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Transmission of seed-borne infection of muskmelon by *Didymella bryoniae* and effect of seed treatments on disease incidence and fruit yield

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Abstract

Infected muskmelon plants were collected and a fungus was isolated during field survey of muskmelon conducted in 4th, 5th, and 6th agroclimatic zones of Karnataka state. The pathogen was identified as *Didymella bryoniae* upon incubation on potato dextrose agar plates. The pathogen causes gummy stem blight disease in muskmelon. Spore concentration of $12 \times 10^5\,\text{ml}^{-1}$ was found to be very effective in reestablishment of the pathogen upon artificial inoculation. The pathogen was located both externally and internally on the seed. Naturally infected seeds were subjected to transmission studies in vitro and in vivo. Four fungicides and two biological agents were evaluated for their efficacy against gummy stem blight disease incidence and fruit yield in field conditions. In water agar, primary seedling infection occurred on hypocotyls and cotyledons while pycnidia on ungerminated seeds and stunted seedling were also noticed due to severity of the infection. Typical symptoms expressed from 35 to 67 days after sowing until harvest experimentally, the fungus was more prevalent at the collar region of the plant. Mean disease incidence from all the cultivars significantly reduced except Bavistin (Carbendazim 50% WP), among which fungicides Dithane M-45 0.2% (Mancozeb 75% WP) and Wanis 0.3% significantly ($P = 0.001$) reduced the disease incidence where only 10.2 and 13.0%, disease was recorded, respectively and severity of gummy stem blight compared with Captaf 0.3% (Captan 50% WP) with 24.2% disease, whereas Bavistin (Carbendazim 50% WP) seed treatment was par with the control. Among antagonists, *Pseudomonas fluorescens* applied as pure culture ($1 \times 10^8\,\text{cfu ml}^{-1}$) and formulation of ($26 \times 10^7\,\text{cfu g}^{-1}$) at the rate of 8 and $10\,\text{g kg}^{-1}$ significantly ($P = 0.001$) reduced the disease incidence, which showed 17.7, 21.5, and 20.5%, disease respectively. On the contrary pure culture of *Trichoderma harzianum* ($1 \times 10^8\,\text{cfu ml}^{-1}$) recorded 18.2% *D. bryoniae* incidence followed by its formulation ($21 \times 10^7\,\text{cfu g}^{-1}$), which recorded 24.0 and 21.2% disease in 8 and $10\,\text{g kg}^{-1}$, respectively. Mean fruit weight from all the tested cultivars were increased at higher concentration (0.3%) by as much as 265 g in Dithane M-45, 154 g in Captaf and only 55 g in Bavistin treated seeds, while Wanis treatment resulted in decreased fruit weight when compared to untreated control. Seeds treated with *P. fluorescens* both as pure culture and formulation significantly ($P = 0.001$) effective in increasing the fruit yield by 370, 350, and 363 g, respectively. Though slight decrease in the yield was noticed in *T. harzianum* both as pure culture and formulation were significantly ($P = 0.001$) effective in increasing the fruit yield. However, both *P. fluorescens* and *T. harzianum* treatments showed significant increase in fruit weight over fungicides and untreated seeds.
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Keywords: Muskmelon (*Cucumis melo* L.); Gummy stem blight; *Didymella bryoniae*; Seed transmission; Seed treatment

1. Introduction

Sowing of infected seeds can reduce germination, vigour and potential yield by transmitting pathogen from seed to plants. The most adverse effect of seed-borne pathogen is contaminating disease free areas, thus seed-borne pathogens acts as primary source of inoculum for disease development. Though, the level of seed-borne inoculum might be extremely low, the rate of its increase may be extremely high when exposed with favourable epidemiological factors, including local agricultural practices (Neergard, 1977). Seed infection usually occurs

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during three distinct physiological phases in the seed production, seed development and seed maturation, the pathogen can be involved in all these stages of growth and can transmit from planted to the new crop thus developing a systemic infection that can colonize the seed (McGee, 1995). Gummy stem blight caused by *Didymella bryoniae*[1] anamorph *Phoma cucurbitacearum* (Fr. Fr) Sacc. is a highly prevalent disease on Cucurbits world wide (Sitterly and Keinath, 1996). *Cucurbita* spp. are especially susceptible to black rot, which directly reduces both pre and post-harvest yields (Keinath et al., 1995; Zitter and Kyle, 1992). Gummy stem blight causes severe defoliation in late production stages and is the second most important Cucumber Pathogen in North Carolina, following root–knot nematode (Wehner and Amand, 1993). The pathogen is located on and in the seed coat and transmits from seed to seedling in Cucumber and Pumpkin (Lee et al., 1984). Cotyledons and young leaves of watermelon and muskmelon are equally susceptible to gummy stem blight infection. The leaves of young Squash and Cucumber plants are resistant but become susceptible with age, especially at high temperature and humidity (Prasad and Norton, 1967). Epidemiology of *D. bryoniae* and the incidence of ascospores in glasshouses, outdoors and under controlled conditions were investigated by Vansteekelenburg (1983). Influence of humidity on incidence of *D. bryoniae* on cucumber leaves and growing tips under controlled environment studied by Vansteekelenburg (1985), he also reported that 10-fold conidial concentrations were needed to get equal infection as with leaf wetting. Disease is known to spread in the greenhouse during the growth season by means of airborne ascospores and by conidia transported by water on plant surfaces, by contact between plants or between plant and man or high tools to the host plant (De Neergaard, 1989). Fletcher and Preece (1966) reported that disease could be transmitted on a pruning knife at any node of plant. Optimization and fungicide application timing for optimum management of gummy stem blight epidemics on watermelon was evaluated by (Keinath, 1995). Yield losses of 30% and more can occur in early season crops that often encounter showery weather conditions conducive to the disease (Keinath, 2000).

The primary objective of these experiments is disease monitoring in muskmelon crop grown in farmers' field and to evaluate seed-borne nature and transmission from naturally infected seed to seedling both at in vitro and in vivo. A secondary objective was to determine the effect of different chemical and biological agents against *D. bryoniae* incidence and fruit yield.

[1] *Abbreviations used: D. bryoniae*, *Didymella bryoniae*; *P. fluorescens*, *Pseudomonas fluorescens*; *T. harzianum*, *Trichoderma harzianum*; NUV, near ultra violet; PDA, potato dextrose agar; OD, optical density.

2. Materials and methods

2.1. Field survey and collection of seed samples

The survey was conducted in 4th (Central dry zone), 5th (Eastern dry zone), and 6th (southern dry zone) agroclimatic zones, which includes Hassan, Bangalore, Chamarajanagar, Mandya, and Mysore districts of Karnataka state (Ramachandra et al., 2004) during the month of October–December. The plants were diagnosed as infected on the basis of typical symptoms of gummy stem blight viz., stem necrosis with an exudation of gummy material, angular water-soaked lesions on the leaves and rotten fruits. The percent infection was calculated based on the number of plant infected in total area of crop growth. From each fields 10 healthy fruits and 7–11 rotten fruits which were completely decayed, symptomatic stems and leaves showing typical gummy stem blight disease were collected separately from the field, seeds from rotten fruits were separated and air dried. The collected materials were stored in polythene bags at 5 °C.

2.2. Isolation of D. bryoniae

400 seeds collected from infected rotten fruits of four cultivars viz., NHM-7, CGH-3, Green Ring and AML-65 seeds and their symptomatic plant parts each isolated from different zones were surface sterilized with 0.2% NaOCl for 5 min, followed by three wash and plated on potato dextrose agar plates and incubated under 12/12 h alternate cycles of Near Ultra Violet light and darkness at 23 ± 2 °C (ISTA, 1999).

2.3. Pathogenicity test

To confirm pathogenicity of the four isolates of *D. bryoniae* isolated from NHM-7, CGH-3, Green Ring and AML-65, 30-day-old muskmelon plants were randomly selected and wounded using a syringe needle and inoculated at the collar region with different spore suspension of 3.7×10^5, 7×10^5, 9.3×10^5, and 12×10^5 conidia ml^{-1} in sterile distilled water. Plants inoculated with sterile distilled water served as control. The plants were covered with plastic bags for 2 days and kept at 23 ± 2 °C and 90% relative humidity, with a 12 h photoperiod under greenhouse conditions. Plants were assessed for the disease, 7 days after inoculation and continued up to 30 days. Experiments were conducted in four replicates of 10 plants each and repeated thrice for all the four isolates.

2.4. Component plating

400 seeds each from rotten fruits from all the four cultivars were surface sterilized as described above and were soaked in distilled water for 24 h. The soaked seeds were then dissected into three parts viz., cotyledons, seed coat and embryo using sterilized forceps and needles under

aseptic conditions. The components were placed on potato dextrose agar plates. The plates were incubated under 12/12 h alternate cycles of NUV light and darkness at 23 ± 2 °C (ISTA, 1999). Experiments were conducted in four replicates consisting 100 seeds each per cultivar.

2.5. Disease development in seedling from naturally infected seeds

2.5.1. In vitro seed transmission of D. bryoniae by water agar method

Ten percent of water agar was prepared and 10 ml of the medium was poured into each sterilized test tube, seeds were surface sterilized with 0.2% NaOCl for 5 min followed by three wash and one seed was placed in each test tube. Four replicates of 25 seeds each for all the infected fruits of cultivars viz., NHM-7, CGH-3, Green Ring and AML-65 were maintained, these sets were incubated for 21 days under 12/12 h alternate cycles of NUV light and darkness. Seedlings were regularly examined for the occurrence of disease symptoms. Data was scored up to 19 days of incubation and tabulated (Mathur and Kongsdal, 1998).

2.5.2. Seed transmission of D. bryoniae in field

The experiment was conducted in disease free experimental plot of Applied Botany, Seed Pathology, and Biotechnology, Manasagangotri, university of Mysore, where soil from the field were collected randomly and tested for the presence of *D. bryoniae* by serial dilution technique. Experimental plot consisted of 12 m rows with 5 plants per row. Each having 7 cm deep and 2.5 cm in diameter, plants within rows were spaced 1.5 m apart, rows were spaced 2.5 m apart. Likewise four cultivars with four replicates were sown in the field. The experimental plot was randomly designed and maintained according to standard commercial practice for muskmelon crop production by following normal agronomical practices and observation was made for natural induction of disease incidence, symptoms were analyzed after emergence of the seedlings up to fruiting stage at regular intervals of 5 days. Infected leaves and stem pieces were collected and subjected to seed transmission test by plating on PDA agar and incubated under NUV light and darkens at 23 ± 2 °C to observe the re-establishment of *D. bryoniae* in all the four cultivars.

Infected rotten fruits from each cultivar were randomly picked from the plots, seeds from these fruits were separated cleanly and air dried. 400 seeds from each cultivar were plated on PDA plates and incubated under NUV light and darkens at 23 ± 2 °C to assess the impact of *D. bryoniae* seed transmission and percent re-establishment of *D. bryoniae* in seeds was observed under sterobinucular microscope.

2.5.3. Mass multiplication of biological agents

The antagonistic strains of *Pseudomonas fluorescens* and *Trichoderma harzianum* were isolated from the native soil, maintained on nutrient medium and then used as a potential biocontrol agents. *P. fluorescens* was mass multiplied by inoculating on King's B medium and incubated at 35 ± 2 °C for 2 days in a BOD incubator. After 48 h of incubation, culture broth was centrifuged at 10,000 rpm for 10 min. The pellets were resuspended in sterile distilled water and washing was repeated thrice. The washed bacterial pellet was made into a turbid solution with sterile distilled water. The optical density (OD) of the solution was adjusted to 0.45 (A_{610} nm) to obtain 1×10^8 cfu ml^{-1} for the seed treatment (Mortensen, 1992). *P. fluorescens* formulation (26×10^7 cfu g^{-1}) was prepared by mixing 100 ml of suspension and 25 g of purified talcum powder under sterile conditions. Carboxy methyl cellulose (2.5 g) was added to 250 g formulation, packed in polythene bags and stored in the form of talc under ambient conditions (Umesha et al., 1998).

Fungal biological agent *T. harzianum* was mass multiplied on PDA plates and incubated at 23 ± 2 °C under 12/12 h alternate cycles of NUV light and darkness for 7 days. After 7 days of incubation, culture broth was centrifuged at 10,000 rpm for 10 min. The pellets were resuspended in sterile distilled water and washing was repeated thrice. The washed fungal pellet was made into a turbid solution with sterile distilled water. The OD of the solution was adjusted to 0.45 (A_{610} nm) to obtain 1×10^8 cfu ml^{-1} for the seed treatment. *T. harzianum* formulation (21×10^7 cfu g^{-1}) was prepared by mixing 100 ml conidial mass with talcum powder (1:10 w/w); packed in polythene bags and stored under ambient conditions of 23 ± 2 °C and used (Raju et al., 1999).

2.5.4. Seed treatment with biological agents

Infected seeds with varied level of *D. bryoniae* of four cultivars viz., NHM-7, CGH-3, Green Ring and AML-65 were treated with pure culture of *P. fluorescens* and *T. harzianum* at the rate of 1×10^8 cfu ml^{-1} by mixing 400 seeds with 5 ml cell/conidial suspension. Formulations of *P. fluorescens* (26×10^7 cfu g^{-1}) and *T. harzianum* (21×10^7 cfu g^{-1}) in the form of slurry treatment at the rate of 8 and 10 g kg^{-1} of seeds, respectively. After 24 h treated seeds were air dried and further tested for their efficacy in field conditions against *D. bryoniae* disease incidence and fruit yield.

2.5.5. Seed treatment with fungicides

Infected seeds with varied level of *D. bryoniae* of four cultivars viz., NHM-7, CGH-3, Green Ring and AML-65 were treated with different fungicides viz., Bavistin (a.i. Carbendazim 50% WP) a product of BASF India Ltd., Captaf (a.i. Captan 50% WP) from Rallis India, Dithane M-45 (a.i. Mancozeb 75% WP) manufactured by De-Nocil crop protection, India and Wanis a product of SPIC India. Seeds were treated with different concentrations (0.1–0.3%) of chemicals and biological agents both pure culture (1×10^8 cfu ml^{-1}) and talc formulation of 8 and 10 g kg^{-1} were screened in the laboratory, later tested for their efficacy in field conditions against *D. bryoniae* disease incidence and fruit yield.

2.5.6. Effect of seed treatment

The experimental plot was designed and maintained according to standard practices for muskmelon production, likewise four replicates were maintained for each treatment and sterile distilled water treated seeds as control block was also maintained.

2.5.7. Fruit yield

Fruit weight was calculated for each treatment based on average weight of randomly harvested five healthy fruits.

3. Results

Among the muskmelon fields surveyed (Fig. 1), cultivar NHM-7 in 5th zone (Dodaballapura field) showed severe 39% disease incidence followed by cultivar CGH-3 in 6th zone (Nanjangud field) with 17%, cultivar AML-65 recorded 15% disease incidence in 4th zone (Arasikere field) and cultivar Green Ring recorded the least *D. bryoniae* incidence of 13% in 6th zone (Malavalli field).

3.1. Isolation of D. bryoniae

On PDA plates, the mycelium was olivaceous green and few pycnidia were observed from 9th day after incubation. White aerial mycelium was also produced (Fig. 2). Conidia were hyaline, cylindrical with rounded ends, mostly single septate, but a few 1- septate, and 6–11 × 3–5 μm in size (Fig. 3), based on the symptoms, morphological and conidial characteristics, the fungus was identified as *D. bryoniae* which causes gummy stem blight disease. The pathogen was recorded and reported for the first time in India on the host muskmelon (Sudisha et al., 2004).

3.2. Pathogenicity test

All the four isolates upon inoculation with different concentrations of *D. bryoniae* showed varied degree of infection. Doddaballapura (NHM-7) isolate upon inoculation with higher concentrations 12×10^5 spores ml^{-1} was found to be an effective and highly virulent, where inoculated

Fig. 1. Map showing different agroclimatic zones of Karnataka state, India, where present studies was carried out.

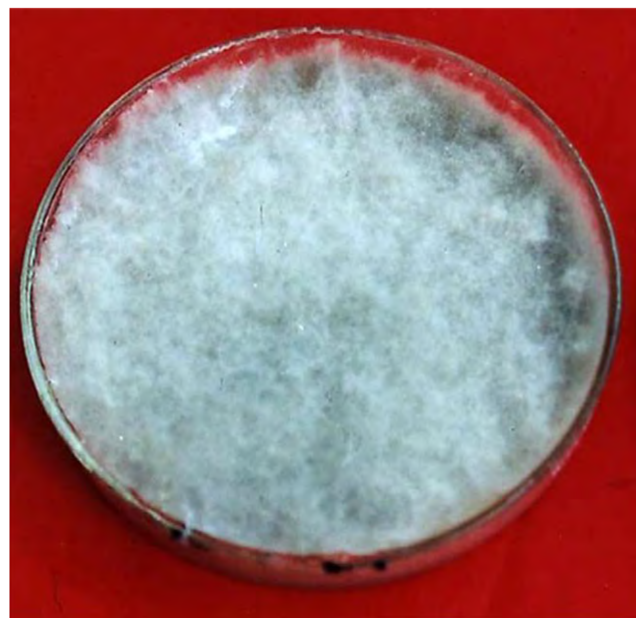

Fig. 2. Cultural morphology of *D. bryoniae*.

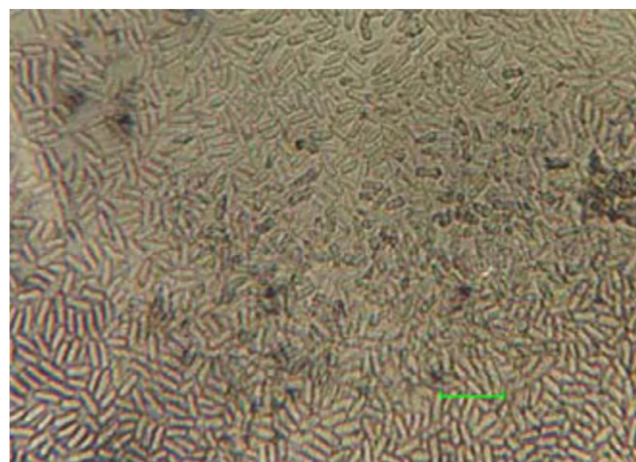

Fig. 3. Conidia of *D. bryoniae* (bar = 20 μm).

plants expressed severe infection of papery lesions on leaves and pycnidia on stem were noticed on 7th day after inoculation. On the contrary Arasikere (AML-65) isolate with less concentration of 3.7×10^5 and 7×10^5 spores ml^{-1} the re-establishment and severity of the pathogen was very low in inoculated plants. In contrast control plants were completely free from disease.

3.3. Component plating method

Different components of seed was evaluated, results indicated that the mean incidence of *D. bryoniae* pathogen was more dominant in seed coat with 31%, followed by cotyledons with 11% and embryo with 4% in tested cultivars.

3.4. Seed transmission of D. bryoniae

3.4.1. Water agar method

Seedling symptom test showed varied incidence of black spot on hypocotyls, lesions on cotyledon, pycnidia on ungerminated seed and stunted growth (Fig. 4). Among four cultivars screened, the isolate from NHM-7 was highly virulent, where typical symptoms of gummy stem blight initiated from 8th day of incubation, which is followed by CGH-3 and Green Ring, interestingly in AML-65 the pathogen was able to express only on 15th and 16th day of incubation. Whereas, no disease symptoms was noticed on controlled seedlings.

3.4.2. Field

The disease progressed rapidly from 35 to 67 days after sowing until harvest. Initially the pathogen expressed on leaves showing angular lesions that dry and drops out giving the lesion a ragged appearance (Fig. 5). As the disease progresses oozing brown exudation in the crown of infected plants dotted with black pycnidia on stems (Fig. 6) expressed after 47th-day-old plants. Severe infection was observed where vine cankers are found near the soil line producing gummy brown ooze resulting blackening or rotting of fruits at the ripening stage. During the observation the disease was more prominent at the colar region of the plant (Fig. 7) resulting poor plant stand in the field. Similar typical symptoms were observed in all the four cultivars used. On the other hand control plants were asymptomatic. The mean of four cultivars with four replicates recorded 41% of natural disease transmission (Fig. 8). Subsequently, *D. bryoniae* was re-isolated from the harvested seeds in all the four cultivars.

Fig. 5. Infected leaves showing water-soaked lesions and pycnidia within the affected tissues.

3.4.3. Effect of chemical fungicide and biological agents on D. bryoniae

In the present investigation seed treatment with higher concentrations (0.2 and 0.3%) in all the tested fungicides proved to be highly significant ($P = 0.001$) in reducing the *D. bryoniae* incidence. In NHM-7 among tested fungicides Dithane M-45, both at 0.2 and 0.3% stood superior in reducing the disease incidence where only 10.2 and 13.0% disease was recorded, respectively. Seed treatment with Wanis at 0.3% also significantly inhibited the disease which recorded 15.7% incidence, followed by Captaf 0.3% with 24.2% disease. In case of Bavistin seed treatment was par with the control (Fig. 9). On contrary in biological agents *P. fluorescens* pure culture at 1×10^8 cfu ml^{-1} showed greater level of disease reduction with 17.7%, whereas the formulation of the same at

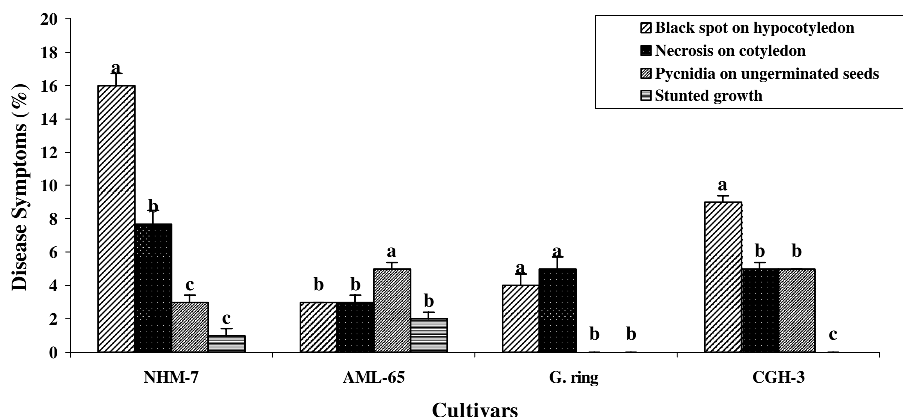

Fig. 4. Seed transmission of *D. bryoniae* by water agar method.

Fig. 6. Symptoms showing brown, oozing lesions with black pycnidia on the stem of muskmelon.

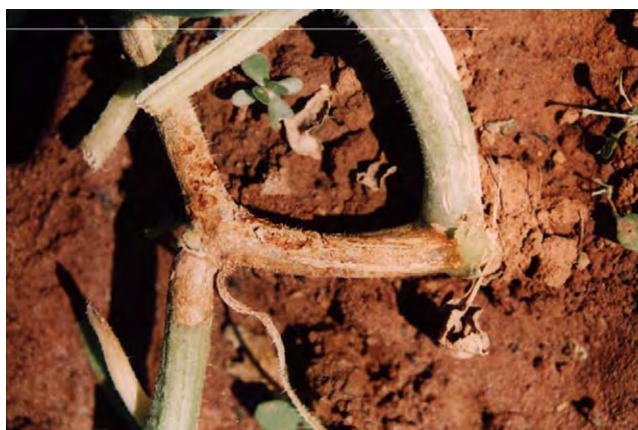

Fig. 7. Severe infection on collar region of stem.

8 and $10\,g\,kg^{-1}$ treated seed, recorded disease of 21.5 and 20.5%, respectively. On the other hand pure culture of *T. harzianum* $1 \times 10^8\,cfu\,ml^{-1}$ treated seed recorded disease incidence of 18.2% and seed treated with the formulation of the same at 8 and $10\,g\,kg^{-1}$ recorded 24.0 and 21.2% disease incidence, respectively (Fig. 10). Interestingly, a similar trend in disease protection was evident when seed treatments with above fungicides and biological agents were tested to CGH-3, Green Ring and AML-65 cultivars.

3.4.4. Effect of seed treatment on fruit yield

In all the tested cultivars, Dithane M-45 0.3% treated seeds showed 265 g increase in fruit weight, 154 g increase in captaf (0.3%), and 55 g increase in fruit weight was noticed in Bavistin (0.3%) treated seeds over control. On the contrary, though Wanis treated showed significant reduction in disease it failed to enhance the fruit yield compared to untreated (Fig. 11). In case of biological agents seed treatment pure culture of both bacteria and fungal agent stood superior in enhancing the fruit weight significantly ($P = 0.001$) compared to its formulations. Where *P. fluorescens* Pure culture of $1 \times 10^8\,cfu\,ml^{-1}$ significantly increased the fruit weight by 370 g followed by its formulations 8 and $10\,g\,kg^{-1}$ which showed 350 and 363 g increase in fruit yield over untreated control. On the other hand pure culture of *T. harzianum* at $1 \times 10^8\,cfu\,ml^{-1}$ showed 334 g increase in fruit weight followed by its formulations 8 and $10\,g\,kg^{-1}$ which recorded 311 and 319 g increase in fruit weight over control (Fig. 12).

4. Discussion

The occurrence of fungi associated with muskmelon crop in commercial fields of Karnataka state was surveyed over past 3 years. A Fungus most frequently isolated from infected twigs and leaves on PDA medium was reported to be *D. bryoniae* which causes gummy stem blight disease in muskmelon for the first time in India on host muskmelon (Sudisha et al., 2004) and the disease was highly severe with an average incidence of 39% in cultivar NHM-7 at Doddaballapura field. The pathogen directly reduces both pre and post-harvest yields (Zitter and Kyle, 1992). Since many non-related plant pathogens produce similar types of symptoms, our studies strongly indicated that preliminary diagnosis of the fungi under controlled conditions proved to be very efficient method where NHM-7 isolate upon inoculation with $12 \times 10^5\,spores\,ml^{-1}$ resulted significantly virulent, however the spore concentrations tested for all the four isolates under controlled conditions showed varied degree of disease infection. Sattar et al. (2002) suggested that inoculating young leaves and twigs of Geranium with anthracnose fungi and evaluated the infected plants 3–5 days after inoculation was found as an effective mode of pathogenecity test in Geranium.

The present investigation clearly revealed that *D. bryoniae* is both external and internal seed-borne pathogen, which can carry the inoculum from the seed to plant, as evidenced from the present work, *D. bryoniae* was found lying dominant in the seed coat, similar report have been well documented by Lee et al. (1984) in Cucumber and Pumkin. A study from elsewhere indicates that *D. bryoniae* in watermelon invaded epidermis, cotyledons and embryos (Rankin, 1954). Agarwal and Sinclair (1997) reported that seed-borne pathogen can be transmitted either by infection of embryo, endosperm or by contamination of seed coat. The presence of *D. bryoniae* on or in the seed may provide an unsuspectual and potentially dangerous source of infection.

In the present studies seed transmission by water agar method, clearly indicated transmission of *D. bryoniae* from seed to seedling. Typical symptoms of the disease like black spot on hypocotyledon, lesions on cotyledon, pycnidia on

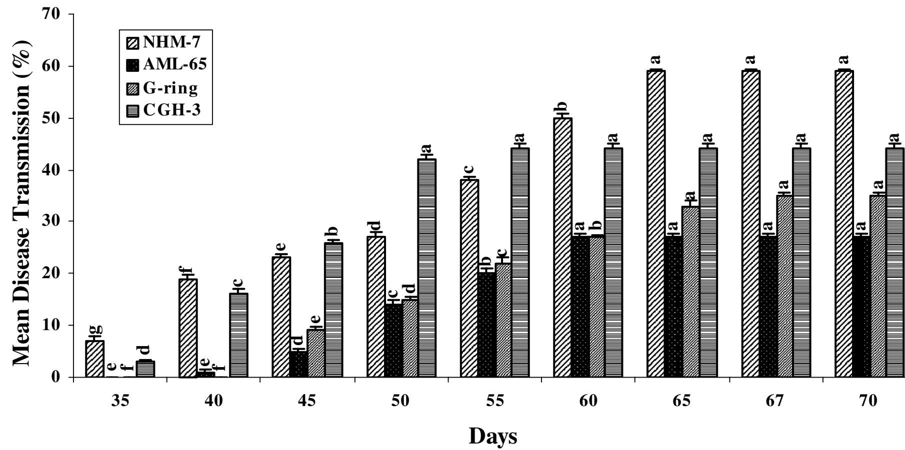

Fig. 8. Seed transmission of *D. bryoniae* in field conditions.

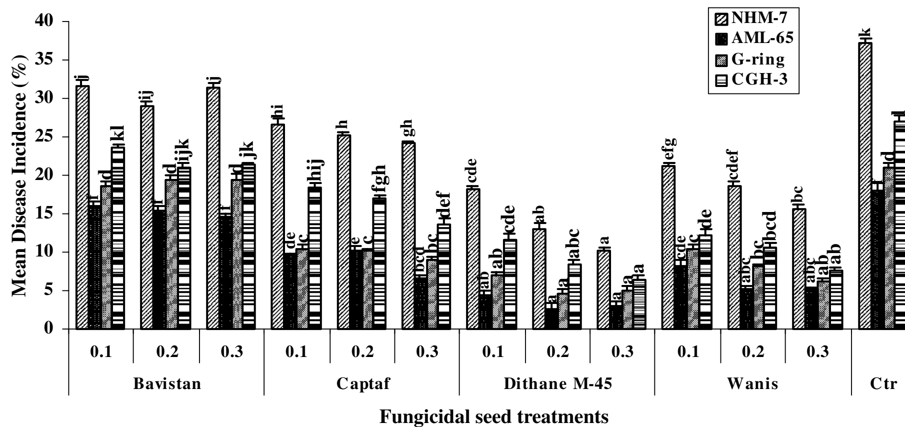

Fig. 9. Effect of fungicidal seed treatments on *D. bryoniae* in field conditions.

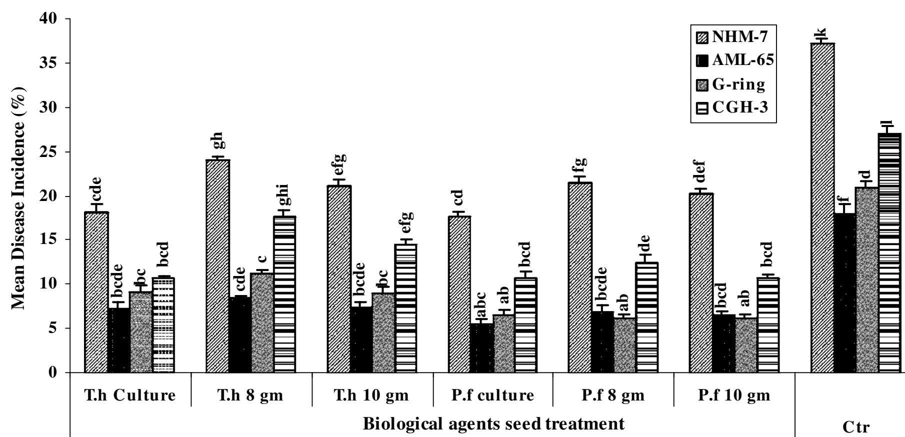

Fig. 10. Effect of biological agents seed treatments on *D. bryoniae* in field conditions.

ungerminated seed, and stunted growth were also noticed due to severity of infection, which is similar to the observation of Lee et al. (1984).

In field conditions, symptoms were easily manifested. Primary infection on leaf begins with irregular spots that dry and drop out giving the lesion a ragged appearance and on stem appears as pale green and irregular shaped patches around the nodes and gradually become grey or blackish grey with exuded gum sometimes turns blackish-brown with dotted pycnidia which exactly correlated to studies of Hemmi (1922). In addition as noticed the disease development was more severe at the collar region of the plant. The present work revealed that *D. bryoniae* during the mycelial development it causes foliar symptoms only at its maturity, disease severity occurs after the formation of ascospores and causes complete collapse of the

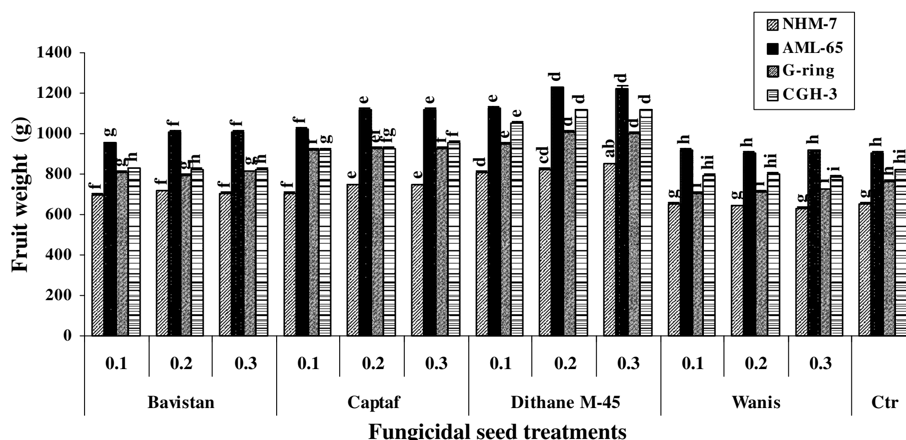

Fig. 11. Effect of fungicidal seed treatments on fruit yield.

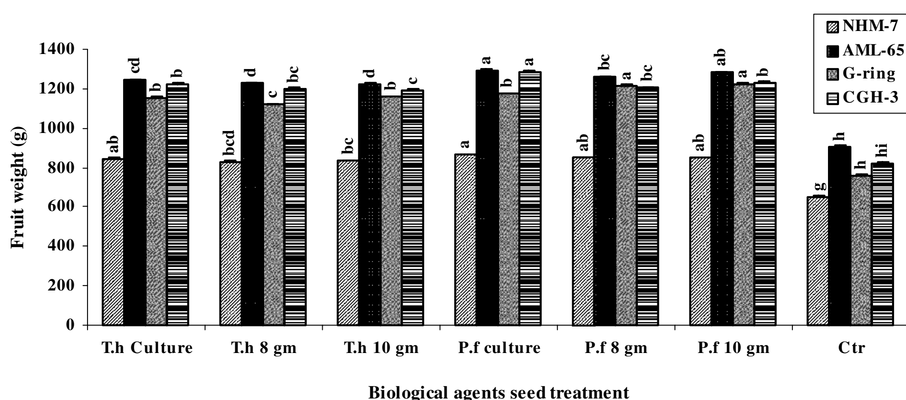

Fig. 12. Effect of biological agents seed treatments on fruit yield.

crop. During development and maturation of seeds, there are all the chances for the spores of *D. bryoniae* to spread, germinate and invade the seeds systemically (Rankin, 1954). Fletcher and Preece (1966) noticed that disease could be transmitted on a pruning knife at any node of the plant. Schenck (1968) reported that the ascospores were the primary source of inoculum based on the distribution pattern of infected plant in the field and the association of ascospores with initial infection. Environmental factors influences infection of *D. bryoniae* in attached Cucumber leaves in laboratory and hydathodes region close to main vein is the most susceptible areas of the intact leaf to *D. bryoniae* infection as reported by Svedelius and Unestam (1978). In addition they also observed that mechanical injury increases the susceptibility to conidial infection. The role of insect injury and powdery mildew in the epidemiology of gummy stem blight disease of Cucurbits both in Laboratory and field experiments was carried out by Bergstrom et al. (1982). The effect of temperature and duration of surface wetness on spore production and infection of cucumber by *D. bryoniae* was reported by Arny and Rower (1991).

Many attempts on chemical control of *D. bryoniae* in cucurbits were evaluated by several workers (Johnson et al., 1995; Keinath, 2000; Keinath et al., 1998; Vanstee-kelenburg, 1978; Vawdrey, 1994). Although, crop rotation away from cucurbits for at least two years can reduce the incidence and severity of gummy stem blight, fungicides applications remains the primary control method (Keinath, 1996; 2000). There is hardly any literature available on the effect of selected fungicides viz., Bavistin (Carbendazim 50% WP), Captaf (Captan 50 WP), Dithane M-45 (Mancozeb 75% WP) and Wanis and biological agents *P. flourescens* and *T. harzianum* against *D. bryoniae* and enhancing fruit yield in muskmelon. In the current findings fungicides tested except bavistin all other fungicides gave fair to good control of gummy stem blight disease, it is clear from the present investigation that Dithane M-45 and Wanis stood superior in complete overcoming of gummy stem blight followed by Captaf. Though Wanis treatment significantly inhibited *D. bryoniae* but failed to increase fruit yield and was par with the control. This might be due to phytotoxins present in the fungicide, which targets directly over the pathogen that might result in low yield or some pathogen may enter through the soil, which intern colonize the plant and inhibit its growth and causes major effect on decrease in yield. On the other hand, the increase in the fruit yield is due to some metabolites produced by fungicides that induces resistance into the host responsable for stimulating growth and yield.

Among biological agents pure culture of both *P. flourescens* and *T. harzianum* (1×10^8 cfu ml^{-1}) were the best in controlling *D. bryoniae* compare to its formulation (8 and 10 g Kg^{-1}) and also resulted highly significant ($P = 0.001$) increase in fruit yield, which stood superior over all fungicides treatments, seeds treated with *T. harzianum* developed less gummy stem blight infection suggested that the rhizosphere competence permitted it to persist on muskmelon roots at effective levels, similarly treatment of bean seeds with *T. harzianum* increased yields and decreased the root rot of dry beans (Venette and Gross, 1990). This indicates the effectiveness of *T. harzianum* on root colonization in rhizosphere of muskmelon. Also increase in the fruit yield is due to hormones like gibberellins, cytokinins and indole acetic acid or hormone like substances which inhibit the fungi and enhance the plant growth. The use of *Trichoderma* and *Pseudomonas* species in controlling the seed and soil-borne diseases has been well documented (Bankole and Adebanjo, 1996; Expert and Digat, 1995; Handelsman and Eric, 1996; Umesha et al., 1998; Vidhyasekaran and muthamilan, 1995). According to Liu et al. (1995) the controlling mechanism might be due to the production of antibiotics or related substances by *P. flourescens*. Burr et al. (1978) in their studies observed the increase in growth and yield of Potato when *P. flourescens* was applied. Several studies indicate that yellow-green siderophores produced by *P. flourescens* are responsible for enhanced plant growth and yield (Kloepper et al., 1980). Our studies indicated that both *T. harzianum* and *P. flourescens* have offered maximum disease protection against *D. bryoniae* and enhanced fruit weight.

This research demonstrate that *D. bryoniae* recorded for the first time in India on muskmelon crops is an seed borne infection that transmit from seed to seedling and seed can carry the inoculum from the plant to seed in muskmelon and regular use of fungicides and biological agents controls gummy stem blight disease and increase the yield of healthy fruit. Our studies also indicate that some of the alternative treatments Dithane M-45 and Wanis can reduce the overall incidence of gummy stem blight. On contrary pure culture of both *P. flourescens* and *T. harzianum* (1×10^8 cfu ml^{-1}) showed potential for controlling gummy stem blight. However, more research is required to determine that fungicide Wanis give similar yields as those obtained with biological agents.

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