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DEFENSE RESPONSES IN COWPEA ELICITED BY ENTOMOPATHOGENIC FUNGI AGAINST *APHIS CRACCIVORA* KOCH

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ABSTRACT

Use of entomopathogenic fungi for controlling the aphids is well attempted and further substantiation of interaction of entomopathogenic fungus in cowpea plants against aphid were studied in detail. Bioassays were performed with five fungal isolates against cowpea aphid *Aphis craccivora* Koch. The results revealed that the isolate *Beauveria bassiana* (NBAIR) recorded the highest mortality of 94.67% at 120 hr (at 5 days after treatment- DAT). The foliar application of its crude formulation @ 10^8 spores ml^{-1} had significant influence on the induction of defense related enzymes and components against *A. craccivora* infestation in cowpea. Resistant mechanism mediated by entomopathogenic fungi in cowpea against *A. craccivora* showed sustained and timely induction/ accumulation of defense enzymes. On 4 DAT the enzymes viz., peroxidases, polyphenol oxidases, phenylalanine ammonia lyase, chitinase, catalase and phenolics revealed maximum activity.

Key words: Cowpea, *Beauveria bassiana*, *Metarhizium anisopliae*, *Lecanicillium lecanii*, *Aphis craccivora*, defense, peroxidases, polyphenol oxidases, phenyl alanine ammonia lyase, chitinase, catalase, phenolics

Cowpea is the fourth most widely produced pulse crop in India after chickpea, pigeon pea and black gram. It can be grown during both rainy and summer seasons. The crop fits well into traditional rice-wheat cropping systems as a short duration crop and offers farmers with additional revenue. As a legume plant, it can play a significant role in fixing nitrogen from 20-80 kg/ ha (Hayat et al., 2008). Leguminous crops generally attract insect pests because of their high nutritive value (Mogotsi, 2006); therefore, proper and effective pest management methods should be adopted to minimize the losses caused by them (Singh et al., 2009). Aphid *Aphis craccivora* (Koch) is a major threat to cowpea from seedling to pod bearing stage. The nymphs and adults cause serious damage by sucking the plant sap. Young seedlings die as a result of high infestation, whilst older plants display signs such as stunted growth, crinkling, curling, late blooming and pods shriveling accompanied with yield loss. Aphid feeding damage to cowpea cultivars can result in production losses of up to 90% in susceptible varieties and up to 30% in intermediate resistance types. In modern agriculture, more selective and less polluting products are increasingly being employed by farmers for the effective control of insect pests (Siegwart et al., 2015).

Entomopathogenic fungi are the most abundant type of microorganisms that infects insect bodies through contact rather than ingestion and are widely distributed in nature across a wide host range and has immense significance because of environmental and food safety concerns. Furthermore, they are known to be non-toxic to non-target organisms like predators and parasitoids, and are easily cultured (Wu et al., 2015). They are especially significant in aphid management because aphids have morphological, biological, and ecological traits that render them vulnerable to fungal infections, which can produce epizootics that substantially lower aphid populations (Steinkraus, 2006). Among these fungi, *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metchnikoff) Sorokin and *Lecanicillium lecanii* (Zimmermann) Zare and Gams received major attention (Kalsbeek et al., 1995, Diaz et al., 2009). Increased interests in the use of entomopathogenic fungi in pest management necessitate the selection of fungal isolates with high virulence that show significant enzyme activities on target insects (Gebremariam et al., 2022). Keeping this in view, the current study evaluated entomopathogenic fungi for the control of *A. craccivora,* as well as the interaction between the effective entomopathogenic fungus in cowpea plants and *A. craccivora.*

MATERIALS AND METHODS

The study was carried out at the Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore (11° 07' - 3.36" N, 76° 59' -

39.91" E). Cowpea cultivar 'Vamban 2 (VBN 2)' was used in all experiments for evaluating the efficacy of entomopathogens against *A. craccivora*. The entomopathogenic pure cultures strains of *Beauveria bassiana* (Bb NBAIR and Bb SBI), *Metarhizium anisopliae* (Ma NBAIR, and Ma SBI) and *Lecanicillium lecanii* (Ll NBAIR) were collected from the Department of Agricultural Entomology and Plant Pathology, Tamil Nadu Agricultural University (TNAU), National Bureau of Agricultural Insect Resources (NBAIR), Bangalore and Sugarcane Breeding Institute (SBI), Coimbatore. Sabouraud maltose agar with yeast extract (SMA+Y) medium (maltose 40 g, yeast extract 5 g, peptone 10 g, agar 15 g, distilled water 1l) were used to maintain the obtained colonies. To avert bacterial contamination, the antibiotics 0.05 mg ampicillin, 0.02 mg chloramphenicol and 0.2 mg streptomycin were added/ ml to the SMA+Y. The pure slants in test tubes were maintained at - 20°C as a stock culture. The apterous aphids' culture was established in the laboratory by collecting adults from the cowpea fields. To initiate the assay, cowpea plants were raised in mud pots containing potting mixture with sand, soil and dried cow dung in the ratio of 1:1:1. The aphids collected from the fields were relocated on the trifoliate stage of cowpea seedlings of VBN 2 variety. The pots were covered with netted cage and thereby the aphid colonies were kept free from natural enemies. Once in three weeks, new seedlings were raised and infested aphid twigs from the old plants were tied with them. Thus, aphid culture was maintained throughout the period of study (Saranya, 2012).

To test the pathogenicity of entomopathogens against aphid under laboratory conditions, fifteen days old VBN 2 seedlings were raised in paper cups measuring 7.5 x 7.5 cm. Six different spore concentrations ($1x10^8$, $1x10^7$, $1x10^6$, $1x10^5$, $1x10^4$, $1x10^3$ spores ml^{-1}) for each pure culture of entomopathogenic fungi (B. *bassiana, M. anisopliae, and L. lecanii*) were maintained individually. Each concentration was replicated thrice. For bioassay experiment, the apterous aphids were released on the cowpea seedlings @ 10 aphids/ seedling using camel hairbrush. Each treatment used a total of 30 aphids. After the aphids had been inoculated, an atomizer was used to spray the various concentrations ($1x10^8$, $1x10^7$, $1x10^6$, $1x10^5$, $1x10^4$, $1x10^3$ spores ml^{-1}) of fungal spore solutions on the seedlings. As a control, aphids were sprayed with a 0.05% Tween 80 solution (Butt and Goettel, 2000). Aphid mortality was monitored at 24 hr intervals for up to seven days. On daily basis, the dead aphids were collected and placed in a petridish with wet filter paper, which was then stored in a humid chamber. The mortality count included the deceased aphids that produced mycelial growth. Using Abbott's method, mortality data was adjusted for control mortality (Abbott, 1925).

Further substantiation of interaction of entomopathogenic fungus in cowpea plants were studied and the activity of defense enzymes i.e., biochemical alteration in plants were recorded at different time intervals. Cowpea leaves pretreated with a crude preparation of entomopathogenic fungal strains and challenged with aphids were collected for the estimation of defense related enzymes. Each replication of the treatment had four plants sampled separately. For comparison, untreated control (without spray, but infected with aphids) and absolute control (no aphid infestation and healthy) were also maintained. The leaves were collected at 24 hr interval initiating from 0 hr to 9 days. The obtained leaves were blended in liquid nitrogen and stored at -80°C. The homogenized sample was extracted with 2 ml of sodium phosphate buffer 0.1 M (pH 7.0) at 4°C. The obtained sample was centrifuged for 20 min at 10,000 rpm and the extract was used for estimation of defense enzymes. The chitinase (EC 3.2.1.14) colorimetric assay was performed according to the Boller and Mauch (1988). The enzyme activity was expressed as n moles GlcNAc equivalents min^{-1} g^{-1} fresh weight. The CAT activity (EC 1.11.1.6) was measured spectrophotometrically as reported by Chaparro-Giraldo et al. (2000). Activity was determined using the extinction coefficient (ε_{240nm} = 40 mM^{-1} cm^{-1}) for H2O2 and expressed in mmol min^{-1} g^{-1} of sample. The assay of phenol was calculated according to a standard curve obtained from a folin-ciocalteau reagent with a phenol solution (C6H6O) and represented as catechol equivalents g^{-1} tissue weight (Zieslin and Ben-Zaken, 1993).

RESULTS AND DISCUSSION

Pathogenicity of fungal isolates: Aphid mortality in treatments were corrected with respect to control mortality. Table 1 shows the data for corrected % mortality at various time periods. The highest mortality of 94.67% was obtained in the highest spore concentration of 10^8 spores ml^{-1} in *B. bassiana* (NBAIR) followed by *B. bassiana* (SBI) (92.44%) and *M. anisopliae* (NBAIR) (85.89%) at 120 hr (5 days) after treatment. At 144 hr (6 days) after treatment there were marked increases in the mortality. Among the fungal isolates, *B. bassiana* (NBAIR) and *B. bassiana* (SBI) recorded 100% mortality at 10^8 spores

Table 1. Effect of entomopathogenic fungal isolates on the mortality of *A. craccivora*

Entomopathogenic fungal Isolates	Spores ml^{-1}	% corrected mortality in hr					
		24	48	72	96	120	144
B. bassiana (Bb NBAIR)	10^8	7.89	34.22	58.67	88.78	94.67	100.00
	10^7	5.44	8.78	35.22	57.56	85.78	100.00
	10^6	0.00	6.56	23.56	52.89	78.56	80.00
	10^5	0.00	2.11	15.78	28.78	37.67	39.67
	10^4	0.00	0.00	14.33	18.67	22.00	28.89
B. bassiana (Bb SBI)	10^8	4.33	12.00	46.56	65.00	92.44	100.00
	10^7	0.00	5.56	24.44	43.33	82.22	85.56
	10^6	0.00	2.22	20.00	32.22	66.67	70.00
	10^5	0.00	0.00	12.22	21.11	30.00	33.33
	10^4	0.00	0.00	6.67	10.00	23.33	25.56
M. anisopliae (NBAIR)	10^8	0.00	15.33	28.78	54.11	85.89	94.22
	10^7	0.00	9.87	23.00	32.33	79.22	83.22
	10^6	0.00	4.33	12.11	28.56	54.78	64.33
	10^5	0.00	0.00	8.78	16.33	17.67	27.56
	10^4	0.00	0.00	4.56	8.67	14.33	18.78
M. anisopliae (SBI)	10^8	0.00	10.33	17.78	38.11	58.87	72.25
	10^7	0.00	7.48	13.00	28.33	32.22	65.22
	10^6	0.00	4.73	7.11	15.56	23.78	43.33
	10^5	0.00	0.00	5.78	12.33	16.67	25.56
	10^4	0.00	0.00	3.56	5.67	13.33	17.78
L. lecanii (Ll NBAIR)	10^8	0.00	11.33	25.78	41.11	58.89	62.22
	10^7	0.00	7.89	23.00	33.33	42.22	55.22
	10^6	0.00	0.00	11.11	25.56	37.78	43.33
	10^5	0.00	0.00	0.00	7.78	13.33	16.67
	10^4	0.00	0.00	0.00	6.67	12.33	15.78

ml^{-1}. With increased spore concentration, the duration of reproduction was significantly reduced. Within 24 hr of infection, the fertility of pea aphids, *A. pisum,* infected with *B. bassiana* and *P. neoaphidis* decreased (Baverstock et al., 2006). Smitha (2007) observed increase in mortality of banana mealy bug *Geococcus* sp (90 %) with *Hirsutella thompsonii* (10^8 spores ml^{-1}) after three days of inoculation. Ekesi et al. (2000) observed 91 and 93% mortality of *A. craccivora* at 7 days post treatment. Loureiro and Moino (2006) obtained 100% mortality of *Myzus persicae* by *B. bassiana* and *M. anisopliae* @10^6 and 10^7 spores ml^{-1}, respectively.

Plant defense induced by *B. bassiana* (NBAIR) against *A. craccivora*: The activitiy of the enzyme's phenylalanine ammonia lyase, peroxidase, polyphenol oxidase, catalase and chitinase increased in the entomopathogenic fungal pathogens treated cowpea against *A. craccivora*, as demonstrated by biochemical analysis.

Peroxidase (PO): In the present study increased activity of PO was documented in *B. bassiana* (NBAIR) treated cowpea plants infested with *A. craccivora* and it was significantly different from all other treatments after 120 hr. The PO activity increased up to 120 hr of inoculation and thereafter, declined. No significant increase was noted in the untreated healthy plants (Table 2). PO's direct post-ingestive toxicity against insects has been related to production of semiquinone free radicals and ensuing synthesis of quinones (Zhu Salzman et al., 2008; Barbehenn et al., 2010). Peroxidases have been involved in different physiological functions that boosts plant resistance against herbivore and pathogen attack (Duffey and Stout, 1996); the process includes oxidation of hydroxyl cinnamyl alcohol, oxidation of phenol, cross linking of polysaccharide and extension monomers, lignification, production and polymerization of phenolics, hypersensitive response and suberization (Zhang et al., 2008; Chen et al., 2009). These phenomena in turn lead to the production of anti-nutritive compounds against the biotic stress (Gulsen et al., 2010; He et al., 2011).

Polyphenol oxidase (PPO): Accumulation of polyphenol oxidase was higher in *B. bassiana* (NBAIR)

Table 2. Induction of peroxidase (PO) and poly/ phenol oxidase (PPO) activity against aphid in *B. bassiana* treated cowpea

S. No.	Treatments	PO activity (changes in A420/ min/ g of fresh tissue) Hours after challenge inoculation						
		0 h	24 h	48 h	72 h	96 h	120 h	168 h
1.	Bb (NBAIR) (In)	1.37^{a}	1.57^{a}	1.63^{a}	1.75^{a}	1.97^{a}	2.47^{a}	2.32^{a}
2.	*Beauveria* talc based (commercial formulation Racer, check) (In)	1.24^{ab}	1.45^{ab}	1.57^{ab}	1.74^{ab}	1.83^{b}	2.35^{ab}	2.28^{a}
3.	Chemical (dimethoate, check) (In)	0.92^{b}	1.32^{ab}	1.36^{b}	1.56^{b}	1.62^{b}	2.16^{b}	1.87^{bc}
4.	Control (Inoculated)	0.52^{cd}	0.57^{c}	0.62^{cd}	0.68^{cd}	0.68^{d}	0.68^{cd}	0.69^{de}
5.	Bb NBAIR (UI)	0.78^{bc}	0.72^{b}	0.82^{c}	0.93^{c}	1.24^{bc}	1.32^{c}	1.28^{bc}
6.	*Beauveria* talc based (commercial formulation Racer) (UI)	0.73^{bc}	0.78^{b}	0.85^{c}	0.96^{bc}	0.82^{cd}	1.23^{e}	0.87^{cd}
7.	Chemical (dimethoate) (UI)	0.65^{bc}	0.83^{b}	0.97^{bc}	0.94^{bc}	1.08^{c}	1.54^{bc}	1.47^{b}
8.	Healthy control	0.32^{d}	0.37^{cd}	0.38^{e}	0.45^{d}	0.52^{de}	0.57^{cd}	0.42^{e}
S. No.	Treatments	PPO activity (changes in A490/ min/ g of fresh tissue) Hours after challenge inoculation						
		0 h	24 h	48 h	72 h	96 h	120 h	168 h
1.	Bb (NBAIR) (In)	0.23^{d}	1.27^{a}	1.37^{a}	1.58^{a}	1.67^{a}	1.92^{a}	1.82^{a}
2.	*Beauveria* talc based (commercial formulation Racer, check) (In)	0.85^{a}	1.12^{b}	1.18^{b}	1.48^{a}	1.59^{a}	1.73^{b}	1.68^{ab}
3.	Chemical (dimethoate, check) (In)	0.63^{b}	0.81^{bc}	0.84^{cd}	0.96^{b}	1.18^{b}	1.23^{bc}	0.97^{bc}
4.	Control (Inoculated)	0.52^{bc}	0.55^{c}	0.55^{d}	0.69^{bc}	0.76^{cd}	0.87^{d}	0.78^{cd}
5.	Bb NBAIR (UI)	0.44^{c}	0.53^{cd}	0.58^{d}	0.64^{c}	0.92^{c}	0.92^{cd}	0.94^{bc}
6.	*Beauveria* talc based (commercial formulation Racer) (UI)	0.45^{c}	0.52^{cd}	0.54^{d}	0.62^{c}	0.96^{bc}	0.95^{bc}	0.92^{c}
7.	Chemical (dimethoate) (UI)	0.38^{cd}	0.45^{d}	0.44^{e}	0.45^{d}	0.43^{e}	0.42^{e}	0.38^{e}
8.	Healthy control	0.32^{cd}	0.34^{de}	0.48^{e}	0.47^{d}	0.67^{de}	0.78^{de}	0.75^{cd}

In a column means followed by a common letter (s) not significantly different (p = 0.05, DMRT); In- Inoculated, UI- Uninoculated

treated plants challenge inoculated with *A. craccivora* and supreme activity was observed at 120 hr and thereafter started to decline whereas in uninoculated control plants, there was a slight induction of PPO (Table 2). PPO is an antinutritional enzyme that helps plants defend themselves against insect herbivory (Mahanil et al., 2008; Bhonwong et al., 2009). The process involves the formation of the highly reactive and toxic quinones from the phenols that interact with the amino acids chain nucleophilic side and causes cross-linking and restricts the protein supply to insect pests (Zhang et al., 2008; Bhonwong et al., 2009). PPOs create melanin, which improves the digestibility and palatability of plant tissues while also increasing cell wall confrontation to insects and pathogens (Zhao et al., 2009).

Phenylalanine ammonia lyase (PAL): In current study, entomopathogenic fungal bioformulation treated cowpea plants inoculated with *A. craccivora* induced the plant to synthesize higher level of PAL. The enzyme activity induced by *B. bassiana* (NBAIR) bioformulation reached the maximum at 120 hr and thereafter, it decreased (Table 3). In phenyl propanoid metabolism, phenyl alanine ammonia lyase (PAL) plays a crucial part in the production of several defensive compounds (Daayf et al., 1997). Plant-pathogen and plant-pest interactions may trigger PAL activity (Ramanathan et al., 2000; Radja Commare, 2000; Kandan et al., 2002; Bharathi et al., 2004; Harish, 2005; Saravanakumar, 2006). Corroborating to the above findings, when compared to an untreated healthy control, PAL activity was considerably greater in entomopathogenic fungal pathogen treatments supported by Harish (2005) and Saravanakumar (2006).

Chitinase (PR3): Chitinase activity was greater in the leaves of cowpea plants that had been pretreated with the *B. bassiana* (NBAIR) bioformulation and the enzyme activity peaked at 120 hours after infection (Table 3). Plants with high chitinase levels have been linked to pest and disease resistance (Maurhofer et al., 1994; Van Loon, 1997). It can also serve as an amylase inhibitor, preventing plant components from

Table 3. Induction of Phenylalanine Ammonia Lyase (PAL) and Chitinase activity against aphid in *B. bassiana* treated cowpea plants

S. No.	Treatments	PAL activity (n mol trans cinnamic acid/ min/ g of fresh tissue) Hours after challenge inoculation						
		0 h	24 h	48 h	72 h	96 h	120 h	168 h
1.	Bb (NBAIR) (In)	7.44^{a}	7.87^{a}	8.25^{a}	11.32^{a}	14.28^{a}	18.02^{a}	18.32^{a}
2.	*Beauveria* talc based (commercial formulation Racer, check) (In)	7.33^{a}	7.38^{a}	8.09^{a}	10.09^{b}	12.14^{b}	15.19^{b}	15.24^{b}
3.	Chemical (dimethoate, check) (In)	6.22^{b}	6.27^{b}	6.62^{ab}	5.85^{c}	5.86^{cd}	7.15^{c}	8.26^{c}
4.	Control (Inoculated)	4.83^{c}	4.85^{cd}	5.17^{b}	3.24^{d}	4.63^{d}	3.28^{d}	3.22^{d}
5.	Bb NBAIR (UI)	1.38^{e}	1.58^{ef}	1.52^{cd}	0.53^{ef}	0.81^{e}	0.88^{f}	0.78^{g}
6.	*Beauveria* talc based (commercial formulation Racer) (UI)	1.35^{e}	1.53^{f}	1.48^{de}	0.50^{ef}	0.49^{ef}	0.99^{ef}	0.92^{ef}
7.	Chemical (dimethoate) (UI)	1.74^{d}	1.89^{e}	1.82^{c}	0.84^{e}	0.25^{f}	1.38^{e}	1.34^{e}
8.	Healthy control	1.57^{de}	1.56^{ef}	1.49^{d}	0.51^{ef}	0.46^{ef}	0.63^{fg}	0.58^{gh}
S. No.	Treatments	n mol GlcNac min^{-1} mg^{-1} of protein (Hours after inoculation)						
		0 h	24 h	48 h	72 h	96 h	120 h	168 h
1.	Bb (NBAIR) (In)	6.42^{a}	5.42^{a}	5.76^{a}	6.74^{a}	6.34^{a}	6.34^{a}	6.12^{a}
2.	*Beauveria* talc based (commercial formulation Racer , check) (In)	5.15^{b}	5.37^{ab}	4.44^{b}	5.45^{b}	4.73^{b}	5.23^{b}	4.87^{b}
3.	Chemical (dimethoate, check) (In)	4.27^{bc}	4.28^{bc}	3.34^{c}	4.67^{bc}	3.92^{c}	3.86^{c}	3.82^{c}
4.	Control (Inoculated)	3.34^{cd}	3.22^{c}	2.36^{d}	3.45^{de}	2.65^{d}	2.75^{d}	2.74^{d}
5.	Bb NBAIR (UI)	3.12^{d}	3.20^{cd}	2.27^{d}	3.38^{d}	2.54^{de}	2.67^{de}	2.62^{d}
6.	*Beauveria* talc based (commercial formulation Racer) (UI)	2.29^{ef}	2.61^{d}	1.83^{e}	2.52^{f}	1.84^{e}	2.24^{e}	2.24^{e}
7.	Chemical (dimethoate) (UI)	2.26^{f}	2.39^{de}	1.56^{ef}	2.79^{ef}	1.73^{f}	1.84^{f}	1.82^{f}
8.	Healthy control	2.32^{e}	2.23^{e}	1.26^{f}	2.26^{g}	1.27^{g}	1.23^{g}	1.24^{fg}

In a column means followed by a common letter (s) not significantly different (p = 0.05, DMRT); In- Inoculated, UI- Uninoculated

being digested (Ary et al., 1989). Inducing chitinase activity might hinder the pest growth, alimentation and eventually kill it (Shapira et al., 1989; Wang et al., 1996).

Catalase (CAT): In response to *A. craccivora* inoculation, induction of catalase was higher in *B. bassiana* (NBAIR) bioformulation treated plants. The catalase activity was comparatively lower in the untreated plants challenged with *A. craccivora.* The extreme activity was noticed at 120 hr of infestation in *B. bassiana* (NBAIR) pretreated plants. In healthy plants, the expression was low (Table 4).

Phenolics: Increased phenolic accumulation was observed after *A. craccivora* was inoculated into *B. bassiana* (NBAIR) treated plants. On comparison, in the untreated healthy controls, phenol activity was rather modest. At 120 hr after infection, the highest accumulation was seen (Table 4).

Plants have evolved various defensive systems to defend themselves against herbivores and diseases. Insects are the most common herbivores, causing significant damage to plants, and protecting plants from these herbivores is critical for plant survival. Furthermore, the entomopathogenic fungal infections and their interaction readily influenced the sluggish growth of these insect pests and induce resistance. Feeny's slow-growth, high-mortality theory is supported by this observation (Feeny, 1976). Hence, it is concluded that slow development and high mortality is required, and certain entomopathogens should be considered as key natural enemies of insect pests.

CONFLICT OF INTEREST

No conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

SP executed the research, B.M: Draft the paper, whereas PS supervised the work. All authors have read and agreed to the published version of the manuscript.

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Table 4. Induction of catalase and total phenolics activity against aphid in *B. bassiana* treated cowpea plants

S. No.	Treatments	Catalase activity (µmol of H_2O_2 consumed/ min/ g of fresh tissue) Hours after challenge inoculation						
		0 h	24 h	48 h	72 h	96 h	120 h	168 h
1.	Bb (NBAIR) (In)	2.86^{a}	2.84^{a}	3.23^{a}	3.45^{a}	3.97^{a}	3.53^{a}	3.24^{a}
2.	*Beauveria* talc based (commercial formulation Racer, check) (In)	2.74^{ab}	2.76^{b}	3.14^{b}	3.21^{b}	3.33^{bc}	3.25^{b}	2.96^{b}
3.	Chemical (dimethoate, check) (In)	2.35^{b}	2.38^{b}	2.95^{c}	2.97^{c}	2.98^{c}	2.25^{c}	1.92^{c}
4.	Control (Inoculated)	2.26^{bc}	2.25^{c}	2.36^{d}	2.56^{d}	2.75^{d}	1.36^{d}	1.34^{cd}
5.	Bb NBAIR (UI)	1.44^{d}	1.56^{d}	1.58^{e}	1.67^{e}	1.85^{de}	1.12^{de}	0.92^{d}
6.	*Beauveria* talc based (commercial formulation Racer) (UI)	1.47^{d}	1.48^{de}	1.64^{e}	1.75^{e}	1.87^{ef}	0.94^{e}	0.85^{de}
7.	Chemical (dimethoate) (UI)	1.43^{d}	1.48^{de}	1.49^{f}	1.54^{e}	1.67^{f}	0.86^{f}	0.78^{e}
8.	Healthy control	1.48^{d}	1.45^{e}	1.47^{f}	1.48^{e}	1.54^{f}	0.68^{f}	0.54^{e}
S. No.	Treatments	Phenol content (µg of catechol/ g of fresh tissue) Hours after challenge inoculation *						
		0 h	24 h	48 h	72 h	96 h	120 h	168 h
1.	Bb (NBAIR) (In)	92.18^{a}	104.45^{a}	108.37^{a}	118.95^{a}	124.47^{a}	128.44^{a}	121.43^{a}
2.	*Beauveria* talc based (commercial formulation Racer, check) (In)	86.72^{b}	97.44^{a}	98.24^{ab}	114.44^{a}	112.37^{b}	117.75^{b}	113.67^{b}
3.	Chemical (dimethoate, check) (In)	66.58^{d}	76.35^{b}	83.42^{c}	94.21^{bc}	92.22^{c}	97.86^{cd}	92.14^{cd}
4.	Control (Inoculated)	48.13^{e}	47.37^{c}	52.84^{d}	62.46^{d}	57.64^{e}	62.72^{e}	51.28^{e}
5.	Bb NBAIR (UI)	86.73^{b}	96.34^{a}	98.73^{ab}	108.15^{b}	103.75^{bc}	98.82^{cd}	99.85^{c}
6.	*Beauveria* talc based (commercial formulation Racer) (UI)	75.21^{c}	84.77^{c}	86.75^{bc}	92.98^{c}	87.23^{d}	82.75^{d}	87.87^{d}
7.	Chemical (dimethoate) (UI)	67.19^{d}	75.45^{b}	87.52^{bc}	98.84^{bc}	98.66^{c}	108.26^{c}	98.82^{c}
8.	Healthy control	40.31^{e}	42.36^{d}	45.83^{e}	52.82^{e}	43.83^{f}	41.65^{f}	38.24^{f}

In a column means followed by a common letter (s) not significantly different (p = 0.05, DMRT); In- Inoculated, UI- Uninoculated

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