for its efficacy on enhancement of plant growth under greenhouse conditions. The results clearly showed that the treatment NBP-53 was significantly effective in enhancement of plant growth recording Mean shoot length (MSL) of 7.42 cm, Mean root length (MRL) of 12.5 cm, Fresh shoot weight (1.5 g), Fresh root weight (FRW) of 0.17 g when compared to control which showed Mean shoot length (MSL) of 5.35 cm, Mean root length (MRL) of 4.67 cm, Fresh shoot weight (1.12 g), Fresh root weight (FRW) of 0.1 g respectively. Dithane M-45 fungicide at 0.1% concentration recorded maximum Mean shoot length (MSL) of 13.87 cm, Mean root length (MRL) of 14.75 cm, Fresh shoot weight (1.9 g), Fresh root weight (FRW) of 1.15 g (Table 2).

Effect of NBP-53 isolate in offering protection against chilli anthracnose disease under greenhouse conditions

Pathogen inoculated 30 day old NBP-53 raised chilli plants were evaluated for the protection studies under greenhouse conditions wherein it showed maximum protection of 58%. Whereas control plants were all found to be diseased/ infected and no % protection was observed. The positive treatment (Dithane M-45) recorded highest protection of 63.7% against *C. capsici* under greenhouse conditions (Fig. 1).

Table 1 Effect of seed treatment with NBP-53 isolate on per cent germination and seedling vigor in chilli under *in vitro* conditions

Treatments	MSL (cm)	MRL (cm)	% Germination	Vigor index
Control	2.16 ± 0.06^c	3.11 ± 0.04^c	62.0 ± 0.40^c	327.07 ± 6.69^c
NBP-53	3.65 ± 0.06^b	4.27 ± 0.11^b	66.0 ±0.40^b	522.97 ± 10.11^b
Dithane M-45 (0.1%)	5.72 ± 0.08^a	6.12 ± 0.42^a	75.0 ± 0.40^a	889.15 ± 33.41^a

Note: Values are means of individual four replicates ± standard error. Means followed by different alphabets are significantly different ($p \leq 0.05$) according to Tukey's-B HSD

Table 2 Effect of seed treatment with NBP-53 isolate on growth of chilli plants under greenhouse conditions

Treatments	MSL (cm)	MRL (cm)	FSW (g)	FRW (g)
Control	5.35 ± 0.53[c]	4.67 ± 0.11[c]	1.12 ± 0.07[c]	0.10 ± 0.00[b]
NBP-53	7.42 ± 0.66[b]	12.5 ± 0.64[b]	1.50 ± 0.04[b]	0.17 ± 0.04[b]
Dithane M-45 (0.1%)	13.87 ± 0.42[a]	14.75 ± 0.15[a]	1.90 ± 0.16[a]	1.15 ± 0.11[a]

Note: Values are means of individual four replicates ± standard error. Means followed by different alphabets are significantly different ($p \leq 0.05$) according to Tukey's-B HSD

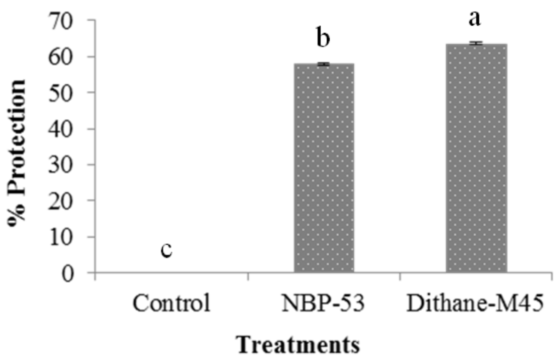

Fig 1 Effect of NBP-53 isolate on protection against anthracnose disease of chilli under greenhouse conditions.

DISCUSSION

Enhancing plant's own defense mechanism against broad spectrum of phytopathogens appears to be one of the most promising and eco-friendly strategies for plant disease management and crop improvement (Edreva, 2004). The rhizosphere is a zone of intense microbial activity (Westover *et al*., 1997). Organic nutrients of plant roots favor the growth of microbes (Lynch, 1990). A good number of rhizospheric microbes present in, on and around the root system may provide a variety of benefits to the plants (Nihorimbere *et al*., 2011).

Use of Plant growth promoting fungi (PGPFs) could be a good approach in the sustainable management of fungal disease as it improved crop yield and enhance resistance in the host plant. Beneficial micro-organisms which reside in soil were antagonistic to other pathogenic micro-organisms thereby preventing the host plant against pathogen infections. Earlier reports on PGPFs belonging to the genera *Trichoderma* sp., *Fusarium* sp., *Penicillium* sp., *Phoma* sp., enhanced plant growth and resistance in various crop plants (Chowdappa *et al*., 2013).

The present study was aimed to assess the effect PGPF isolate *Aspergillus* sp., (NBP-53) in enhancement of the growth and resistance via protection against anthracnose disease. The seed quality parameters revealed that the isolated NBP-53 strain recorded maximum germination (66%) and seedling vigour (522.97) in comparison to the control when treated at 1×10^8 CFU/ mL under *in vitro* conditions. The positive control Dithane M-45 showed increased seed germination and vigour in compared to control and NBP-53. Similar reports on seed treatment with *Aspergillus* sp., (PGPFYCMAf) reported to increase per cent seed germination of 83 and vigour 1632 in Sunflower (Nagaraju *et al*., 2013). Likewise, seed treatment with the conidial suspension of *Aspergillus niger* enhanced the growth of Chick pea (Yadav *et al*., 2011). Other PGPF such as seed treatment with *T. harzianum* enhanced seed germination and seedling vigor in tomato, chilli (Azarmi *et al*., 2011). Dithane M-45 increased growth and development in case of okra (Buts *et al*., 2014).

The treatment of the study NBP-53 was also effective in increasing shoot length, root length, fresh shoot weight and fresh root weight in chilli compared to control under greenhouse conditions. The maximum effect was observed in Dithane M-45 in comparison to NBP-53, and control. Similar correlations were made with the studies of Yadav *et al*., 2011 wherein the treatment with *Aspergillus niger* strain BHUAS01 recorded increased shoot length (13.61 cm), root length (6.21 cm), shoot dry weight (8.23 g) root dry weight (6.21 g) but the effect was increased when used in combination with other PGPFs like *P. citrinium*, *T. harzianum*. Similarly, in case of wheat, treatment with *Aspergillus niger*, *Aspergillus flavus*, individually enhanced the growth height (88.9 cm, 90.5 cm), dry weight (3.5 g, 4.3 g) and yield (638.82, 538.89). However, combination treatment of *Trichoderma harzianum* with two *Aspergillus* sp., have increased height, dry weight and yield in wheat (Matrood and Al-Taie, 2017).

Finally, the disease protection studies was assessed in chilli plants raised with seed treatment with NBP-53 and the result indicated that the treatment stood superior in offering protection of 58% in chilli plants whereas Dithane M-45 recorded highest protection of 63.7% when compared to control in which all the plants were observed diseased upon pathogen inoculation. Similar type of studies wherein the treatment with *Trichoderma* sp., (PGPFYCMTh) recorded maximum protection of 63% in sunflower susceptible seeds treated with conidial suspension at 1×10^8 CFU/mL followed by *Penicillium* sp., (PGPFYCMPo) and *Trichoderma* sp., (PGPFYCMTv) which showed 60% and 55% protection against downy mildew disease caused by *Plasmopara halstedii* (Nagaraju, *et al*., 2013). Likewise, *Penicillium* isolate GP15-1 offered maximum protection of 58.15% against anthracnose disease of cucumber. The same isolate also stood best in offering highest protection of 73.53% at 1% concentration against damping-off in cucumber seedlings caused by *R. solani* (Hossain *et al*., 2014). Dithane M-45 reported to inhibit the growth of *C. capsici* causing anthracnose disease of chilli (Haq *et al*., 2013) and also reduced collar rot of tomato up to 98.5% against *Sclerotium rolfsii* (Dutta and Das, 2002).

CONCLUSIONS

Overall, the study concluded that the seed treatment with NBP-53 was significant in maximizing the growth and resistance in chilli plants under *in vitro* and green house conditions against anthracnose disease as compared to that of control. Moreover, the treatment could be used as substitute to present available fungicides as the treatment is environmental friendly, cost effective and considerably good bio control agent in sustainable management of plant disease.

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