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Developing novel approaches for the control of stored-grain insect pests: laboratory assays of insecticide, diatomaceous earths and entomopathogenic fungi and their implementation on small-scale farms in Pakistan

Entwicklung neuer Verfahren zur Kontrolle von Schädlingen gelagerten Getreides: Laboruntersuchungen von Insektiziden, Kieselerden und entomopathogenen Pilzen sowie Implementierung auf kleinbäuerlichen Betrieben in Pakistan

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This dissertation has been accepted by the Faculty of Geography/Geosciences of Trier University to obtain the academic degree Doktor der Naturwissenschaften (Dr. rer. nat.).

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# Erklärung

Hiermit erkläre ich, dass die vorliegende Arbeit mit dem Titel "Developing novel approaches for the control of stored-grain insect pests: laboratory assays of insecticide, diatomaceous earths and entomopathogenic fungi and their implementation on smallscale farms in Pakistan" von mir selbständig verfasst wurde und bisher weder im Ganzen noch in Teilen in diesem Fachbereich oder in einem anderen akademischen Institution eingereicht worden ist. Alle für die Arbeit genutzten Hilfsmittel wurden genannt und die Ergebnisse etwa beteiligter anderer Autoren klar gekennzeichnet. Ich versichere zudem, dass mir die Promotionsordnung in der gültigen Fassung bekannt ist.

## Declaration

Herewith I declare that the here presented thesis with the title "Developing novel approaches for the control of stored-grain insect pests: laboratory assays of insecticide, diatomaceous earths and entomopathogenic fungi and their implementation on small-scale farms in Pakistan" was written by myself and was, neither in total nor in parts, submitted to this faculty or any other academic institution. All help used for this work was acknowledged by me and the results of eventual co-authors are clearly maked. I also declare that I am aware of the promotion order in its valid form.

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# 1 Introduction

Agriculture is the mainstay of Pakistan's economy contributing more than 24% to the Gross Domestic Product (GDP) (Akhtar and Aslam, 2003), and food crops hold an important position in the economy as they account for 68% of the total cultivated area of the country (Chowdhry et al., 1998). The most important crops in Pakistan belong to the world's major agricultural food commodities including wheat, rice, maize and sugarcane (Akhtar and Aslam, 2003).

Wheat (*Triticum aestivum* L.) (Poaceae) is the third largest food crop of the world, and Pakistan is the eighth largest wheat producing country globally (Shuaib et al., 2007). In Pakistan, wheat occupies 37% of the total cultivated area, represents 76% of the total food production (Jilani, 2007), adding 13.8% to the value added in agriculture and 3.4% of the GDP (Hussain et al., 2006). The total area under wheat cultivation in Pakistan is approximately 8.45 million hectares from which 72% (6.48 Mio. ha) is located in Punjab (Khan, 2007). Punjab is the largest province of Pakistan in terms of population density and number, accounts for 45% of the total agricultural production of this province. The wheat cultivation is economically sustainable, and it gives good quality and quantity of grain with excellent storage properties. Wheat is the main food crop in Pakistan and contains 13% protein (Ahmad and Sheikh, 2003). Consequently, it is the largest grain crop of the country.

Rice (*Oryza sativa* L.) (Poaceae) is the 2nd most important staple food of Pakistan after wheat and is grown in all provinces of the country (Chaudhry, 1994). In terms of acreage, it occupies more than 2.5 million hectares and accounts for more than 11% of the food crop production. Besides being an important staple food for millions of people, rice is also the second largest source of earning foreign exchange after cotton. Presently, the total annual rice production in Pakistan amounts about 4.99 million tons (Anonymous, 2004-05). Pakistan is also the largest producer of aromatic quality fine rice (Basmati) in the world.

Maize (*Zea mays* L.) (Poaceae) is the world's top ranking food crop even before wheat and rice. In Pakistan, maize is grown over an area of about one million hectares with production of 1.76 million tons/annum (GOP, 2003). Maize also occupies over 345

thousand hectares and accounts for over 3% of the food crop production of Punjab. It is a multipurpose crop which not only serves as a food source for human beings, but also provides feed for animals, livestock and poultry. It is important mainly due to its nutritional value and contains starch (72%), protein (10%), oil (4.8%), fiber (8.5%), sugar (3%) and ash (1.7%) (Chaudhary, 1993).

The harvested products are stored in different types of storage structures from small to large scale as storage of food is inevitable in both, the times of deficit and surplus production (Lal, 1996). In Pakistan, the harvested grains have to be stored for a period of ten to twelve months for the regular food supply throughout the year (Syed et al., 2001). About 65-75% of the total produced wheat in Pakistan is stored at farms not only as food but also for seed purpose (Choudhry and Anwar, 1988). However, storage periods of more than three months make the commodity more vulnerable to insect attacks.

Being a living entity, grains get affected by several biotic and abiotic factors resulting in quantitative and qualitative losses during storage (Singh et al., 1990; Ahmad, 1995). Among the biotic factors, insect pests are the major agents, which cause serious losses to grains in term of quantity and quality. The environmental conditions like in the Punjab province of Pakistan are highly favourable for insect growth and development. Therefore, grain storage losses of just 5% in developed countries can reach up to 10-15% in developing countries like Pakistan (Ahmad, 1980), and heavy losses ranging up to 25.5% have been recorded (Irshad et al., 1988).

The role of insect pests as a threat to stored commodities has been discussed many times by various researchers (Golebiowska, 1969; Singh et al., 1990; Rees, 1996). About 23 species of stored grain insect pests are most important in infesting commodities during storage. About 75% of these pest species belongs to one single insect order, i.e. the Coleoptera (Viñuela et al., 1993).

Among these species, *Rhyzopertha dominica* (Fabricius, 1792) (Coleoptera: Bostrychidae), the lesser grain borer, is amongst the most devastating insect species of stored cereals and legumes all around the world (Aslam et al., 1994; Hagstrum and Flinn, 1994). *R. dominica* is the only species of the genus *Rhyzopertha* (Edde, 2012). It seems to be originated from the Indian subcontinent, like many other species of the bostrichids (Potter, 1935). The lesser grain borer is a reddish brown to dark brown coloured beetle,

varying in size, and its head is invisible when viewed from above (Edde, 2012). It feeds on the grains by boring irregularly shaped holes into undamaged kernels, thus being recognized as primary feeder or primary pest (Aitken, 1975). The adult females deposit eggs in crevices or on the rough surface of the grain. The emerged larvae bore into grains, feed internally and can survive even at 8% moisture contents (Birch, 1945). They are able to complete their life cycle remaining inside the grain (Mayhew and Phillips, 1994). The larvae and adult beetles both are capable of feeding on the germ and the endosperm of grains (Dal-Bello et al., 2001). Thus, the germination rate of infected seeds is highly affected. The degree of damage caused by adult feeding varies with the age of the beetle, and greater damage is caused by the young imagos (Rao and Wilbur, 1972).

The red flour beetle, *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) is a cosmopolitan stored product insect pest species that causes serious damage in stored grains, ware houses, food mills etc. Although, this species is feeding on a large variety of different foods, but generally it is abundant in processed products for instance flour (Aitken, 1975). Consequently, *T. castaneum* is more known as secondary insect pest, but it can also damage the whole grains, nevertheless developing better in processed products (Aitken, 1975). The optimum temperature for its development ranges from 22 to 40°C, and the species is capable of completing its life cycle within 20 days (Rees, 2004). Adults are very active and capable of accomplishing their development even in small patches of food (Campbell and Runnion, 2003). *T. castaneum* is important because in addition to individuals of each life stage the infested materials may also have insect remains and benzoquinones (Baur, 1984).

*Cryptolestes ferrugineus* (Stephens, 1831) (Coleoptera: Laemophloeidae), the sawtoothed grain beetle infests the stored cereals and their derivatives by feeding on the germ of the grain kernels (Sinha, 1965). They are not capable of boring whole kernels but can be linked with primary insect pests like *Sitophilus* spp. and *R. dominica* (Tuff and Telford, 1964). The adults are good fliers (Mason, 2003), and the larvae grow individually inside the seed coat of the cereal seeds. Thus, they are protected from cannibalism or predation when e.g. occurring together with *T. castaneum* (Suresh et al., 2001). The grubs are white in color, 3 mm in length having two brown projections at the posterior end of their body. During feeding, they enter through the germ-end and pupate.

Infestations do not develop in the grains with <12% moisture content or <40% relative humidity. The optimum temperature for the growth ranges from 20 to 40°C (Mason, 2003). *C. ferrugineus* avoids light, generally searching for shelther which may provide physical contact with the body of the insect from all sides.

Currently, the stored product psocids belonging to the genus *Liposcelis* (Motschulsky, 1852) (Psocoptera: Liposcelididae) are emerging pests in stored grains (Nayak, 2006). They have often been considered as secondary pests, which have been assumed to be unable to infest whole grains. However, recent studies indicated the abilities of psocids to infest sound kernels as well (Nayak et al., 2005). Thus, they may develop local populations in the infested commodity as primary and even sole pest species (Athanassiou et al., 2010 b). Several psocid species have been recorded in steel bins holding wheat as well as in empty bins (Throne et al., 2006). Psocid populations have been found to be abundant under conditions of higher temperature and moisture content of the grains (Opit et al., 2009). Under laboratory conditions, *L. bostrychophila* may cause about 10% grain loss after three months of infestation (Kučerová, 2002). In addition to the quantitative and qualitative losses of the stored grains (Obr, 1978), several psocid species may also act as a vector for fungal spores and other microorganisms and hence, for example, might be responsible for the development of allergies in humans (Turner and Ali, 1996).

For the stored grain insect pests' management, the utilization of synthetic residual insecticides has been the method of choice to preserve stored product since the 1950's. However, these chemicals are being discouraged mainly due to three different reasons: their toxicity to mammals (as food get contaminated with insecticidal residues), the development of insect pest resistance to these substances and the need of quality environment (Pacheco et al., 1990; Arthur, 1996).

For example, phosphine has been extensively used as an insecticidal fumigant against stored grain insect pest since the 1930's (Chaudhry, 1997), but a highest level of resistance has already been reported against this fumigant in Pakistan (Alam et al., 1999) and various other countries all over the world (Tyler et al., 1983; Rajendran and Narasimhan, 1994; Collins et al., 2002; Pimentel et al., 2010). Genetic assays demonstrated that two or more genes might be responsible for this type of resistance in *R*.

*dominica* and some other stored grain insect pests (Ansell et al., 1990). Under the international agreement of the Montreal Protocol, the worldwide phase-out and ban of methyl bromide (MB), an effective compound for the control of post-harvest insect pests has motivated research to find suitable alternatives for the replacement of methyl bromide (Fields and White, 2002).

One promising future tactic could be the application of insecticides depicting reduced toxicity on mammals. In this regards, pyrethroids being considered among such group of insecticides are considered potential substitute to the presently excerised organophosphorous grain protectants. Many pyrethroids have been successfully assessed against a broad range stored-grain insect pest species, some of these agents are now registered for direct application on the commodities (White and Leesch, 1995; Arthur, 1996).

As environment related issues are receiving more and more attention, agricultural practices also have to be standardized according to the rules and regulations set by the World Trade Organization (WTO). In the same context, the resistances of many insect pests to a larger number of the conventionally used chemicals and the demand of the consumers' for residue free food paved the way for researchers not only to introduce the safe use of the prevailing control measures, but also to develop and evaluate new and alternative control methods. The utilization of viruses, bacteria, protozoa and entomopathogenic fungi are powerful tools of biological control, and thus represent effective choices in the replacement to chemical-based insecticides (Moore et al., 2000). As an integral part of Integrated Pest Management (IPM), the utilization of microbes in biocontrol methods has been evolved as an innovative strategy against damaging arthropods. These methods are not only successful to combat insect pest's resistance and to cope with environmental issues, but also their enhanced performance at lower rates is one key point for their successful application (Starnes et al., 1993).

The true fungi (Kingdom: Mycota) are classified into four distinct divisions, i.e. Chytridiomycota, Zygomycota, Ascomycota and Basidiomycota (Hawksworth et al., 1995). The entomopathogenic fungi belong to the two classes Entomophthorales in the Zygomycota and the class Hyphomycetes of Ascomycota (Shah and Pell, 2003). The important genera of this latter class are *Aspergillus, Beauveria, Culicinomyces, Hirsutella*,

*Metarhizium*, *Nomuraea*, *Paecilomyces*, *Tolypocladium* and *Verticillium* (Inglis et al., 2001).

The entire procedure of infection by entomopathogenic fungi generally accomplishes five to six steps including; (i) adhesion of the fungal conidia to the insect's cuticle, (ii) germination of the conidia, (iii) penetration to host's cuticle, (iv) defeating the host's defence responses, (v) vegetative growth inside the insect body and (vi) post mortal sporulation (Narayanan, 2004; Zimmermann, 2007). The host insects are penetrated by the fungi either through the cuticle or their alimentary canal (digestive tract) (Broome et al., 1976). However, most fungal invasions have been observed through the cuticle (Akbar et al., 2004) because specific long chain hydrocarbons of the insect's cuticle facilitate conidial attachment through hydrophobic interaction in conidial cell wall (Boucias et al., 1991). Certain enzymatic processes are also adopted by Hyphomycetous fungi to penetrate insect cuticles, for example exoproteases, endoproteases, esterases, lipases, chitobiases and chitinases (Boucias and Pendland, 1998; Butt et al., 1998).

After reaching into the hemocoel, the fungus produces hyphal bodies, which initially degrade the fat and gut tissues, then the malphigian tubules undergo the depletion process (Pekrul and Grula, 1979). In this way, a multiple action of the fungus inside the insect body leads to the death of the target organism. These entomopathogenic fungi exert their toxic effect with various toxic substances, which they produce at the entrance into the host's body e.g. *Beauveria bassiana* generates beauvericin (Zizka and Weiser, 1993), bassianolide and oosporein (Eyal et al., 1994). In the same way, some of them cause tetanic paralysis (Dumas et al., 1996), whereas others may suppress the immune system of an insect (Cerenius et al., 1990).

Entomopathogenic fungi are a diverse group with a large variety of genotypes (Inglis et al., 2001). Thus, they are equally effective against field crop as well as stored grain insect pests. As more emphasis has been laid on the utilization of biological control measures for the protection of plants and stored commodities, the entomophagous fungi have recently received great and still increasing attention (Hluchý and Samšiňáková, 1989). In this regard, the most extensively studied species is *Beauveria bassiana* sensu lato (Balsamo) Vuillemin (Ascomycota: Hypocreales: Clavicipitaceae) (Moino et al., 1998; Dal-Bello et al., 2001; Akbar et al., 2004; Vassilakos et al., 2006), followed by

*Metarhizium anisopliae* sensu lato (Metschnikoff) Sorokin (Ascomycota: Hypocreales: Clavicipitaceae) for controlling stored products insect pests (Dal-Bello et al., 2001; Batta, 2003, 2004; Michalaki et al., 2006).

Diatomaceous Earth's (DE's) have also been considered as natural, non-toxic and stable alternative to synthetic chemicals. DEs originate from the fossils of unicellular algae, i.e. phytoplanktons or diatoms (Korunic, 1998). The major portion of its composition is comprised by hydrated silica, which dehydrates the insect body by both, cuticle lipid absorption and abrasion when getting in contact with the insect's body (Ebeling, 1971; Quarles and Winn, 1996).

Prior to the discovery of these desiccating properties, there were different schools of thought about the mode of action by the inert dusts including (i) the ingestion of the inert dust particles in to digestive system of the insects (Richardson and Glover, 1932), (ii) chemical reactions induced by these dust particles on the insect's cuticle (Shafer, 1915) and (iii) mechanical actions (Germar, 1936), which resulted in insect mortality. Initially, the disruption of the epicutilce by the dust particle was supposed to be the main reason of water losses from the insect body, but later on the absorption of cuticular was layers by the porous dust particles was established (Kalmus, 1944; Mewis and Ulrich, 2001). The wax layer around the insect cuticle is the most important barrier against water loss from the insect body. However, when this layer is absorbed by DE or other inert dust particles, this leads to dehydration of the insect body, a process which accelerates under dry conditions (Alexander, 1944 a, b). The use of DEs against insects and mites has been discussed in many reviews and research articles (e.g. Fields and Korunic, 2000, Chanbang et al., 2007; Vardeman et al., 2007; Wakil and Ashfaq, 2008; Wakil and Javed, 2008).

These dusts are applied without any special equipment and are directly mixed with the grains (Athanassiou et al., 2004 b). The low mammalian toxicity of DEs (Subramanyam et al., 1994) along with its easy removal from the treated material and its wide ranged effectiveness (Golob, 1997; Subramanyam and Roesli, 2000) are some key points for the successfulness of this compound. On the other hand, reduced grain flowability (Jackson and Webley, 1994), bulk density and dusty appearance of the grains (Korunic et al., 1997) when treated with higher dose rates of DEs are some limiting

factors in the even distribution and acceptability of DE treated grains at the international markets.

The use of various environment friendly control strategies including the use of microbes and inert dusts are without any doubt considered as one of the most promising alternatives to chemical control, but chemical control could not be replaced completely due to its uncontestable performance over biological control measures against insect pest species. The application of new chemistry insecticides is propagated all over the world as they possess low mammalian toxicity and provide an excellent control of insect pests.

Imidacloprid (1-(6-Chloro-3-pyridylmethyl)-Nitroimidazolidin-2-ylidenamine) is a second generation neonicotinoid and has emerged as a more effective and stable compound of its class (Bai et al., 1991). It was patented in 1985 by Bayer and was the first chemical of the neonicotinoid class that was widely marketed since 1991 (Liu et al., 2008; Zhang et al., 2008). Later on, it was registered in 56 countries for foliar and soil application (Leicht, 1996). Mullins (1993) stated its effectiveness against Coleopteran, Dipteran, Homopteran and Lepidopteran insect pests. Imidacloprid has also effectively been used against various insect pests of field crops (Harvey et al., 1996; Yue et al., 2003; Razaq et al., 2005; Wang et al., 2005; Byrne et al., 2009). Nayak and Daglish (2006) evaluated the efficacy of Imidacloprid against four different species of stored grain psocids (Psocoptera: Liposcelididae). More recently, Daglish and Nayak (2012) also testified the effect of a neonicotinoid formulation Imidacloprid and an oxadiazine formulation Indoxacarb against five major stored grain insect pest species i.e. *R. dominica, Sitophilus oryzae, T. castaneum, Oryzaephilus surinamensis* and *C. ferrugineus*.

The integrated use of different control measures is the best strategy to overcome shortcomings, difficulties and problems particularly related with high application rates of different control agents, which are evident in case of their individual use (Arthur, 2003). Numerous researchers have already evaluated the integrated effects of either DE with entomopathogenic fungi (Lord, 2001, 2005; Athanassiou and Kavallieratos, 2005; Athanassiou and Palyvos, 2006), DE with certain chemical insecticides (Stathers, 2003; Arthur 2004; Athanassiou, 2006; Athanassiou et al., 2007; Chintzoglou et al., 2008; Kavallieratos et al., 2010; Korunic and Rozman, 2010) or entomopathogenic fungi with

certain chemical insecticides (Purwar and Sachan, 2006; Santos et al., 2007; Brito et al., 2008; Srivastava et al., 2009; Russell et al., 2010). Several factors like dose rate, grain type, tested insect species, exposure intervals and length of the storage period significantly influence the efficacy of the control agents, either in separate or combined use. By keeping all of this in mind, the here presented project was designed to address the following specific objectives:

- (i) Identifying the combined effects of DEs, *B. bassiana* and Imidacloprid against four major stored grain insect species i.e. *R. dominica*, *T. castaneum*, *C. ferrugineus* and *Liposcelis paeta*
- (ii) Checking the susceptibility of different populations/strains of each test insect species to three grain protectants
- (iii) Determining the effect of grain type on the insecticidal efficacy of each grain protectant against the test insect species
- (iv) Investigating the long-term effectiveness of DE, entomopathogenic fungi and insecticide treatments in stored wheat
- (v) Examining the effectiveness of DE, entomopathogenic fungi and insecticide during on-farm trials

#### 2 Materials and Methods

# 2.1 Tested pest insect species

The adults of *R. dominica* (F., 1792) used in the experiments were obtained from a laboratory colony developed on wheat at  $25\pm2^{\circ}$ C and  $65\pm5\%$  rh *C. ferrugineus* (Stephens, 1831) and *T. castaneum* (Herbst, 1797) were reared on wheat flour at  $25\pm2^{\circ}$ C and  $65\pm5\%$  rh; however, 5% brewer's yeast was added to the flour in the later species. The culture of *L. paeta* (Pearman, 1942) was maintained at 30°C and 75% rh, on a mixture of 97% cracked wheat, 2% rice krispies and 1% brewer's yeast (Athanassiou et al., 2009 a). Two weeks old adults of each species were used in the bioassays.

#### 2.2 Commodities

The test grain commodities used in the bioassays were untreated and free of infestation wheat (var. Lasani-2008), rice (var. Basmati-2000) and maize (var. DK-6142). The moisture contents of all commodities were estimated using Dickey-John moisture meter (Dickey-John Multigrain CAC II; Dickey-John Co., USA). The moisture contents measured were 11.15% for wheat, 10.35% for rice and 11.20% for maize. However, the freshly harvested infestation-free wheat grains used in the field trials had moisture contents ranging from 10.75-11.85%.

# 2.3 Tested grain protectants

#### **2.3.1 DE formulations**

The diatomaceous earth formulations tested in the experiments were Protect-It and DEBBM. Protect-It (Hedley Technologies Inc., Mississauga, Ontario, Canada) is a fresh water DE that contains 83.7% SiO<sub>2</sub>, 5.6% Al<sub>2</sub>O<sub>3</sub>, 2.3% Fe<sub>2</sub>O<sub>3</sub>, 0.9% CaO, 0.3% MgO, 1.9% other oxides like TiO<sub>3</sub> and P<sub>2</sub>O<sub>3</sub>, 3-5% moisture content, and <1% crystalline silica. The DEBBM (supplied by Dr. Zlatko Korunic, Diatom Research and Consulting Inc., Guelph, Canada) contains 90% diatomaceous earth, 9.95% inert material and 0.05% active ingredient of Chinese plant extract (bitterbarkomycin).

#### 2.3.2 Insecticide formulation

Imidacloprid is a nicotine acetylcholine receptor agonist/antagonist, the commercial liquid formulation Confidor 200 SL containing 200 g/litre of active ingredient (Bayer CropScience Limited, Pakistan) was used in the trials. Its effectiveness has been stated against the insect pests belonging to different orders.

## 2.3.3 Entomopathogenic fungi

For our experiments, we used a *Beauveria bassiana* sensu lato (Balsamo) Vuillemin (Ascomycota: Hypocreales: Clavicipitaceae) isolate, which was obtained from the fungal culture maintained in the laboratory. The strain was primarily isolated from an infected insect cadaver by using the single spore method suggested by Choi et al. (1999). The species was identified based on morphological characters using the keys (Barnett and Hunter, 1998). It then was sub-cultured on Saboraud Dextrose Agar (SDA) plates for the mass culturing of the fungal conidia by incubation at  $25\pm5^{\circ}$ C and 70% rh. The fungal conidia were harvested with sterilized scalpel after 14 days of incubation. The fungal conidia were suspended by diluting the conidial powder in sterile 0.05% Tween-80 solution, enumerated with a haemocytometer and then adjusted to achieve the required concentrations (conidia/ml). The rate of germination for *B. bassiana* conidia was measured approximately as 93% prior to each bioassay.

# 2.4 Combined effects of DE and Imidacloprid

Imidacloprid was diluted in distilled water to prepare three concentrations 1.25, 2.5 and 5.0 ppm so as to achieve 1.25, 2.5 and 5.0 mg/kg of active ingredient (Imidacloprid) on grains. Eight lots of 1 kg each were prepared in plastic jars for wheat, rice and maize (i.e. 24 lots in total). Out of eight treated lots, six from each grain type were treated independently with Imidacloprid at the dose rate of 1.25, 2.5, and 5.0 mg/kg, i.e. two lots for each concentration. One lot of each grain treated with respective concentrations of Imidacloprid was admixed with diatomaceous earth (Protect-It) at the dose rate of 150 mg/kg (for combine treatment), while the second lot was left as such to represent the alone treatment of Imidacloprid. Among the remaining two lots, one was treated with Protect-It alone (150 mg/kg), and the other lot (eighth) left without treatment

for the purpose of control treatment for wheat, rice and maize, respectively. In order to receive the uniform distribution of Imidacloprid, the grain lots were spread in trays in form of thin layers, and then 10 ml of Imidacloprid from each solution or water for controls was sprayed per kg of each grain type with the help of a hand sprayer. The grains were allowed to dry by keeping them in incubators adjusted at  $25\pm1^{\circ}$ C and 65% rh for 48 hours to equilibrate the excessive moisture (Kavallieratos et al., 2009). On the other hand, jars having Imidacloprid treated lots (for each of three concentrations from each grain type) were admixed with Protect-It (150 mg/kg) and shaken for 5-7 min manually to achieve an even distribution of the DE particles. Afterwards, four 100 g samples from each of these 24 treated grain lots were taken out and placed in small vials provided with a hole at the top covered with wire gauze fitted caps for proper aeration.

Then, fifty adults of each insect species were introduced into each vial, which were then kept in incubators set at  $27\pm2^{\circ}$ C and 70% rh. The required relative humidity level was maintained using a saturated salt solution (Greenspan, 1977). The adult mortality counts were performed 24, 48, 96 and 168 h post-exposure, all dead and live adults were taken away from the vials after the last count, and the vials were kept under the same conditions for 62 days for beetles and 30 days for psocids, respectively, to record the emergence of progeny. Each treatment was replicated three times by preparing new lots each time for each grain commodity (3 repetitions x 4 vials x 8 treatments x 4 species x 3 commodities = 1,152 vials).

## 2.5 Combined effects of entomopathogenic fungi and DEs

Nine lots of wheat, rice and maize (i.e. 27 in total), each weighed 1 kg, were prepared and placed in plastic jars. From these nine lots for each test grain type, six were admixed with two *B. bassiana* dose rates; three lots with  $1.5 \times 10^8$  and three with  $1.5 \times 10^{10}$  conidia kg<sup>-1</sup> of grains. Among the remaining three lots of each commodity, one was treated with Protect-It at a dose rate of 150 mg/kg, one with DEBBM at 50 mg/kg, respectively, and the last lot was left for control treatment. The treated material in all treated jars was mixed thoroughly with the help of a glass rod to ensure the uniform distribution of the treated substance. For each *B. bassiana* dose, 150 mg/kg of Protect-It was added into the first lot and DEBBM in the second lot; no DE was added to the third

lot. The DE was directly added to the respective lot (already treated with *B. bassiana*) and was mixed manually for 5-7 min for the homogeneity of the material in the grains. We obtained nine treatments in total: two fungi alone, two DEs alone, four fungus/DE combinations and one control. After treating the grains, four samples of 100 g each were taken from each jar and placed in small plastic vials, with a hole of 1.5 cm in diameter at the top covered with muslin cloth.

Fifty adults from each of the four insect species, respectively, were released in each vial, and the vials were placed in incubators at  $27\pm2^{\circ}$ C and 70% rh. The specific relative humidity level was attained by using saturated salt solution as recommended by Greenspan (1977). After having the mortality counts after 7, 14 and 21 days, the dead adults were removed successively after each exposure interval. At the end, the treated material was retained under the same conditions for 62 days for the three beetle species and 30 days for the psocids to record their progeny emergence. Each treatment was independently replicated three times for each insect species on each grain commodity (3 repetitions x 4 vials x 9 treatments x 4 species x 3 commodities = 1,296 vials).

# 2.6 Long-term effects of *B. bassiana*, DE and Imidacloprid

#### 2.6.1 Germination test

Imidacloprid was tested at five dose rates (1.25, 2.5, 5.0, 7.5 and 10 ppm; i.e. 1.25, 2.5, 5.0, 7.5 and 10  $\mu$ l/litre) for estimating its inhibitory effect on the conidial germination of *B. bassiana*. The insecticide was dissolved in distilled water (100 ml) containing 0.05% Tween-80. Conidia of *B. bassiana* were suspended in the respective aqueous solution; conidia in distilled water served as control. After one hour, 1 ml aliquots of each concentration were spread on Petri plates containing a thin film of SDA media. The plates were then incubated at 20±5°C and 70% rh in the dark. After 24 hours, the number of germinated conidia was counted under a microscope.

# 2.6.2 Grain treatment

We tested nine treatments of wheat: DEBBM alone (50 mg/kg), Imidacloprid alone (5.0 ppm; i.e. 5.0 mg/kg wheat), *B. bassiana* alone 2.8 x  $10^8$  and 2.8 x  $10^{10}$  conidia kg<sup>-1</sup> of wheat, DEBBM with Imidacloprid, DEBBM with the lower and the higher fungal

dose rate as well as Imidacloprid with the lower and the higher fungal dose rate. Various lots of wheat (1 kg each) were prepared for the treatments of the grains with the given grain protectants. The respective quantities of DEBBM, Imidacloprid and *B. bassiana* alone as well as in combination were mixed into each batch of wheat. An additional lot of wheat grain remained untreated and served as control. Imidacloprid was first diluted in water to prepare a solution containing the aforementioned dose rate of active ingredient (5.0 ppm). This solution was applied to the grains spread in plastic trays using a hand sprayer. Prior to the addition of DEBBM to the Imidacloprid treated grains, trays were placed in an incubator adjusted at  $25\pm1^{\circ}$ C and 65% rh for 48 hours (Kavallieratos et al., 2009) in order to normalize the increased moisture contents due to the insecticidal spray. The lots were placed in plastic jars and shaken manually for 5-7 min to achieve a uniform distribution of the test materials among the grains. Later on, the jars were kept in incubators set at  $27\pm2^{\circ}$ C and 70% rh for the whole period of experimentation.

# 2.6.3 Bioassays

The effect of all treatments was determined for a period of six months by conducting bioassays every 30 days. At the day of treatment, four samples of 100 g each were taken from each treated and control jars to conduct the first bioassay. Each 100 g grain sample was put in a vial, and then 50 adult beetles and psocids were liberated, respectively, per vial. The vials were kept in incubators set at  $25\pm2^{\circ}$ C and  $65\pm5^{\circ}$ % rh, and the specific relative humidity level was adjusted using saturated salt solution recommended by Greenspan (1977). Mortality counts were performed 7 and 14 days post-exposure, and all dead and alive adults were removed from the grains after the last mortality count. The vials were incubated at the same conditions to record the emergence of F1 beetles and psocids for 62 and 30 days, respectively. The procedure was the same for the following six bioassays started every 30 days along the six months' duration of experiment. The whole procedure was replicated three times in parallel, each based on separately treated wheat lots.

# 2.7 Field trials

# 2.7.1 Trial sites

The field trials were conducted in four districts Faisalabad; Sahiwal (Chichawatni); Chiniot and Multan of the Punjab province, Pakistan over a period of six months.

#### 2.7.2 Grain treatments and trials

We tested the treatments: DEBBM at a rate of 150 mg/kg, Imidaclporid at 5.0 ppm (5.0 mg insecticide/kg grain), *B. bassiana* at a rate of 3 x  $10^{10}$  conidia kg<sup>-1</sup> of wheat, their respective combinations and an untreated control. The grains were treated by thorough mixing of grain protectants in the wheat grains spread on polyethylene sheets with the help of a neat shovel. Each treatment was applied to a 40 kg lot of wheat grains per replication and experimental site. After the grain treatment, each lot was divided into four equal parts weighing 10 kg each, filled into polypropylene bags and labeled properly. Each treatment was replicated independently for three times and was arranged under randomized blocks on the four selected sites in the respective districts. Thus, a total of 336 lots of 10 kg of wheat were assayed (4 test species x 7 treatments x 3 replications x 4 districts). The treated grains in each bag were artificially infested by introducing 15 g of infested wheat grains containing an average number of  $20\pm5$  live adults of *C. ferrugineus*, *R. dominica*, *T. castaneum* and *L. paeta*.

# 2.7.3 Grain sampling

The sampling was carried out taking 200 g of wheat grains from the centre and four corners of each bag, respectively, every month for six months. The data recorded after every month of stoarge from the samples included the number of living and dead adults and the percentage of damaged grains (i.e. with one or more emergence holes).

# 2.8 Statistical analysis

The control mortalities were adjusted with Abbott's formula [Corrected mortality (%) = (1 - insect population in treated unit after treatment / insect population in control)

unit after treatment) x 100] (Abbott, 1925). Repeated-measure analyses were used to analyse the adult mortality data for each species with treatment and commodity as main effects and mortality was the response variable in the laboratory experiments. The General Linear Model (GLM) was used for the analysis of progeny production; in this case, the mean number of emerging adults per vial was the response variable, whereas dose and commodity were the main effects. The analyses were done with Minitab 13.2 (Minitab, 2002 Software Inc., Northampton, MA). The Tukey-Kramer (HSD) test at the 5% significance level (Sokal and Rohlf, 1995) was used to compare the means of adult mortality and progeny production. In the field trails, the mean percentages of damaged grains were estimated for each treatment on each site and plotted against the storage period. The pair-wise comparison between treatments was made over the entire period of storage using LSD (Least Significant Difference) tests with 5% error rate as the significance level.

#### 3 Results

# 3.1 Combined effects of DE and Imidacloprid

#### 3.1.1 Adult mortality

The mortality rates of the four tested insect species were significantly affected by the length of exposure (*C. ferrugineus*:  $F_{3,251} = 116$ , P < 0.01; *R. dominica*:  $F_{3,251} = 108$ , P < 0.01; *T. castaneum*:  $F_{3,251} = 127$ , P < 0.01; *L. paeta*:  $F_{3,251} = 153$ , P < 0.01) and increased in all cases. However, the mortality rates differed among species ( $F_{3,1007} = 66$ , P < 0.01), grains ( $F_{2,1007} = 14$ , P < 0.01) and treatments ( $F_{6,1007} = 55$ , P < 0.01). Nevertheless, the interaction between commodity and treatment was not significant in any of the cases (Table 1).

Table 1ANOVA parameters for adult mortality of C. ferrugineus, R. dominica, T.<br/>castaneum and L. paeta on three commodities (maize, rice and wheat)<br/>treated with DE and Imidacloprid

Species	Source		24 h		48 h		96 h		168 h	
		-	F	Р	F	Р	F	Р	F	Р
C. ferrugineus	Commodity	2	16.09	< 0.01	19.83	< 0.01	13.86	< 0.01	15.90	< 0.01
	Treatment	6	33.60	< 0.01	69.08	< 0.01	67.54	< 0.01	72.16	< 0.01
	Commodity × Treatment	12	0.06	1.00	0.32	0.98	0.15	0.99	0.10	0.99
R. dominica	Commodity	2	17.93	< 0.01	16.32	< 0.01	13.38	< 0.01	14.58	< 0.01
	Treatment	6	29.21	< 0.01	57.28	< 0.01	56.98	< 0.01	66.59	1.00
	Commodity × Treatment	12	0.23	0.99	0.27	0.99	0.23	0.99	0.06	0.11
T. castaneum	Commodity	2	18.22	< 0.01	27.05	< 0.01	20.38	< 0.01	21.28	< 0.01
	Treatment	6	24.26	< 0.01	41.95	< 0.01	35.74	< 0.01	73.71	< 0.01
	Commodity × Treatment	12	0.39	0.96	0.46	0.92	0.34	0.97	0.050	1.00
L. paeta	Commodity	2	16.88	< 0.01	14.43	< 0.01	23.41	< 0.01	13.02	< 0.01
	Treatment	6	45.41	< 0.01	78.18	< 0.01	85.35	< 0.01	43.34	< 0.01
	Commodity × Treatment	12	0.07	1.00	0.11	0.99	0.31	0.98	0.60	0.83

For *C. ferrugineus*, the mortality was highest on wheat, intermediate on rice and lowest on maize in all experimental combinations (Table 2; Appendix 1). The treatments only with Protect-It had mortality rates between 49 and 58% at the end of the experiment after 168 h, conforming commodity effect. Applying only Imidacloprid resulted in mortality rates not significantly different from these at low concentrations of 1.25 mg/kg, but higher concentrations performed significantly better. The combinations of Protect-It and Imidacloprid resulted in no significant differences against the sole applications of

Imidacloprid at low concentrations of 1.25 mg/kg, but performed significantly better at the two higher concentrations.

The mortality of *R. dominica* beetles was also higher on wheat as compared on maize and rice (Table 3; Appendix 2). After 168 h, the observed mortality ranged from 44 and 53% with Protect-It alone, while in case of Imidacloprid the highest mortality levels ranging from 71 to 82% were observed at the dose rate of 5.0 mg/kg. Application of Imidacloprid alone at the dose rate of 1.25 mg/kg yielded significantly higher mortality rates than Protect-It alone in most of the cases analysed. The combination of Protect-It with all dose rates of Imidacloprid resulted in higher mortalities than the respective sole treatments, and the highest mortality level (45%) observed after 24 h of exposure reached 98% after 168 h of exposure with the highest dose combination.

For *T. castaneum*, the mortality levels of beetles were also higher on wheat, intermediate on rice and lower on maize (Table 4; Appendix 3). In case of the noncombination treatments, Protect-It resulted in mortality rates between 35 and 47% at the end of the experiment after 168 h, whereas the highest mortality rates between 62 and 75% were examined when Imidacloprid was applied at the rate of 5.0 mg/kg. Imidacloprid applied at the dose rate of 1.25 mg/kg produced significantly higher mortalities than Protect-It alone in all experimental combinations with the exception on maize after 168 h of treatment where both treatments were not significantly different from each other. The mortalities were higher in the grains treated with their alone treatments, and the highest mortalities ranging from 79 to 91% were observed on all grains treated with Protect-It and 5.0 mg/kg of Imidacloprid.

The mortality of *L. paeta* was highest on wheat and lowest on maize, while intermediate on rice (Table 5; Appendix 4). The treatments with Protect-It had mortality rates from 61 to 72% after 168 h. On the other hand, Imidacloprid had the highest mortality rates between 92 and 100% when applied as 5.0 mg/kg at the end of experiment. Applying 5.0 mg/kg of Imidacloprid mixed with Protect-It resulted in mortality rates not significantly different from the sole treatment of Imidacloprid after 96 h and 168 h on wheat and all grains, respectively.
Table 2 Mean percentage mortality ( $\pm$  SE) of *C. ferrugineus* in maize, rice and wheat treated with Protect-It (150 mg/kg) and Imidacloprid (1.25, 2.5 and 5.0 mg/kg) applied alone or in combination. Means followed by the same letters within each row are not significantly different; HSD test at *P*<0.05. Mortality rates were corrected by the mortality rate of untreated controls

Dose rate	Exposure interv	val											
(mg/kg)	24 h			48 h			96 h				168 h		
	Maize	Rice	Wheat	Maize	Rice	Wheat	Maize	Rice	Wheat	Maize	Rice	Wheat	
150/0	9.56±3.60a	13.20±2.88a	17.27±2.63a	21.07±3.12a	24.52±2.83a	30.63±3.09a	32.73±4.14a	38.39±4.35a	42.14±4.32a	49.43±1.64a	53.67±3.44a	58.06±4.11a	
0/1.25	16.22±1.48ab	20.09±2.36ab	25.34±2.72ab	24.37±4.07ab	29.23±3.43ab	34.47±2.64a	37.53±3.65a	43.16±3.95a	46.10±3.82a	51.57±4.06ab	59.67±4.04ab	64.46±3.47ab	
0/2.50	23.14±1.46bcd	27.69±2.44bcd	31.69±2.81abc	36.34±1.97bc	41.23±3.17bcd	45.27±3.00ab	48.30±4.03abc	52.32±2.47ab	54.39±2.98abc	67.48±3.63bc	74.77±1.70bc	78.30±3.91bc	
0/5.00	28.24±3.84bcd	34.25±2.77cd	37.26±1.86bcd	45.39±2.61c	50.16±3.15cd	53.31±3.12bc	56.32±4.15bc	64.41±2.02bc	67.31±4.55bc	82.15±3.98cd	86.29±3.03cd	92.07±2.45cd	
150/1.25	21.61±2.66abc	25.67±2.27abc	29.08±4.34abc	33.65±3.36abc	38.68±2.60abc	42.26±3.70ab	44.69±3.02ab	51.25±1.56ab	50.39±3.49ab	68.16±2.52bc	72.17±2.35bc	76.18±3.53b	
150/2.50	32.28±1.89cd	36.10±3.75cd	40.52±3.07cd	46.56±2.70c	55.43±4.47de	64.47±2.96cd	63.09±2.51cd	67.47±3.19c	72.17±2.38c	86.01±3.25d	91.38±3.21d	95.40±3.37d	
150/5.00	36.19±3.10d	41.38±3.53d	47.27±2.96d	62.48±3.23d	69.32±1.69e	73.38±3.65d	78.59±2.06d	83.21±2.72d	91.26±3.77d	90.30±5.02d	97.34±2.65d	100±0.00d	

Table 3 Mean percentage mortality ( $\pm$  SE) of *R. dominica* in maize, rice and wheat treated with Protect-It (150 mg/kg) and Imidacloprid (1.25, 2.5 and 5.0 mg/kg) applied alone or in combination. Means followed by the same letters within each row are not significantly different; HSD test at *P*<0.05. Mortality rates were corrected by the mortality rate of untreated controls

Dose rate	Exposure interv	val										
(mg/kg)	24 h			48 h		96 h				168 h		
	Maize	Rice	Wheat	Maize	Rice	Wheat	Maize	Rice	Wheat	Maize	Rice	Wheat
150/0	7.49±2.27a	11.43±2.01a	14.16±2.01a	13.49±2.91a	18.49±4.67a	24.43±3.20a	27.02±4.09a	30.46±3.83a	35.31±3.30a	44.31±3.76a	49.28±3.01a	53.47±3.38a
0/1.25	12.45±3.58ab	18.97±1.30ab	21.41±2.51ab	17.32±2.88a	22.16±3.57ab	26.17±3.41a	31.23±2.58a	36.15±2.55a	39.51±2.86a	48.42±4.20ab	52.62±2.40ab	57.55±5.14ab
0/2.50	18.26±3.62abc	23.29±2.80ab	28.23±2.78abc	29.58±3.69a	34.55±1.77bc	37.49±2.37ab	38.26±2.90ab	45.30±2.73ab	47.36±4.29ab	62.20±3.52bc	68.53±1.65c	73.36±4.79bcd
0/5.00	25.13±2.47bc	29.48±3.41bc	34.30±3.04bcd	38.51±3.62b	43.29±3.08c	48.03±2.47bc	54.12±3.83bc	58.43±3.72b	61.35±3.66b	71.24±2.67cd	77.79±2.13cd	82.15±4.9cde
150/1.25	17.24±1.16abc	24.12±3.59abc	26.58±2.85abc	30.26±3.98a	32.38±3.47abc	36.28±3.30ab	41.02±2.73ab	43.94±2.42ab	48.07±2.71ab	56.19±2.84abc	64.49±3.85bc	67.11±3.29abc
150/2.50	29.34±3.72c	31.19±2.93bc	37.74±3.60cd	37.28±4.41bc	48.03±3.02cd	52.53±3.13c	52.37±4.62bc	57.38±3.61b	63.49±3.13b	82.37±3.21d	86.53±3.04de	91.15±3.24de
150/5.00	31.58±3.56c	37.37±3.33c	45.38±3.54d	54.24±3.93c	62.28±2.97d	67.44±3.37d	66.38±4.05c	75.65±3.95c	82.30±3.96c	87.53±4.39d	92.44±3.39e	98.09±1.90e

Table 4 Mean percentage mortality ( $\pm$  SE) of *T. castaneum* in maize, rice and wheat treated with Protect-It (150 mg/kg) and Imidacloprid (1.25, 2.5 and 5.0 mg/kg) applied alone or in combination. Means followed by the same letters within each row are not significantly different; HSD test at *P*<0.05. Mortality rates were corrected by the mortality rate of untreated controls

Dose rate	Exposure interv	val										
(mg/kg)	24 h			48 h		96 h				168 h		
	Maize	Rice	Wheat	Maize	Rice	Wheat	Maize	Rice	Wheat	Maize	Rice	Wheat
150/0	3.57±2.56a	5.09±1.79a	8.32±1.86a	9.08±2.61a	11.26±3.36a	15.51±3.50a	16.15±4.48a	20.34±3.47a	25.48±5.00a	35.12±3.29a	42.08±4.32a	47.01±3.11a
0/1.25	7.34±2.99ab	11.39±1.95ab	15.39±3.12ab	13.13±2.69ab	17.11±3.17a	23.49±3.40ab	23.26±2.73ab	28.75±2.86ab	32.40±2.72ab	38.08±2.82ab	43.91±1.93a	49.34±2.70ab
0/2.50	12.48±2.48abc	14.20±3.58ab	19.09±2.18abc	18.48±3.29ab	23.08±2.87ab	28.03±0.73abc	31.46±3.75abc	33.47±2.94abc	39.06±2.73abc	53.21±3.53bc	60.36±2.73b	64.60±2.68bc
0/5.00	16.52±3.70abc	20.54±2.71bc	25.13±2.59bcd	27.41±2.57bc	35.56±3.90bc	41.15±1.63cde	36.04±4.13bc	40.16±3.26bc	48.02±3.78bc	62.46±4.58cd	68.28±2.37bc	75.23±4.28cd
150/1.25	10.47±2.14abc	13.31±2.46abc	17.04±2.08abc	21.48±3.34ab	26.87±2.73ab	32.40±3.14bcd	30.23±3.61abc	34.12±3.40abc	43.15±2.71bc	45.49±3.75abc	53.44±3.49ab	56.37±1.27ab
150/2.50	18.51±1.75bc	21.04±3.53bc	28.30±1.94cd	26.21±2.92bc	37.02±3.37bc	43.04±3.14de	39.42±3.73bc	45.05±3.88cd	54.09±3.15cd	71.35±4.11d	77.23±4.06cd	84.34±4.23de
150/5.00	22.29±2.68c	26.20±2.27c	34.11±3.84d	37.41±3.03c	45.25±3.90c	54.35±3.33c	47.53±3.53c	58.31±3.36d	65.31±3.02d	79.47±3.07d	86.02±3.62d	91.08±3.22e

Table 5 Mean percentage mortality ( $\pm$  SE) of *L. paeta* in maize, rice and wheat treated with Protect-It (150 mg/kg) and Imidacloprid (1.25, 2.5 and 5.0 mg/kg) applied alone or in combination. Means followed by the same letters within each row are not significantly different; HSD test at *P*<0.05. Mortality rates were corrected by the mortality rate of untreated controls

Dose rate	Exposure interval											
(mg/kg)	24 h			48 h			96 h			168 h		
	Maize	Rice	Wheat	Maize	Rice	Wheat	Maize	Rice	Wheat	Maize	Rice	Wheat
150/0	18.39±2.09a	21.11±1.42a	25.16±3.44a	27.59±3.14a	32.69±3.56a	39.30±2.67a	45.24±3.68a	48.23±2.38a	53.30±3.30a	61.40±4.79a	68.38±3.39a	72.08±4.10a
0/1.25	24.16±1.40ab	27.30±3.36ab	32.21±2.46ab	36.16±2.55ab	40.61±2.83ab	43.05±3.81ab	49.06±4.04ab	54.76±4.43a	59.03±4.37a	74.38±3.44ab	82.42±4.08ab	87.15±4.19b
0/2.50	31.47±2.77bcd	36.49±3.16bcd	40.26±2.67abc	43.36±3.09bc	48.62±2.80bc	52.15±5.03abc	65.26±4.01bc	71.49±3.12b	74.44±2.10b	85.32±3.12bc	91.18±3.13bc	96.15±3.06bc
0/5.00	38.15±2.32cde	42.01±1.45cde	46.39±3.69bcd	57.24±2.86cd	62.01±2.33cd	65.02±2.41cd	78.16±3.53cd	84.18±2.19bcd	93.62±1.96c	92.16±2.33c	97.28±2.71c	100±0.00c
150/1.25	26.45±3.54abc	31.08±2.31abc	35.48±2.96abc	46.01±3.57bc	51.36±3.70bc	57.29±4.06bcd	67.28±3.13c	72.05±3.59bc	78.26±3.49b	88.43±4.78bc	93.11±3.92bc	100±0.00c
150/2.50	41.58±2.18de	47.31±2.37de	51.35±3.51cd	62.52±2.60d	68.48±3.16de	73.21±3.91de	81.37±3.32cd	86.62±2.02cd	97.46±2.53c	95.39±4.60c	100±0.00c	100±0.00c
150/5.00	47.39±3.28e	52.43±2.28e	58.20±4.33d	76.43±2.04e	81.31±2.56e	84.05±3.44e	87.54±3.59d	94.16±3.25d	100±0.00c	100±0.00c	100±0.00c	100±0.00c

#### **3.1.2** Progeny production

The application of Protect-It and Imidacloprid in all concentrations and combinations significantly reduced the mean number of progeny production in all four species (Table 6; Appendix 5). We obtained significance for all main effects (commodity: *C. ferrugineus*  $F_{2,71} = 3.23$ , P = 0.05; *R. dominica*  $F_{2,71} = 6.80$ , P < 0.01; *T. castaneum*  $F_{2,71} = 10.55$ , P < 0.01; *L. paeta*  $F_{2,71} = 5.52$ , P = 0.01; dose: *C. ferrugineus*  $F_{7,71} = 672.47$ , P < 0.01; *R. dominica*  $F_{7,71} = 352.70$ , P < 0.01; *T. castaneum*  $F_{7,71} = 200.77$ , P < 0.01; *L. paeta*  $F_{7,71} = 352.70$ , P < 0.01; *T. castaneum*  $F_{7,71} = 200.77$ , P < 0.01; *L. paeta*  $F_{7,71} = 352.70$ , P < 0.01; *T. castaneum*  $F_{7,71} = 2.23$ , P = 0.02; *T. castaneum*  $F_{14,71} = 4.37$ , P < 0.01; *L. paeta*  $F_{14,71} = 8.95$ , P < 0.01). The progeny emergence was declined significantly more on wheat compared on maize in most of the cases. The mixed application of Protect-It with 1.25, 2.5 or 5.0 mg/kg Imidacloprid had considerably reduced progeny emergence than the respective components on all three grain types.

Table 6Mean number ( $\pm$ SE) of *C. ferrugineus*, *R. dominica*, *T. castaneum* and *L. paeta* adult progeny in maize, rice and wheat<br/>treated with Protect-It (150 mg/kg) and Imidacloprid (1.25, 2.5 and 5.0 mg/kg) applied alone or in combination. Means<br/>followed by the same letters within each row are not significantly different; HSD test at P < 0.05

Dose rate	Insect species											
(mg/kg)	C. ferrugineus			R. dominica			T. castaneum			L. paeta		
	Maize	Rice	Wheat	Maize	Rice	Wheat	Maize	Rice	Wheat	Maize	Rice	Wheat
150/0	14.25±1.52b	12.08±2.08b	8.08±1.78b	21.58±2.56b	19.25±1.80b	15.16±1.30b	33.08±2.25b	28.16±1.67b	21.75±2.02b	11.08±1.38b	7.25±1.50b	3.25±0.57b
0/1.25	12.41±2.31bc	7.25±1.29bc	5.41±1.16bc	18.41±1.58bc	13.08±1.85bc	9.25±1.80bc	27.58±1.76bc	21.58±1.87bc	16.58±2.45bc	6.58±1.01c	4.16±1.15bc	1.25±0.21b
0/2.50	8.08±1.48bcd	6.58±1.02bc	3.25±0.52bc	12.25±1.39bcd	7.58±1.20bcd	4.41±1.69bc	20.50±2.03cd	14.47±1.96cde	11.08±2.35bcd	3.16±0.79cd	1.58±0.18bc	0.00±0.00b
0/5.00	5.16±1.08cd	4.16±1.15bc	1.16±0.21c	8.16±1.08cd	3.41±1.73cd	2.58±1.45bc	16.41±2.20cde	10.08±1.45def	8.41±2.14cd	1.75±0.15d	0.00±0.00c	0.00±0.00b
150/1.25	9.25±1.44bcd	5.25±1.25bc	4.08±1.16bc	19.08±2.10bc	14.25±1.23bc	11.50±1.61bc	21.00±1.84bcd	17.41±1.36cd	13.50±1.98bcd	0.00±0.00d	0.00±0.00c	0.00±0.00b
150/2.50	3.00±1.56d	2.41±1.02c	0.00±0.00c	10.25±1.15bcd	6.16±1.22cd	2.25±1.29c	13.16±1.67de	8.25±1.52ef	4.25±1.52cd	0.00±0.00d	0.00±0.00c	0.00±0.00b
150/5.00	2.25±1.37d	0.00±0.00c	0.00±0.00c	4.08±1.64d	1.08±0.16d	0.00±0.00c	7.25±1.70e	3.58±2.02f	1.25±0.72d	0.00±0.00d	0.00±0.00c	0.00±0.00b
0/0 Control	67.25±3.03a	73.58±4.05a	82.91±1.85a	78.25±4.93a	82.08±5.67a	91.41±6.35a	56.25±5.13a	67.41±2.59a	74.75±5.39a	97.50±2.98a	156.25±6.08a	103.50±10.24a

#### **3.2** Combined effects of entomopathogenic fungi and DEs

#### 3.2.1 Adult mortality

The mortality rates of the four tested insect species were all significantly affected by the length of exposure (*C. ferrugineus*:  $F_{2,215} = 35.6$ , *P* <0.01; *R. dominica*:  $F_{2,215} =$ 36.7, *P* <0.01; *T. castaneum*:  $F_{2,215} = 44.6$ , *P* <0.01; *L. paeta*:  $F_{2,215} = 34.2$ , *P* <0.01). Other main effects i.e. insect species ( $F_{3,863} = 139.34$ , *P* <0.01), grain type ( $F_{2,863} = 50.73$ , *P* <0.01) and treatments ( $F_{7,863} = 184.71$ , *P* <0.01) also significantly affected the adult mortality rates, but their associated interactions were not significant in their effect (Table 7).

Table 7	ANOVA parameters for adult mortality of C. ferrugineus, R. dominica, T.
	castaneum and L. paeta on three commodities (maize, rice and wheat)
	treated with <i>B. bassiana</i> , Protect-It and DEBBM (total $df = 287$ )

Source	df	7 d		14 d		21 d	
		F	Р	F	Р	F	Р
Commodity	2	59.08	< 0.01	55.90	< 0.01	62.88	< 0.01
Species	3	176.06	< 0.01	191.16	< 0.01	122.93	< 0.01
Treatments	7	198.97	< 0.01	233.72	< 0.01	220.66	< 0.01
Commodity x Species	6	0.06	0.99	0.23	0.96	0.33	0.92
Commodity x Treatments	14	0.13	0.99	0.09	>0.99	0.27	0.99
Species x Treatments	21	1.06	0.39	0.75	0.78	2.18	< 0.01

The mortality rates for *C. ferrugineus* were highest on wheat and lowest on maize under the influence of all treatments and exposure intervals (Table 8; Appendix 6). The treatments with Protect-It and DEBBM alone had mortality rates between 47 to 76% and 63 to 87%, respectively after 21 days, exhibiting the commodity effect. *B. bassiana* alone yielded significantly reduced mortality rates at the lower dose rate compared to the higher dose rates if exposed for seven days. The combinations of Protect-It and DEBBM with *B. bassiana* resulted in significant differences against the sole applications of each component, but the mortality levels were not significantly different for Protect-It and DEBBM with 1.5 x  $10^{10}$  conidia kg<sup>-1</sup> in wheat and rice after seven and in wheat after 21 days of exposure.

For *R. dominica* beetles, the mortality rates were also higher on wheat than on maize and rice (Table 9; Appendix 7). At the end of the experiment, the examined

mortality rates ranged from 38 to 67% with Protect-It alone, 51 to 84% with DEBBM alone, while in the case of the sole application of *B. bassiana* (dose rate:  $1.5 \times 10^{10}$  conidia kg<sup>-1</sup> of grain) the mortalities at the end of the experiment ranged from 45 to 76%. DEBBM alone yielded significantly higher mortality rates than Protect-It alone in all cases. The combined treatments of Protect-It and DEBBM with the higher dose rate of *B. bassiana* resulted in higher mortalities than the respective individual treatments. The highest mortality levels (68 and 76%) for Protect-It and DEBBM, respectively, observed after seven days of exposure, reached 96 and 100%, respectively, after 21 days of exposure with the highest dose combination.

The highest mortality levels of *T. castaneum* beetles were also observed on wheat, intermediate on rice and lowest on maize (Table 10; Appendix 8). In the case of sole treatments, Protect-It produced mortality rates ranging from 32 to 61%, DEBBM from 45 to 78% and *B. bassiana* from 16 to 68%. In all cases with one single exception, the application of the higher dose rate of *B. bassiana* (1.5 x  $10^{10}$  conidia kg<sup>-1</sup> of grain) yielded significantly higher mortality rates than the application of the lower dose rate. *B. bassiana* used at its higher dose rate yielded higher mortality rates than Protect-It alone, but lower ones than DEBBM alone in all experimental combinations. The mortalities were higher in the treatments with the combined application of Protect-It and DEBBM, respectively, with the higher dose rate of *B. bassiana* compared with their alone treatments. Mortality rates after 21 days ranged from 75 to 87% on all grains treated with the higher dose rate of *B. bassiana* and Protect-It, while it ranged from 87 to 100% when this treatment was applied in combination with DEBBM.

For *L. paeta*, mortality rates were again higher on wheat than on rice and maize (Table 11; Appendix 9). The treatments with Protect-It had mortality rates from 52 to 81%, with DEBBM from 67 to 94% during 21 d of exposure, and *B. bassiana* had mortality rates ranging from 63 to 88% at the end of experiment when applied at its higher dose rate. Admixing  $1.5 \times 10^{10} B$ . *bassiana* conidia kg<sup>-1</sup> of grain with Protect-It and DEBBM, respectively, resulted in higher mortality rates than in the sole treatments, however, adding *B. bassiana* to DEs only resulted in significant differences for Protect-It (for all cases) while it was not significant for DEBBM (for all cases).

Table 8Mean percentage mortality ( $\pm$  SE) of *C. ferrugineus* in three commodities (maize, rice, wheat) treated with one dose<br/>rate of Protect-It (150 mg/kg), DEBBM (50 mg/kg), two dose rates of *B. bassiana* (1.5 x 10<sup>8</sup>; 1.5 x 10<sup>10</sup> conidia kg<sup>-1</sup>)<br/>applied alone or in combination. Means followed by the same letters within each row are not significantly different;<br/>HSD test at *P*<0.05. Mortality rates were corrected by the mortality rate of untreated controls</th>

Exposure	Commodity	Dose rate B. bas	<i>siana</i> (conidia kg <sup>-1</sup> ),	Protect-It/DEBBN	A (mg/kg)				
interval		1.5 x 10 <sup>8</sup> /0/0	1.5 x 10 <sup>10</sup> /0/0	0/150/0	0/0/50	1.5 x 10 <sup>8</sup> /150/0	1.5 x 10 <sup>10</sup> /150/0	1.5 x 10 <sup>8</sup> /0/50	1.5 x 10 <sup>10</sup> /0/50
7 d	Maize	27.63±4.49a	52.34±3.24bcd	47,55±3.34bc	63.66±4.46cde	42.50±2.44ab	67.39±3.44ab	56.50±3.46bcde	70.51±2.02e
	Rice	32.41±4.78a	57.74±5.15bcd	51.36±4.07abc	69.37±3.90cd	45.39±3.68ab	74.25±4.41d	61.30±2.88bcd	73.58±5.76d
	Wheat	36.52±2.88a	65.76±2.81bcd	56.18±4.29bc	72.06±4.41cd	49.04±4.53ab	76.37±3.82d	64.40±3.90bcd	79.52±3.65d
14 d	Maize	43.26±5.14a	62.19±4.89abcd	57.58±3.49abc	73.35±5.46cde	51.76±3.91ab	82.70±3.33de	66.30±5.17bcd	91.68±2.81e
	Rice	49.36±4.44a	67.50±2.53abc	62.41±4.85abc	76.54±3.77cd	57.46±3.58ab	87.53±4.94de	69.40±3.10bcd	96.65±2.84e
	Wheat	54.25±5.10a	73.47±4.06bcd	66.11±3.50abc	80.55±3.25cd	61.11±4.25ab	92.14±4.45de	74.26±3.96bcd	100.00±0.00e
21 d	Maize	57.14±5.58a	72.73±3.36abc	65.36±3.78ab	78.46±3.10bcd	62.49±5.56ab	87.42±3.76cd	73.63±4.00abc	96.55±3.44d
	Rice	63.15±3.69a	78.17±2.44abc	72.37±5.07ab	84.57±4.24bcd	67.13±4.97ab	94.24±4.03cd	77.65±2.97abc	100.00±0.00d
	Wheat	68.16±3.06a	81.03±3.42ab	76.25±3.63ab	87.62±3.65bc	72.44±2.46a	100.00±0.00c	80.23±3.74ab	100.00±0.00c

Table 9Mean percentage mortality ( $\pm$  SE) of *R. dominica* in three commodities (maize, rice, wheat) treated with one dose rate<br/>of Protect-It (150 mg/kg), DEBBM (50 mg/kg), two dose rates of *B. bassiana* (1.5 x 10<sup>8</sup>; 1.5 x 10<sup>10</sup> conidia kg<sup>-1</sup>)<br/>applied alone or in combination. Means followed by the same letters within each row are not significantly different;<br/>HSD test at *P*<0.05. Mortality rates were corrected by the mortality rate of untreated controls</th>

Exposure	Commodity	Dose rate B. bas	ose rate B. bassiana (conidia kg <sup>-1</sup> ), Protect-It/DEBBM (mg/kg)										
interval		1.5 x 10 <sup>8</sup> /0/0	1.5 x 10 <sup>10</sup> /0/0	0/150/0	0/0/50	1.5 x 10 <sup>8</sup> /150/0	1.5 x 10 <sup>10</sup> /150/0	1.5 x 10 <sup>8</sup> /0/50	1.5 x 10 <sup>10</sup> /0/50				
7 d	Maize	23.35±3.71a	45.17±3.13bc	38.61±3.47ab	51.09±4.58bcd	33.78±4.16ab	59.54±3.25cd	44.15±4.61bc	67.50±4.11d				
	Rice	27.73±4.65a	52.12±3.20bcd	43.65±4.99ab	56.08±4.50bcd	35.53±2.71ab	65.31±4.82cd	48.59±4.05abc	71.83±4.58d				
	Wheat	32.35±4.65a	57.40±2.93bcd	49.38±2.79abc	62.87±3.32cde	40.18±4.48ab	68.29±3.86de	54.74±3.40bcd	76.62±3.60e				
14 d	Maize	35.25±2.46a	61.59±2.97cde	48.16±3.52abc	67.12±2.41de	43.53±2.60ab	75.22±3.36ef	57.42±4.48bcd	84.59±3.46f				
	Rice	44.14±5.62a	63.74±4.27ab	51.13±2.57a	73.44±3.44bc	47.31±4.81a	78.12±4.33bc	60.33±3.33ab	87.32±3.50c				
	Wheat	47.29±3.21a	68.45±4.34bcd	57.51±4.69abc	76.69±3.31cde	52.12±4.30ab	82.35±5.14de	61.49±3.81abc	93.36±4.07e				
21 d	Maize	44.18±2.30a	65.27±4.51bc	57.30±3.07ab	76.40±4.14cd	52.36±3.91ab	87.18±4.18d	68.36±3.61bc	93.64±4.11d				
	Rice	52.38±2.50a	73.65±4.18bcd	62.55±4.06abc	79.37±3.56abc	57.72±3.73ab	92.08±4.71de	75.28±4.83bcd	97.34±2.65e				
	Wheat	56.38±3.91a	76.60±4.76bc	67.56±3.65abc	84.14±3.24cde	62.36±4.08ab	96.37±2.08de	79.27±4.28bcd	100.00±0.00e				

Table 10 Mean percentage mortality ( $\pm$  SE) of *T. castaneum* in three commodities (maize, rice, wheat) treated with one dose rate of Protect-It (150 mg/kg), DEBBM (50 mg/kg), two dose rates of *B. bassiana* (1.5 x 10<sup>8</sup>; 1.5 x 10<sup>10</sup> conidia kg<sup>-1</sup>) applied alone or in combination. Means followed by the same letters within each row are not significantly different; HSD test at *P*<0.05. Mortality rates were corrected by the mortality rate of untreated controls

Exposure	Commodity	Dose rate B. bas	<i>siana</i> (conidia kg <sup>-1</sup> ),	Protect-It/DEBBN	I (mg/kg)				
interval	-	1.5 x 10 <sup>8</sup> /0/0	1.5 x 10 <sup>10</sup> /0/0	0/150/0	0/0/50	1.5 x 10 <sup>8</sup> /150/0	1.5 x 10 <sup>10</sup> /150/0	1.5 x 10 <sup>8</sup> /0/50	1.5 x 10 <sup>10</sup> /0/50
7 d	Maize	16.12±2.35a	38.04±3.53bc	32.40±3.78abc	45.24±3.26cd	26.15±3.74ab	47.29±4.07cd	34.29±4.07bc	56.21±3.36d
	Rice	22.58±2.73a	41.23±4.89abc	37.31±2.84abc	49.04±4.57bcd	31.14±3.07ab	54.35±4.81cd	40.26±3.99abc	64.18±4.35d
	Wheat	25.38±5.00a	47.14±4.35bc	42.35±4.44abc	53.45±2.72cd	34.69±1.84ab	56.32±2.57cd	45.51±4.32bc	68.42±2.06d
14 d	Maize	29.48±2.98a	52.60±2.08cd	42.90±3.50abc	55.23±5.07cde	34.50±1.69ab	65.73±4.88de	48.63±1.72bcd	71.18±4.21e
	Rice	31.53±2.42a	55.41±2.87bcd	48.17±1.76bc	61.01±4.44cde	39.45±3.63ab	68.04±3.77de	52.15±3.39bcd	76.48±3.85e
	Wheat	37.56±2.83a	59.28±4.80bcd	51.48±3.34abc	64.11±2.34cd	45.61±3.30ab	73.11±2.52de	56.46±3.38bc	82.03±3.67e
21 d	Maize	36.48±3.26a	60.15±4.26bcd	53.72±2.45abc	67.29±3.94cd	46.65±4.31ab	75.83±3.81de	59.15±3.22bcd	87.50±3.87e
	Rice	43.56±2.15a	64.48±3.71bc	56.40±4.61ab	75.29±3.28cd	50.36±4.67ab	83.31±2.29de	65.31±4.88bcd	94.31±3.62e
	Wheat	48.06±2.37a	68.15±3.45bc	61.30±4.25abc	78.35±3.76cd	56.45±4.77ab	87.15±3.96de	73.28±4.22bcd	100.00±0.00e

Table 11 Mean percentage mortality ( $\pm$  SE) of *L. paeta* in three commodities (maize, rice, wheat) treated with one dose rate of Protect-It (150 mg/kg), DEBBM (50 mg/kg), two dose rates of *B. bassiana* (1.5 x 10<sup>8</sup>; 1.5 x 10<sup>10</sup> conidia kg<sup>-1</sup>) applied alone or in combination. Means followed by the same letters within each row are not significantly different; HSD test at P < 0.05. Mortality rates were corrected by the mortality rate of untreated controls

Exposure	Commodity	Dose rate B. bas	ssiana (conidia kg <sup>-1</sup> ), l	Protect-It/DEBBM	(mg/kg)				
interval		1.5 x 10 <sup>8</sup> /0/0	1.5 x 10 <sup>10</sup> /0/0	0/150/0	0/0/50	1.5 x 10 <sup>8</sup> /150/0	1.5 x 10 <sup>10</sup> /150/0	1.5 x 10 <sup>8</sup> /0/50	1.5 x 10 <sup>10</sup> /0/50
7 d	Maize	32.41±4.63a	63.56±3.91bcde	52.63±4.35bc	67.44±4.41cde	46.23±3.04ab	73.55±3.27de	56.06±3.39bcd	78.35±3.84e
	Rice	37.22±3.50a	68.18±4.06bcd	56.80±3.33b	75.57±3.48cd	51.65±2.74ab	76.72±3.65cd	62.73±4.20bc	84.62±3.13d
	Wheat	43.27±1.59a	72.36±3.37cde	61.43±3.01bc	78.62±3.48cde	54.49±4.27ab	80.61±3.73de	67.32±4.59bcd	87.29±3.20e
14 d	Maize	50.27±5.11a	75.40±4.03bcde	62.32±4.96abc	78.18±4.57cde	54.61±5.33ab	86.12±3.87de	67.10±4.09abcd	96.26±2.50e
	Rice	56.68±4.14a	81.44±2.58cd	68.34±2.00abc	84.35±3.95cde	61.51±3.69ab	94.16±5.83de	74.06±2.83bc	100.00±0.00e
	Wheat	62.22±3.77a	84.68±4.50cde	73.15±3.46abc	89.36±3.15de	65.04±3.14ab	100.00±0.00e	79.28±3.63bcd	100.00±0.00e
21 d	Maize	62.37±5.22a	76.70±3.23abc	71.14±4.30ab	86.35±3.37bcd	69.48±3.13ab	95.29±4.71cd	76.34±4.48abc	100.00±0.00d
	Rice	66.44±2.84a	83.17±4.18bc	75.79±2.64ab	91.61±3.58cd	74.40±3.88ab	100.00±0.00d	81.52±3.79bc	100.00±0.00d
	Wheat	71.24±4.28a	88.70±2.59bcd	81.43±3.31abc	94.59±3.17cd	78.52±3.28ab	100.00±0.00d	84.10±2.38abc	100.00±0.00d

Table 12 Mean number ( $\pm$  SE) of *C. ferrugineus*, *R. dominica*, *T. castaneum* and *L. paeta* adult progeny in three grain commodities (maize, rice, wheat) treated with one dose rate of Protect-It (150 mg/kg), DEBBM (50 mg/kg), two dose rates of *B. bassiana* (1.5 x 10<sup>8</sup>; 1.5 x 10<sup>10</sup> conidia kg<sup>-1</sup>) applied alone or in combination. Means followed by the same letters within each row are not significantly different; HSD test at *P*<0.05

Exposure	Commodity	Dose rate B. bas	Dose rate <i>B. bassiana</i> (conidia kg <sup>-1</sup> ), Protect-It/DEBBM (mg/kg)									
interval		Control	1.5 x 10 <sup>8</sup> /0/0	1.5 x 10 <sup>10</sup> /0/0	0/150/0	0/0/50	1.5 x 10 <sup>8</sup> /150/0	1.5 x 10 <sup>10</sup> /150/0	1.5 x 10 <sup>8</sup> /0/50	1.5 x 10 <sup>10</sup> /0/50		
C. ferrugineus	Maize	98.58±3.84a	21.25±1.52b	12.41±2.31bcd	17.58±1.76bc	9.25±1.43cde	18.50±1.08bc	7.25±1.15de	14.16±2.02bcd	1.25±0.72e		
	Rice	101.08±3.40a	19.16±1.81b	7.41±1.15cd	13.25±1.89bc	5.91±1.45cd	14.41±2.23bc	3.58±1.20d	8.25±1.14cd	0.00±0.00d		
	Wheat	115.25±3.41a	14.00±2.30b	5.16±1.40bcd	10.58±2.40bc	3.25±1.66cd	12.41±1.74bc	0.00±0.00d	6.25±1.52bcd	0.00±0.00d		
R. dominica	Maize	95.58±3.84a	34.41±1.47b	20.75±2.67cde	24.91±2.02bcd	17.08±2.89def	28.66±1.29bc	11.41±1.42ef	22.16±2.31cde	5.83±1.72f		
	Rice	109.00±4.34a	27.50±3.35b	16.75±2.59bcd	21.33±2.16bc	11.25±1.52cde	25.08±1.87b	8.41±2.14de	16.58±2.45bcd	2.25±1.23e		
	Wheat	124.92±4.91a	23.08±1.68b	12.16±1.40bcd	16.58±2.45bc	7.41±1.36cde	19.00±1.98b	4.25±1.52de	14.25±1.23bcd	0.00±0.00e		
T. castaneum	Maize	72.75±2.41a	48.08±2.64b	32.41±2.38cd	37.16±2.24bc	25.25±2.03de	42.16±2.72bc	21.00±1.84ef	34.25±1.50cd	13.41±2.04f		
	Rice	85.16±4.29a	42.08±2.78b	24.25±1.32cde	32.75±2.32bc	16.16±1.30def	35.58±2.56bc	13.50±2.12ef	27.41±3.27cd	8.08±1.48f		
	Wheat	94.41±3.85a	35.75±2.41b	18.16±2.09cde	25.91±2.59bc	12.25±1.46def	28.08±2.78bc	9.25±1.75ef	21.41±2.24cd	4.25±1.52f		
L. paeta	Maize	122.92±5.81a	16.41±1.50b	7.08±1.45bcd	9.25±1.04bcd	5.75±1.80bcd	11.50±1.50bc	2.16±1.38cd	8.41±1.08bcd	0.00±0.00d		
	Rice	163.25±4.92a	13.08±1.64b	4.16±1.15bc	7.25±1.44bc	2.41±1.72c	8.16±1.30bc	0.00±0.00c	5.50±1.39bc	0.00±0.00c		
	Wheat	158.25±5.22a	8.41±2.00b	2.08±1.30b	5.16±1.10b	$0.00 \pm 0.00 b$	7.16±1.37b	0.00±0.00b	3.00±1.80b	$0.00 \pm 0.00b$		

## **3.2.2** Progeny production

All treatments resulted in a significant reduction of the mean number of F1 adults for all four species (Table 12; Appendix 10). The combined treatments of Protect-It and DEBBM, respectively, with 1.5 x  $10^{10}$  *B. bassiana* conidia kg<sup>-1</sup> performed better than their sole application; however, the difference was not significant in most of the cases for DEBBM. For progeny production, all main effects were significant (commodity: *C. ferrugineus*  $F_{2,80}$  = 9.50, *P* = 0.t05; *R. dominica*  $F_{2,80}$  = 16.79, *P* <0.01; *T. castaneum*  $F_{2,80}$ = 28.52, *P* <0.01; *L. paeta*  $F_{2,80}$  = 3.24, *P* = 0.04; dose: *C. ferrugineus*  $F_{8,80}$  = 871.31, *P* <0.01; *R. dominica*  $F_{8,80}$  = 532.18, *P* <0.01; *T. castaneum*  $F_{8,80}$  = 255.61, *P* <0.01; *L. paeta*  $F_{8,80}$  = 1489.35, *P* <0.01) and their associated interaction, commodity x dose (*C. ferrugineus*  $F_{16,80}$  = 4.65, *P* <0.01; *R. dominica*  $F_{16,80}$  = 41.66, *P* = 0.02; *T. castaneum*  $F_{16,80}$  = 5.75, *P* <0.01; *L. paeta*  $F_{16,80}$  = 14.07, *P* <0.01). Significantly stronger reduction of progeny emergence was observed on wheat compared on maize in most of the cases.

# 3.3 Long-term effects of *B. bassiana*, DE and Imidacloprid

### **3.3.1** Germination test

*B. bassiana* germination was significantly affected by the treatments ( $F_{5,29} = 12.3$ , P < 0.01). The percentage germination observed at 1.25 ppm was not considerably different from control, while germination percentage recorded at 2.5 and 5.0 ppm was not significantly different from each other. On the other hand, the germination rates at 7.5 and 10 ppm of Imidacloprid were significantly reduced than the control (Figure 1; Appendix 11).

## 3.3.2 Adult mortality

For all analyzed species, the main effects and their associated interactions for seven and 14 days post-exposure of wheat were significant (Table 13). Adult mortality rates declined significantly with extending time of storage, the integrated treatments were the more efficient compared with the alone treatments. Combinations including the higher dosage of *B. bassiana* performed best at the end of the storage period, while combinations including Imidacloprid performed best at the beginning. Furthermore,

mortality rates were higher in the assays with 14 days of exposure than in the respective exposures for seven days (Tables 14 to 17).



- Figure 1 Germination ( $\% \pm$  SE) of *B. bassiana* conidia on SDA mixed with 1.25, 2.5, 5.0, 7.5 and 10 ppm of Imidacloprid (Imid) on SDA media (means of bars labeled with the same letters are not significantly different; HSD at *P* = 0.05)
- Table 13ANOVA parameters of main effects and interactions for adult mortality of<br/>C. ferrugineus, R. dominica, T. castaneum and L. paeta in each exposure<br/>interval (for each exposure interval total df = 188)

Species	Source	df	7 d		14 d	
-		-	F	Р	F	Р
C. ferrugineus	Bioassays (i.e. storage time)	6	101.34	< 0.01	148.83	< 0.01
	Treatments	8	162.97	< 0.01	128.56	< 0.01
	Bioassays × Treatments	48	3.27	< 0.01	3.89	< 0.01
R. dominica	Bioassays (i.e. storage time)	6	77.87	< 0.01	115.66	< 0.01
	Treatments	8	116.06	< 0.01	111.19	< 0.01
	Bioassays × Treatments	48	2.72	< 0.01	3.68	< 0.01
T. castaneum	Bioassays (i.e. storage time)	6	133.85	< 0.01	137.16	< 0.01
	Treatments	8	155.72	< 0.01	145.22	< 0.01
	Bioassays × Treatments	48	4.55	< 0.01	4.79	< 0.01
L. paeta	Bioassays (i.e. storage time)	6	109.67	< 0.01	130.67	< 0.01
	Treatments	8	135.29	< 0.01	78.11	< 0.01
	Bioassays × Treatments	48	2.30	< 0.01	2.04	< 0.01

For *C. ferrugineus*, the lowest mortalities of the beetles during the first bioassay at the beginning of the storage were observed at the lower application rate of *B. bassiana*, while the highest mortality (100%) was observed in the grains admixed with the mixed application of DEBBM with Imidacloprid and DEBBM with *B. bassiana* (2.8 x  $10^{10}$  conidia kg<sup>-1</sup> of grain). After 30 days of storage, mean mortality percentage was decreased for all treatments with the exception of *B. bassiana* alone at its higher dose rate in the

seven days exposure treatment and the combined application of DEBBM and Imidacloprid in the 14 days exposure treatment for which the mortality either increased or remained stable, respectively (Table 14; Appendix 12). Adding DEBBM enhanced mortality rates with both, *B. bassiana* and Imidacloprid. In case of combined treatments, *B. bassiana* mixed with Imidacloprid yielded higher mortalities compared to *B. bassiana* mixed with DEBBM, but after 150 days of storage the higher dose of test fungus with DEBBM gave higher mortalities compared to all other treatments.

For *R. dominica*, the adult mortality at the beginning of the storage reached 100% for Imidacloprid in combination with both, DEBBM and the higher dose rate of *B bassiana* if exposure was for a period of 14 days. The lowest mortality rate at the beginning of the bioassay (36%) was in the grains treated with the lower dose rate of *B. bassiana* exposed for seven days (Table 15; Appendix 13). Similar to *C. ferrugineus* and *L. paeta* (Table 14 and 17), there was a gradual, but constant reduction in adult mortality rates in the treatments with DEBBM along the storage period resulting in more than 50% adult mortality even after 180 days of storage and 14 days post-exposure in units treated with DEBBM alone and 70% mortality for the units where it was mixed with the higher concentration of *B. bassiana*.

The adult mortality rates of *T. castaneum* were lower in all treated units than in the other three insect species. The highest adult mortality (100%) was observed for the mixed application of DEBBM and Imidacloprid at the beginning of the bioassay after an exposure of 14 days. On the other hand, the lowest mortality during this bioassay (29%) was again observed in the grains mixed with the lowest dose of *B. bassiana* and an exposure of seven days (Table 16; Appendix 14). After 180 days of storage, the highest beetle mortality (>60%) was observed for the DEBBM and *B. bassiana* (2.8 x 10<sup>10</sup> conidia kg<sup>-1</sup> of grain) combination treatment in the 14 days exposure. The same combination also yielded the highest final mortality rate of all treatments in the seven days exposure.

Table 14 Mean percentage mortality ( $\pm$  SE) of *C. ferrugineus* adults exposed for seven and 14 days on wheat treated with DEBBM (50 mg/kg), Imidacloprid (Imid: 5.0 mg/kg), *B. bassiana* (Bb1: 2.8 x 10<sup>8</sup>; Bb2: 2.8 x 10<sup>10</sup> conidia kg<sup>-1</sup>) and their respective combinations in seven bioassays carried out from 0 to 180 d after grain treatment (values within each exposure interval for each bioassay followed by the same letters are not significantly different; HSD test at P = 5%). Mortality rates were corrected by the mortality rate of untreated controls

Exposure	Treatments	Bioassays (days)							
interval		0	30	60	90	120	150	180	
7 d	DEBBM	68.18±3.47d	63.12±4.36de	58.02±3.25cd	56.31±3.91cd	50.70±4.70bcd	48.16±2.01bc	44.65±3.06bc	
	Imid	87.06±2.16ab	84.07±3.57abc	75.611±2.65b	63.96±4.85bc	49.32±4.01cde	40.78±4.40cd	35.29±3.70cd	
	Bb1	43.18±1.52e	41.43±1.82f	38.87±3.89e	36.23±3.15e	32.72±1.64e	28.13±3.87d	26.38±2.52d	
	Bb2	72.03±2.56cd	74.65±1.81cd	71.62±2.21bc	68.29±2.75abc	64.40±3.08abc	61.09±4.01ab	57.32±3.24ab	
	DEBBM+Imid	100.00±0.00a	97.34±2.15a	90.60±3.62a	79.26±2.89ab	67.04±2.43ab	63.42±3.44ab	54.61±2.69ab	
	DEBBM+Bb1	54.07±1.98e	52.31±2.89ef	47.71±3.12de	43.70±2.69de	42.29±4.39de	39.56±3.23cd	32.39±3.76cd	
	DEBBM+Bb2	82.10±3.29bc	79.15±3.48bcd	78.38±2.83ab	76.51±2.07ab	73.59±4.65a	71.26±2.91a	68.59±3.54a	
	Imid+Bb1	71.18±3.59cd	66.47±3.47de	57.34±2.67cd	52.29±3.37cde	44.64±2.88de	37.15±3.15cd	34.45±3.18cd	
	Imid+Bb2	93.37±3.08ab	91.89±4.76ab	84.55±2.39ab	81.79±3.45a	75.40±2.89a	66.40±2.69a	61.13±4.08ab	
14 d	DEBBM	79.30±2.20cd	76.39±2.89cd	73.59±3.32cde	71.23±2.92bcd	68.35±3.37abc	64.11±3.07a	63.98±1.86a	
	Imid	98.09±1.90ab	95.11±3.21ab	88.50±3.38abc	67.44±2.68cd	56.29±3.54bcd	48.34±3.22b	42.24±2.15b	
	Bb1	64.41±2.40e	60.06±2.25e	57.69±2.55e	52.04±3.49e	47.68±3.37d	41.12±3.12b	36.34±3.46b	
	Bb2	89.41±2.62abc	87.16±1.46abc	84.41±3.39abc	78.61±2.81abc	75.01±2.74a	72.26±3.96a	68.13±2.26a	
	DEBBM+Imid	100.00±0.00a	100.00±0.00a	97.04±2.95a	86.48±3.18ab	73.35±2.86ab	68.14±2.53a	62.75±2.90a	
	DEBBM+Bb1	68.49±4.22de	66.70±3.32de	62.45±3.60de	59.08±3.16de	55.24±4.55cd	46.12±2.61b	45.54±2.87b	
	DEBBM+Bb2	99.13±0.86ab	93.65±3.54ab	91.60±3.17ab	84.29±2.90ab	82.14±3.57a	78.11±3.06a	74.31±2.41a	
	Imid+Bb1	87.79±3.34bc	81.64±3.91bc	76.17±3.92bcd	63.54±2.99cde	51.36±3.87cd	44.72±3.54b	38.58±2.90b	
	Imid+Bb2	100.00±0.00a	97.52±2.22b	93.13±3.84a	89.59±3.44a	84.65±4.20a	74.23±2.19a	71.69±2.40a	

Table 15 Mean percentage mortality ( $\pm$  SE) of *R. dominica* adults exposed for seven and 14 days on wheat treated with DEBBM (50 mg/kg), Imidacloprid (Imid: 5.0 mg/kg), *B. bassiana* (Bb1: 2.8 x 10<sup>8</sup>; Bb2: 2.8 x 10<sup>10</sup> conidia kg<sup>-1</sup>) and their respective combinations in seven bioassays carried out from 0 to 180 d after grain treatment (values within each exposure interval for each bioassay followed by the same letters are not significantly different; HSD test at P = 5%). Mortality rates were corrected by the mortality rate of untreated controls

Exposure	Treatments	Bioassays (days)						
interval		0	30	60	90	120	150	180
7 d	DEBBM	56.83±2.75de	54.64±3.85cde	53.20±3.20bcd	51.65±3.73bcd	46.69±2.70bcde	43.31±3.53bc	41.51±2.64bc
	Imid	82.08±3.40ab	78.31±2.02ab	71.38±5.04ab	60.20±3.47abc	42.70±4.72cde	35.15±3.61cd	28.37±3.37cd
	Bb1	36.08±4.32f	37.61±2.22e	35.49±4.74d	32.68±3.69d	28.45±4.08e	25.19±2.41d	21.63±3.86d
	Bb2	65.18±3.58cd	67.11±5.35bc	65.17±4.82abc	63.35±4.34abc	57.59±2.89abcd	52.55±2.12ab	48.36±3.71ab
	DEBBM+Imid	92.32±3.53a	88.39±4.37a	84.00±3.20a	72.39±4.81a	59.49±4.11abc	53.23±2.78ab	46.62±3.94ab
	DEBBM+Bb1	43.68±2.89ef	42.05±3.14de	41.72±2.46d	37.47±4.37d	33.93±4.20e	31.71±2.94cd	24.15±2.60d
	DEBBM+Bb2	74.07±3.24bc	76.44±3.41ab	73.14±4.02ab	71.68±4.16ab	65.47±3.77ab	63.31±3.62a	59.22±3.47a
	Imid+Bb1	62.13±3.00cd	57.15±3.40cd	50.56±4.50cd	46.63±3.95cd	38.57±4.09de	32.40±3.47cd	26.99±3.34cd
	Imid+Bb2	89.47±3.63ab	83.59±3.42ab	78.34±3.74a	75.18±4.61a	69.17±4.75a	58.05±3.98ab	50.61±3.14ab
14 d	DEBBM	73.80±2.64de	71.46±4.26cd	69.15±2.94bcd	65.30±5.04bcd	63.67±3.61abc	61.29±4.02a	56.49±3.55abc
	Imid	96.52±3.21ab	92.85±2.48°	84.66±3.63ab	62.49±4.00bcde	51.61±4.31cd	44.53±2.48bc	38.54±2.96de
	Bb1	53.04±3.45f	52.05±3.67e	48.69±3.33e	43.88±4.36e	40.51±4.91d	35.67±2.18c	31.33±3.84e
	Bb2	84.17±2.92bcd	86.54±3.91abc	82.66±4.38abc	75.39±2.65abc	71.89±3.60ab	66.18±4.15a	62.38±3.56ab
	DEBBM+Imid	100.00±0.00a	98.04±2.49a	94.11±3.53a	82.35±3.62ab	61.55±4.77bc	57.61±3.63ab	51.18±3.08bcd
	DEBBM+Bb1	61.34±3.82ef	57.34±2.57de	54.29±3.60de	51.02±3.40de	47.76±2.74cd	43.51±2.83bc	42.74±2.55cde
	DEBBM+Bb2	93.29±4.28abc	89.32±2.90ab	87.70±4.64ab	78.56±2.64ab	76.47±3.61ab	72.68±3.76a	70.61±1.94a
	Imid+Bb1	79.51±2.05cd	73.59±3.32bcd	65.62±3.22cde	57.19±5.55cde	45.53±3.49cd	38.60±1.67c	33.12±3.57e
	Imid+Bb2	100.00±0.00a	95.65±3.82a	91.56±4.51a	86.13±4.31a	81.63±4.36a	67.04±3.94a	64.51±3.55ab

The general mortality rates for *L. paeta* were higher than on average in the three beetle species. Thus, mortality rates >90% were frequently reached in the assays exposed for 14 days before storage, and rates from 70 to 80% were still frequent at the end of the storage after 180 days. Consequently, the decrease of mortality rates along storage was less pronounced for *L. paeta* than for the three beetle species. 100% adult mortality rates were recorded after 14 days of exposure in the grains treated with Imidacloprid alone, mixed with DEBBM and *B. bassiana* (2.8 x  $10^{10}$  conidia kg<sup>-1</sup> of grain), and DEBBM mixed with *B. bassiana* (2.8 x  $10^{10}$  conidia kg<sup>-1</sup> of grain).

Table 16 Mean percentage mortality ( $\pm$  SE) of *T. castaneum* adults exposed for seven and 14 days on wheat treated with DEBBM (50 mg/kg), Imidacloprid (Imid: 5.0 mg/kg), *B. bassiana* (Bb1: 2.8 x 10<sup>8</sup>; Bb2: 2.8 x 10<sup>10</sup> conidia kg<sup>-1</sup>) and their respective combinations in seven bioassays carried out from 0 to 180 d after grain treatment (values within each exposure interval for each bioassay followed by the same letters are not significantly different; HSD test at P = 5%). Mortality rates were corrected by the mortality rate of untreated controls

Exposure	Treatments	Bioassays (days)								
interval		0	30	60	90	120	150	180		
7 d	DEBBM	44.39±5.20ef	46.81±4.01c	43.57±3.01c	37.72±3.92bc	35.32±3.89bc	34.47±4.28bc	26.67±3.45cde		
	Imid	73.45±1.96abc	76.41±2.56ab	62.85±3.53ab	38.59±5.12bc	29.44±3.12c	23.79±1.98cd	17.04±2.02e		
	Bb1	29.78±2.67f	34.03±4.16c	31.43±2.22c	25.95±2.52c	21.91±1.93c	17.56±3.50d	12.78±2.10e		
	Bb2	58.83±3.80cde	65.24±3.46b	59.62±2.27b	51.30±3.75ab	46.69±2.70ab	42.07±2.52ab	39.33±2.84abc		
	DEBBM+Imid	87.31 ±2.70a	83.37±3.45a	76.04±2.29a	63.76±4.00a	51.17±3.05a	44.49±1.99ab	32.55±2.51bcd		
	DEBBM+Bb1	35.28±4.39f	36.50±3.13c	33.92±3.31c	28.59±2.32c	23.13±2.88c	20.68±2.73cd	15.30±2.94e		
	DEBBMBb2	66.76±3.10bcd	69.72±2.62ab	65.15±4.20ab	57.98±1.75a	55.69±3.00a	52.21±2.22a	48.77±3.30a		
	Imid+Bb1	53.38±4.31de	41.29±2.86c	36.53±1.70c	31.44±3.63c	26.21±2.92c	21.37±2.10cd	19.26±3.34de		
	Imid+Bb2	82.10±3.28ab	77.46±4.30ab	71.43±2.03ab	65.17±3.67a	58.46±3.43a	47.11±2.90ab	42.62±3.23ab		
14 d	DEBBM	68.32±2.68c	70.41±3.86cde	66.09±1.76bc	62.28±3.80abc	56.78±2.73ab	53.57±2.45ab	51.54±2.87ab		
	Imid	88.14±3.47ab	83.76±2.10abc	76.46±3.66ab	51.02±3.94bcd	38.76±3.46c	32.69±2.70cd	27.89±4.11c		
	Bb1	42.04±2.76d	47.14±4.37f	43.76±4.31d	35.56±2.75d	32.54±3.79c	28.48±3.97d	25.59±3.90c		
	Bb2	71.12±2.42c	76.49±3.73bcd	72.68±1.52ab	67.37±3.55ab	64.39±3.45a	60.55±3.68ab	55.43±2.82ab		
	DEBBM+Imid	100.00±0.00a	96.13±2.47a	87.18±3.55a	76.62±3.54a	58.02±3.15ab	47.61±3.53bc	43.60±3.71bc		
	DEBBM+Bb1	51.43±2.70d	57.34±2.57ef	48.30±3.47d	41.23±3.16d	35.75±3.57c	34.28±2.74cd	30.34±4.85c		
	DEBBM+Bb2	84.24±2.99b	88.27±2.53ab	81.44±3.51ab	74.63±3.49a	71.89±3.60a	67.58±2.60a	64.48±4.54a		
	Imid+Bb1	65.80±1.61c	61.39±3.50def	52.36±2.40cd	47.81±3.28cd	42.32±3.18bc	36.55±1.55cd	31.76±4.21c		
	Imid+Bb2	98.02±1.48a	92.85±2.48a	84.58±3.57a	79.09±3.44a	73.49±3.49a	62.11±4.80ab	58.28±4.23ab		

#### **3.3.3** Progeny production

The significant differences in the progeny production were observed along the time of storage (*C. ferrugineus*:  $F_{6,209}$  = 357.48, *P* <0.01; *R. dominica*:  $F_{6,209}$  = 388.42, *P* <0.01; *T. castaneum*:  $F_{6,209}$  = 419.89, *P* = 0.009; *L. paeta*:  $F_{6,209}$  = 404.21, *P* <0.01), among treatments (*C. ferrugineus*:  $F_{9,209}$  = 1486.59, *P* <0.01; *R. dominica*:  $F_{9,209}$  =

1421.40, P < 0.01; *T. castaneum*:  $F_{9,209} = 790.47$ , P < 0.01; *L. paeta*:  $F_{9,209} = 5154.76$ , P < 0.01) and their associated interaction, bioassay x treatments (*C. ferrugineus*:  $F_{54,209} = 9.62$ , P < 0.01; *R. dominica*:  $F_{54,209} = 8.69$ , P < 0.01; *T. castaneum*:  $F_{54,209} = 6.67$ , P < 0.01; *L. paeta*:  $F_{54,209} = 25.48$ , P < 0.01). The progeny emergence for all four insect species was strongly suppressed in treated grains than untreated grains. The minimum number of the emerged adults was observed for *L. paeta*, while the weakest reduction was obtained for *T. castaneum*. Overall, progeny production was greatly reduced in the grains treated with combined applications of DEBBM + Imidacloprid or Imidacloprid + *B. bassiana* (2.8 x  $10^{10}$  conidia kg<sup>-1</sup> of grain). However, the suppression was declined with the passage of time and more emerged adults were recorded with prolonged storage period (Table 18; Appendices 16 to 19).

Table 17 Mean percentage mortality ( $\pm$  SE) of *L. paeta* adults exposed for seven and 14 days on wheat treated with DEBBM (50 mg/kg), Imidacloprid (Imid: 5.0 mg/kg), *B. bassiana* (Bb1: 2.8 x 10<sup>8</sup>; Bb2: 2.8 x 10<sup>10</sup> conidia kg<sup>-1</sup>) and their respective combinations in seven bioassays carried out from 0 to 180 d after grain treatment (values within each exposure interval for each bioassay followed by the same letters are not significantly different; HSD test at *P* = 5%). Mortality rates were corrected by the mortality rate of untreated controls

Exposure	Treatments	Bioassays (days)							
interval		0	30	60	90	120	150	180	
7 d	DEBBM	75.43±3.92cd	73.10±2.29de	72.28±1.91bc	65.35±4.43bcd	61.14±3.37bcde	56.20±4.23abc	53.59±3.53bcd	
	Imid	97.10±2.89a	92.70±4.72ab	81.36±2.36abc	68.06±2.66abcd	57.32±3.47cde	50.05±3.03bc	46.05±3.15cde	
	Bb1	52.46±2.63e	47.73±3.02f	42.28±3.20e	38.19±2.91e	36.26±2.03f	31.73±1.49d	29.74±3.90e	
	Bb2	83.17±2.71bc	81.12±3.03bcd	77.57±2.81bc	74.22±3.21abc	69.19±2.17abcd	66.82±3.61ab	61.24±4.42abc	
	DEBBM+Imid	100.00±0.00a	100.00±0.00a	94.38±4.17a	83.40±2.86a	72.47±5.41abc	64.07±4.35abc	58.33±4.76abcd	
	DEBBM+Bb1	65.13±3.95de	61.21±2.35ef	57.02±2.97de	54.26±2.97de	51.60±2.67ef	48.32±3.75cd	42.32±2.68de	
	DEBBM+Bb2	91.61±2.40ab	89.02±2.71abc	83.54±2.65abc	81.64±3.56ab	76.64±3.55ab	73.20±3.71a	72.60±2.53a	
	Imid+Bb1	87.39±1.97abc	78.50±2.73cd	70.24±3.54cd	63.33±3.57cd	52.60±3.61def	47.68±2.23cd	45.37±3.69cde	
	Imid+Bb2	100.00±0.00a	97.39±2.60 <sup>a</sup>	86.43±3.14ab	84.57±3.65a	79.56±2.58a	71.34±3.96a	67.25±3.64ab	
14 d	DEBBM	91.89±4.76ab	85.42±3.01bcd	83.34±4.12bc	78.84±3.02abcd	74.92±3.13abcd	68.39±2.31ab	66.57±2.95abc	
	Imid	100.00±0.00a	99.47±0.52 <sup>a</sup>	89.55±3.13abc	74.65±3.01bcde	63.07±3.48bcde	57.65±4.19bc	51.40±2.63cde	
	Bb1	76.02±2.78c	71.64±4.99d	65.41±2.81d	59.57±3.60e	54.40±4.73e	48.28±2.66c	45.58±3.58e	
	Bb2	96.35±3.64a	92.60±3.54ab	87.19±3.63abc	82.35±2.47abcd	80.54±2.86ab	76.31±2.40a	72.19±3.38ab	
	DEBBM+Imid	100.00±0.00a	100.00±0.00a	100.00±0.00a	89.65±3.49ab	78.32±2.92abc	70.16±2.77ab	64.47±3.25bcd	
	DEBBM+Bb1	82.22±3.07bc	78.52±2.91cd	74.26±3.34cd	68.17±2.76de	61.30±3.50cde	53.01±2.48c	49.14±2.45de	
	DEBBM+Bb2	100.00±0.00a	97.52±2.22ab	91.60±3.17ab	86.34±3.18abc	84.47±4.44a	82.27±3.36a	80.20±3.61a	
	Imid+Bb1	94.41±3.87ab	86.44±3.08abc	78.45±3.32bcd	70.76±3.23cde	58.45±3.80de	51.42±3.00c	47.34±3.21e	
	Imid+Bb2	100.00±0.00a	100.00±0.00a	93.13±3.84ab	91.38±4.65a	87.14±3.89a	79.12±2.69a	74.51±2.78ab	

Table 18 Mean number ( $\pm$  SE) of *C. ferrugineus*, *R. dominica*, *T. castaneum* and *L. paeta* offspring produced in wheat treated with DEBBM (50 mg/kg), Imidacloprid (Imid: 5.0 mg/kg), *B. bassiana* (Bb1: 2.8 x 10<sup>8</sup>; Bb2: 2.8 x 10<sup>10</sup> conidia kg<sup>-1</sup>) and their respective combinations in seven bioassays carried out from 0 to 180 d after grain treatment (values within each bioassay and each species followed by the same letters are not significantly different; HSD test at P = 5%)

Species	Treatments	Bioassays (days)						
		0	30	60	90	120	150	180
C. ferrugineus	DEBBM	7.83±1.59bc	10.75±1.84bcd	15.58±2.02bcd	19.16±1.30de	22.25±1.28cd	27.08±1.58de	30.16±1.59de
	Imid	0.00±0.00d	3.58±0.93de	8.17±1.22def	21.50±1.15cde	26.58±1.73bc	31.00±1.70cd	38.08±1.54bcd
	Bb1	12.41±1.76b	15.50±1.98b	22.75±2.09b	32.00±2.41b	34.75±1.70b	40.58±1.74b	46.00±1.84b
	Bb2	4.56±1.17cd	7.83±1.51cd	10.16±1.90cde	13.58±1.80ef	17.41±2.06de	21.75±1.81ef	22.41±1.36ef
	DEBBM+Imid	0.00±0.00d	0.00±0.00e	1.25±0.27f	5.41±1.44fg	21.50±1.73cd	26.50±2.12de	32.75±2.09cd
	DEBBM+Bb1	8.41±1.16bc	12.08±1.74bc	17.41±1.66bc	27.75±1.87bc	29.08±1.74bc	34.41±1.15bcd	39.25±1.23bc
	DEBBM+Bb2	0.00±0.00d	4.91±0.88cde	5.50±1.08ef	8.25±1.28fg	10.91±1.45ef	13.25±1.89f	15.58±1.58f
	Imid+Bb1	5.75±1.18cd	8.83±1.08bcd	14.33±1.52bcd	23.58±1.87cd	30.83±2.11b	36.08±2.45bc	40.25±1.50bc
	Imid+Bb2	0.00±0.00d	0.00±0.00e	19.91±1.72b	4.75±0.80g	5.50±1.23f	16.33±1.73f	19.91±1.74f
	Control	78.50±2.00a	84.50±2.52a	87.08±2.53a	96.41±2.06a	91.58±1.30a	89.08±1.52a	93.25±1.15a
R. dominica	DEBBM	12.91±1.76bcd	17.75±1.60cd	19.08±1.68cde	21.25±2.30def	25.41±1.15d	31.25±1.88def	32.00±1.14ef
	Imid	0.00±0.00f	4.16±1.34fg	11.41±1.60efg	25.58±2.10cde	29.25±1.52cd	36.41±1.58cde	44.58±1.69c
	Bb1	19.58±1.22b	26.50±1.80b	31.50±1.50b	36.50±1.23b	38.50±1.22b	47.50±1.94b	52.75±1.80b
	Bb2	7.75±1.46de	10.58±1.76def	15.75±1.89def	19.41±0.92ef	22.16±1.30d	25.58±1.58fg	29.83±1.81ef
	DEBBM+Imid	0.00±0.00f	0.00±0.00g	3.08±1.97g	8.75±1.04g	26.91±1.44d	29.08±0.92efg	35.50±1.52de
	DEBBM+Bb1	15.83±1.38bc	21.25±1.73bc	25.50±2.18bc	32.41±2.02bc	34.58±1.76bc	38.16±1.76cd	42.08±1.78cd
	DEBBM+Bb2	3.25±1.28ef	7.50±1.37efg	9.16±1.10fg	13.91±1.58fg	14.25±1.60e	16.25±1.89h	19.00±1.70g
	Imid+Bb1	9.50±1.66cde	12.41±1.74de	22.83±1.59bcd	28.66±2.23bcd	37.16±1.72b	42.6±1.52bc	45.25±0.66bc
	Imid+Bb2	0.00±0.00f	2.33±0.91g	5.50±1.01g	6.58±0.88g	9.41±1.15e	20.58±2.10gh	24.75±1.95fg
	Control	82.75±2.67a	87.25±1.75a	83.58±2.47a	91.50±1.50a	86.50±1.70a	94.00±1.52a	95.91±1.76a
T. castaneum	DEBBM	18.75±2.41bc	20.50±1.66cd	24.25±1.60cd	26.58±1.92de	31.08±1.30cd	36.50±1.98de	42.08±1.38de
	Imid	5.14±1.15ef	6.58±1.59ef	13.00±1.46ef	28.50±1.66cde	42.25±1.60b	40.41±1.58cde	47.41±1.20cd
	Bb1	24.75±2.46b	29.08±2.02b	34.08±1.35b	39.16±1.62b	47.16±1.30b	51.16±1.30b	59.16±1.67b
	Bb2	13.91±1.80cde	14.83±1.88d	17.58±1.76de	21.58±2.02ef	24.58±1.35de	32.41±1.45ef	37.00±1.37e
	DEBBM+Imid	0.00±0.00f	2.25±1.23f	4.41±1.16g	10.41±1.58g	29.16±1.41d	33.08±1.45ef	39.50±2.08de
	DEBBM+Bb1	21.16±1.81bc	26.75±1.44bc	29.25±1.70bc	35.83±1.99bc	44.08±2.64b	47.33±2.10bc	51.08±2.10c
	DEBBM+Bb2	9.75±1.84de	12.58±1.45de	14.08±1.74ef	16.41±1.45fg	19.25±2.32ef	21.25±1.70g	25.83±1.86f
	Imid+Bb1	16.58±1.06bcd	17.91±1.65d	26.25±1.60bc	32.75±1.90bcd	39.08±1.58bc	44.66±1.59bcd	46.00±1.39cd
	Imid+Bb2	0.00±0.00f	3.16±1.20f	7.58±1.45fg	9.50±1.44g	14.91±2.16f	26.25±1.46fg	28.50±1.01f
	Control	65.08±2.33a	72.41±2.16a	68.25±1.60a	75.25±1.89a	78.25±2.32a	83.75±1.60a	85.25±1.52a
L. paeta	DEBBM	3.75±1.08bc	5.58±1.08bcd	9.25±1.08cde	11.25±1.84de	18.58±1.10cd	22.16±1.30de	24.83±1.82def
	Imid	0.00±0.00c	0.00±0.00e	4.58±1.01def	15.58±1.62cd	19.25±1.77cd	25.66±2.02cd	29.00±1.37cde
	Bb1	8.50±1.21b	10.16±1.30b	18.25±2.41b	23.83±1.59b	28.41±1.44b	34.50±1.08b	38.41±1.60b
	Bb2	0.00±0.00c	2.25±1.56de	5.41±1.01def	8.16±1.20ef	12.50±1.52de	15.58±1.52ef	17.25±1.89fg
	DEBBM+Imid	0.00±0.00c	0.00±0.00e	0.00±0.00f	3.50±0.90f	15.16±2.11d	19.75±1.04de	23.58±1.60ef
	DEBBM+Bb1	4.41±1.01bc	7.50±1.18bc	13.50±1.42bc	19.66±1.22bc	23.25±1.23bc	27.41±1.90bcd	32.50±2.04bcd
	DEBBM+Bb2	0.00±0.00c	0.00±0.00e	2.33±1.54ef	4.25±0.86f	5.75±1.25ef	7.08±0.87g	10.25±1.42g
	Imid+Bb1	1.16±0.03c	4.50±0.90cde	11.58±1.80bcd	16.16±1.08cd	25.83±1.54bc	31.16±1.30bc	36.91±2.02bc
	Imid+Bb2	0.00±0.00c	0.00±0.00e	1.08±0.02f	2.41±0.82f	4.08±0.87f	9.00±1.32fg	12.75±1.32g
	Control	137.25±2.69a	130.75±1.60a	86.83±2.19a	150.92±1.45a	146.42±2.31a	138.92±2.89a	143.00±1.75a

# 3.4 Field trials

### 3.4.1 Multan district

In the comparison of dead and alive adults among different treatments, higher numbers of dead adults were recorded in the wheat grains admixed with the combined treatments compared with single treatments for all test species. For single treatments, Imidacloprid was more effective in controlling pests over a period of five months than DEBBM and *B. bassiana* alone, while *B. bassiana* proved to be more effective than DEBBM alone for the entire storage period (Figure 2). The highest number of dead adults was observed for *L. paeta* and the lowest for the adults of *T. castaneum*. The ratio of living to dead adults increased with the extending storage period. For the first five months, the combination of DEBBM and Imidacloprid was most effective in controlling all studied pest insects, while it was outperformed in the sixth month by the combination of DEBBM alone treatment after six months of storage (Figure 2). The data for the dead adults of *C. ferrugineus*, *R. dominica*, *T. castaneum* and *L. paeta* is given in the Appendices 20 to 25, however, the one for alive adults is given in the Appendices 26 to 31.

For the entire storage period, significant differences in grain damage were observed among the treatments for each insect species (*C. ferrugineus*:  $F_{6,62} = 25.0$ , *P* <0.01; *R. dominica*:  $F_{6,62} = 24.6$ , *P* <0.01; *T. castaneum*:  $F_{6,62} = 18.0$ , *P* <0.01; *L. paeta*:  $F_{6,62} = 19.3$ , *P* <0.01). The lowest percentage of damaged grains during 120 d (4 months) were recorded for the combination of DEBBM and Imidacloprid, while for the entire storage period (up to 180 d) the least grain damage was observed for the combined DEBBM and *B. bassiana* treatment (Figure 3).



Figure 2 Means (%) of dead and alive adults (a: *C. ferrugineus*, b: *R. dominica*, c: *T. castaneum* and d: *L. paeta*) recorded every 30 d for six months of storage in Multan district on wheat treated with DEBBM (DE: 150 mg/kg), *B. bassiana* (Bb: 3 x 10<sup>10</sup> conidia kg<sup>-1</sup>), Imidacloprid (Imd: 5.0 mg/kg) and their respective dose combinations



Figure 3 Damage ( $\% \pm$  SE) to wheat grains by *C. ferrugineus* (a), *R. dominica* (b), *T. castaneum* (c) and *L. paeta* (d) treated with DEBBM (DE: 150 mg/kg), *B. bassiana* (Bb: 3 x 10<sup>10</sup> conidia kg<sup>-1</sup>), Imidacloprid (Imd: 5.0 mg/kg) and their respective dose combinations during 180 d storage period in Multan district. Bars sharing same letters within each storage period are not significantly different; HSD test at 5%

## 3.4.2 Faisalabad district

The results obtained in the Faisalabad district were quite similar to the ones in Multan district. The DEBBM and Imidacloprid combination also provided the best control of all tested insect species up to 5 months of storage. For this combination, the highest adult mortality rates for *T. castaneum* and *R. dominica* (99 and 100%, respectively) were observed after 60 d of storage, whereas 100% adults of *C. ferrugineus* and *L. paeta* were found to be dead during the first 30 d of storage (Figure 4). In case of all single treatments, the trend for each test substance was also the same as observed for Multan district.



Figure 4 Means (%) of dead and alive adults (a: *C. ferrugineus*, b: *R. dominica*, c: *T. castaneum* and d: *L. paeta*) recorded every 30 d for six months of storage in Faisalabad district on wheat treated with DEBBM (DE: 150 mg/kg), *B. bassiana* (Bb: 3 x 10<sup>10</sup> conidia kg<sup>-1</sup>), Imidacloprid (Imd: 5.0 mg/kg) and their respective dose combinations

For all insect species, the grain damage reduction effect varied significantly among treatments all over the storage period (*C. ferrugineus*:  $F_{6,62} = 34.0$ ; *P* <0.01; *R. dominica*:  $F_{6,62} = 22.3$ , *P* <0.01; *T. castaneum*:  $F_{6,62} = 15.3$ , *P* <0.01; *L. paeta*:  $F_{6,62} = 19.5$ , *P* <0.01). The highest final percentage of damaged grains (28%) was caused by *T.* 

*castaneum* in grains treated with DEBBM alone, while the lowest damage was done by *L. paeta* when DEBBM was mixed with *B. bassiana* (Figure 5).



Figure 5 Damage ( $\% \pm$  SE) to wheat grains by *C. ferrugineus* (a), *R. dominica* (b), *T. castaneum* (c) and *L. paeta* (d) treated with DEBBM (DE: 150 mg/kg), *B. bassiana* (Bb: 3 x 10<sup>10</sup> conidia kg<sup>-1</sup>), Imidacloprid (Imd: 5.0 mg/kg) and their respective dose combinations during 180 d storage period in Faisalabad district. Bars sharing same letters within each storage period are not significantly different; HSD test at 5%

# 3.4.3 Chiniot district

Quite similar results as in the before mentioned districts were also obtained in Chiniot district. However, the adult mortality rates even approached 100% for all insect species when DEBBM was applied in combination with Imidacloprid. For *T. castaneum*, this highest rate of dead adults was recorded up to 60 d; for *R. dominica* and *C. ferrugineus*, this rate was noticed till 90 d, while death of all individuals of *L. paeta* was observed up to 120 d of storage (Figure 6).



Figure 6 Means (%) of dead and alive adults (a: *C. ferrugineus*, b: *R. dominica*, c: *T. castaneum* and d: *L. paeta*) recorded every 30 d for six months of storage in Chiniot district on wheat treated with DEBBM (DE: 150 mg/kg), *B. bassiana* (Bb: 3 x 10<sup>10</sup> conidia kg<sup>-1</sup>), Imidacloprid (Imd: 5.0 mg/kg) and their respective dose combinations

For grain damage, the treatment resulted in strongly varying damage rates, which were highly significantly different for all pest insect species (*C. ferrugineus*:  $F_{6,62} = 39.3$ , P < 0.01; *R. dominica*:  $F_{6,62} = 29.9$ , P < 0.01; *T. castaneum*:  $F_{6,62} = 14.2$ , P < 0.01; *L. paeta*:  $F_{6,62} = 24.0$ , P < 0.01). The lowest percentage of damaged grains was recorded after 60 d of storage (2 months) from the samples taken from combined DEBBM and Imidacloprid treated grains, while after 180 d (6 months), the lowest damage rates were observed in the grains treated with DEBBM combined with *B. bassiana* (Figure 7).



Figure 7 Damage ( $\% \pm$  SE) to wheat grains by *C. ferrugineus* (a), *R. dominica* (b), *T. castaneum* (c) and *L. paeta* (d) treated with DEBBM (DE: 150 mg/kg), *B. bassiana* (Bb: 3 x 10<sup>10</sup> conidia kg<sup>-1</sup>), Imidacloprid (Imd: 5.0 mg/kg) and their respective dose combinations during 180 d storage period in Chiniot district. Bars sharing same letters within each storage period are not significantly different; HSD test at 5%

# 3.4.4 Sahiwal district

The Sahiwal district also showed results in general accordance with the other three districts. However, the percentages of dead adults of each species recorded in this district in all treatments over time were higher than for the other districts, and 100% insect mortality rates were achieved with two or more combination treatments for all tested insect species, with the exception of *T. castaneum*, which only could be eliminated completely with the combination of DEBBM and Imidacloprid, but with this combination as long as four months. Nevertheless, the general tendency for individual treatment effects on all tested pest species were quite similar as observed for the other three districts (Figure 8).



Figure 8 Means (%) of dead and alive adults (a: *C. ferrugineus*, b: *R. dominica*, c: *T. castaneum* and d: *L. paeta*) recorded every 30 d for six months of storage in Sahiwal district on wheat treated with DEBBM (DE: 150 mg/kg), *B. bassiana* (Bb: 3 x 10<sup>10</sup> conidia kg<sup>-1</sup>), Imidacloprid (Imd: 5.0 mg/kg) and their respective dose combinations

The different treatments had significantly varying effects on the percentage of grain damage all over the storage period (*C. ferrugineus*:  $F_{6,62} = 41.8$ , *P* <0.01; *R. dominica*:  $F_{6,62} = 35.3$ , *P* <0.01; *T. castaneum*:  $F_{6,62} = 18.8$ , *P* <0.01; *L. paeta*:  $F_{6,62} = 35.6$ , *P* <0.01). No grain damage was noticed after 60 d (for *R. dominica*) and even 120 d of storage (for *C. ferrugineus* and *L. paeta*) when Imidacloprid in combination with DEBBM was applied. However, no combination was able to avoid damage by *T. castaneum*, but the last mentioned formulation was the most efficient protection. On the other hand, all combination treatments completely avoided damage by *L. paeta* over the first 120 d, and by the end of the storage (after six months), the most effective protection was observed in the combined treatment of DEBBM and *B. bassiana* (Figure 9). The complete data of the damage to wheat grains caused by the four insect species is given in the Appendices 32 to 35.



Figure 9 Damage ( $\% \pm$  SE) to wheat grains by *C. ferrugineus* (a), *R. dominica* (b), *T. castaneum* (c) and *L. paeta* (d) treated with DEBBM (DE: 150 mg/kg), *B. bassiana* (Bb: 3 x 10<sup>10</sup> conidia kg<sup>-1</sup>), Imidacloprid (Imd: 5.0 mg/kg) and their respective dose combinations during 180 d storage period in Sahiwal district. Bars sharing same letters within each storage period are not significantly different; HSD test at 5%

# 4 Discussion

The results of the current studies indicate that *B. bassiana*, DE and the new chemical neonicotinoid insecticide Imidacloprid can be used effectively against the pest species *C. ferrugineus*, *R. dominica*, *T. castaneum* and *L. paeta*, but their effectiveness is determined by various parameters such as dose rate, length of the exposure time, storage period and type of grain commodity. The findings of this work also suggest that the addition of DE enhanced the insecticidal efficacy of Imidacloprid and *B. bassiana* under certain conditions.

## 4.1 Synergistic effect of DE and chemical insecticide

Various studies have demonstrated that DE combined with chemical insecticides gained synergistic effects against different stored grain insect pests (Ceruti and Lazzari, 2005; Athanassiou, 2006; Wakil et al., 2013). Among the initial studies in this field, Arthur (2004) evaluated the new commercial formulation F2 for the protection of stored wheat, maize and rough rice. The formulation was a mixture of three chemical insecticides (i.e. 0.03% deltamethrin, 0.37% piperonyl butoxide, and 0.95% chlorpyriphos-methyl), plus 10% mineral oil, 88% diatomaceous earth Protect-It and 0.65% inert ingredients. Athanassiou (2006) investigated the insecticidal activity of beta-cyfluthrin alone or alongwith a DE formulation SilicoSec against the two pest species *T. castaneum* and *Sitophilus oryzae* (Coleoptera: Curculionidae). Ceruti and Lazzari (2005) also reported similar findings that DE combined with a low dosage of the insecticide deltamethrin provide an efficient control measure against the pest beetle *Sitophilus zeamais* (Coleoptera: Curculionidae).

The study presented here is the first one analysing the potential use of two DE formulations, Protect It and DEBBM, in combination with the new chemical insecticide Imidacloprid. The combination of DEs with Imidacloprid can be explained by the fact that the integration of DE with sublethal doses of chemical insecticides could be the most attractive option to deal with the insect's resistance issues related with the use of conventional insecticides and the environmental problems due to the higher application rates of DE in case of its individual use (Arthur, 2003). In the studies presented here, presence of Imidacloprid enhanced the efficacy of DEs at all of its dose rates (1.25, 2.5).

and 5.0 ppm), thus providing an additive effect at each dose combination. However, as no already published data is available for the application of Imidacloprid with DEs, no comparison with the literature is possible.

In previous studies on the inteagrated use of DE with other insecticides, Athanassiou (2006) reported that the addition of SilicoSec enhanced the insecticidal efficacy of beta-cyfluthrin on *S. oryzae* at the rates of 0.125 and 0.25 ppm, but not at 0.75 ppm. Stathers (2003) also observed that the addition of deltamethrin and permethrin (at the rate of 0.025 mg/kg and 2.0 mg/kg, respectively) to Protect-It did not yield additive effects against the larger grain borer *Prostephanus truncatus* (Coleoptera: Bostrichidae) under field conditions in Zimbabwe. The antagonism between both agents at high doses of the chemical insecticide have to be explained by the knock-down effect of the counterpart insecticides, which may result in a reduced pick-up by insects of DE particles in such DE/insecticide combinations (Ebeling, 1971). The observed differences of the class of insecticide with different modes of actions and, more importantly, due to the reduced application rate of the chemical insecticide if compared with the mentioned studies.

# 4.2 Synergistic effect of DE and entomopathogenic fungi

It is known since some years that DE synergizes with entomopathogenic fungi against several insect pest species of stored grain (Lord, 2001; Akbar et al., 2004). Lord (2001) for the first time observed this effect using *B. bassiana* and Protect-It against lesser grain borer. Later on, Akbar et al. (2004) tested the protective ability of *B. bassiana* alone or in combination with Protect-It against *T. castaneum* and confirmed the initial findings by Lord (2001) about the synergistic interaction of DE and *B. bassiana*. In the following, various scientists investigated such combined applications using different fungal species and DE formulations against a series of stored grain insect pests (Dal-Bello et al., 2006; Vassilakos et al., 2006; Athanassiou and Steenberg, 2007). The results of our bioassays are in line with these previous works, and the simultaneous presence of fungi and DE resulted in higher levels of mortalities than the use of fungi and DE alone.

The mixed use of DE and entomopathogenic fungi is mainly advocated due to the moisture optima of both substances and their mode of action (Lord, 2001). These results underline that there is an additive effect of the tested DEs and *B. bassiana* combinations. Possibly, DE particles, when present on insect cuticle, inactivate the specific epicuticular lipids that possess an inhibitory effect for the attachment, germination and penetration of *B. bassiana* conidia (Lord, 2001). Moreover, the DE also increases the rate of conidia attachment on the body of red flour beetle larvae (Akbar et al., 2004).

On the other hand, we observed an antagonistic effect when DEs were mixed with the lower dose rate of *B. bassiana*. Comparable results have been obtained by Vassilakos et al. (2006) when a commercial product of *B. bassiana* (i.e. Naturalis) was applied along with the SilicoSec against *R. dominica* and *S. oryzae*. In these analyses, a negative effect was also observed for lower dose combinations. Similarly, Michalaki et al. (2007) reported such findings for the fungus *Isaria fumosorosea* (previously known as *Paecilomyces fumosoroseus*) used along with SilicoSec against adults and larvae of *T. confusum* and larvae of *Ephestia kuehniella* (Lepidoptera: Pyralidae). These findings indicate that additive enhancing effects of fungus and DE combinations only occur when specific concentrations of fungal conidia are reached ("critical concentration"). Thus, no additive effect can be observed below these critical levels (Vassilakos et al., 2006).

The enhancing effect of the combined treatment of *B. bassiana* and Imidacloprid might be attributed to the fact that certain neonicotinoid insecticides have no negative effects on conidia germination, conidiogenesis and vegetative growth of *B. bassiana* (Neves et al., 2001). Alizadeh et al. (2007) also confirmed the high usefulness of Imidacloprid for its simultaneous use with *B. bassiana*. The same observation has also been made in our study, and the concurrent presence of Imidacloprid and *B. bassiana* also enhanced the mortality levels of adults as compared with their respective alone treatments, at least during early bioassays.

## 4.3 Effect of dose rates and exposure intervals

It is generally considered that higher mortalities occur at higher application rates of the treatments (Rice and Cogburn, 1999). Sheeba et al. (2001) observed higher mortality percentages for *S. oryzae* when *B. bassiana* was applied at higher dose rates. More recently, Daglish and Nayak (2012) tested Imidacloprid (Premise 200 SC) and the oxdiazine Indoxacarb (Steward 150 EC) for the management of five coleopteran insect pest species of stored grains. Imidacloprid could not offer an absolute control of the test insect species. However, the dose effect was more significant in the case of *C*. *ferrugineus* and *R. dominica* for which higher mortality rates were observed with increasing dose rates. The effects of some insecticides, i.e. pyrethroids (Athanassiou et al., 2004 a) and microbial insecticides (Kavallieratos et al., 2009; Vayias et al., 2009), have also been reported being highly influenced by the dose rates applied. The findings of our studies presented here are in accordance with this established concept, and, for all tested insect species, higher numbers of dead adults were recorded at the respective higher dose rates of *B. bassiana* and Imidacloprid, respectively, if compared with the lower one.

Our study also indicated that the mortality of adult beetles and psocids increased with exposure time, and longer exposure periods are needed to achieve the highest mortality rates. Several previous studies also exhibited the same phenomenon (Athanassiou et al., 2005; Ferizli et al., 2005; Wakil et al., 2006; Ziaee et al., 2007; Shasyeteh et al., 2008; Wakil and Ghazanfar, 2010; Wakil et al., 2010; Riasat et al., 2011). In the case of DE, dust is trapped continuously by the insects' bodies while walking on treated grains as long as they remain in contact with the DE particles, resulting in excessive loss of water and, consequently, death due to desiccation (Arthur, 2000). The same also applies for the fungus, the insecticide, and their combined treatments: with extending exposure interval, more fungal inoculum will be picked up and more toxic residues will be received by the insects when they are likely to remain inside the treated units till the end of the experimental period.

# 4.4 Effects of different DE formulations

It is well established that insecticidal efficacies of different DE formulations vary even against the same insect species. For instance, three DE formulations (PyriSec, Insecto and Protect-It) applied against populations of *T. confusum* revealed considerable variation in the efficacy of these DEs (Kavallieratos et al., 2007). Similarly, Collins and Cook (2006) evaluated the two DEs SilicoSec and Diasecticide in laboratory studies against stored product insect and mite pests, and found SilicoSec the most effective against all pest species analysed. Athanassiou et al. (2005) also reported better effects of PyriSec and Protect-It than for Insecto in controlling three stored-product beetle pest species.

In general, all DEs possess the identical mode of action (Korunic, 1998). Consequently, the additives present in the commercial formulations of DEs render for the observed efficacy differences among various formulations. The two DEs in our study were selected due to difference in their composition and their additives: Protect-It contains silica aerogel and DEBBM is a DE formulation enhanced with bitterbarkomycin (BBM). In the here presented comparative studies, DEBBM proved to be more efficacious than Protect-It, even at highly reduced dose rate (50 ppm), against all tested insect species and on each commodity. The effectiveness of DEBBM against R. dominica (Athanassiou et al., 2007, Wakil et al., 2011), T. castaneum, S. zeamais and C. ferrugineus (Athanassiou et al., 2009 b) has already been reported. The plant derived insecticdes act as feed deterrents and change the insect behaviour, ultimately leading to the insect's starvation. These dead insect bodies may then serve as a suitable substrate for the applied fungal intensification. As certain toxins produced by B. bassiana first suppress the insect immune system, which may already be reduced in starving insects, feed deterrents as an active ingredient strongly enhance the synergistic role of DE hereby directly influencing the fungal pathogenity. BBM possesses such feed deterring properties (Wang et al., 1991); therefore, the enhanced combined effect of these two substances is also reinforced by the toxic effect exerted by the fungal toxins, which suppress the insect immune system of already starving insects.

### 4.5 Effects of grain types

The effectiveness of a DE formulation is greatly influenced by the type of grain or produce (Subramanyam and Roesli, 2000; Athanassiou et al., 2003, 2004 b). The effect of the grain type can be attributed to (i) certain physical and compositional characteristics of the grains that may affect the fecundity and developmental rates of the respective insect species, (ii) the extent of adherence of DE particles to grains, and (iii) specific interactions between grains and DE particles, which may affect the insecticidal efficacy of DEs. For instance, kernel hardness of different varieties of wheat has different adverse

effects on the oviposition of *S. oryzae* (McGaughey et al., 1990). The adherence of DE varies amongst the grain commodities resulting in increased application rates for some commodities over others (Korunic et al., 1997). As DE adherence is higher to wheat grains than to maize, these authors concluded that higher concentration of DE are required on maize to have the same level of effectiveness than for wheat. Athanassiou et al. (2004 b) tested SilicoSec, PyriSec and Insecto on different grain commodities and observed higher adult mortalities of *T. confusum* on rye as compared with oat or triticale. Among eight grain commodities (wheat, oats, peeled barley, whole barley, rye, triticale, maize and rice), adult mortality was >90% after 14 days of exposure interval at the lowest application rates of Insecto on wheat and triticale (Kavallieratos et al., 2005).

Just as for DE (Athanassiou et al., 2003), the efficacy of chemical insecticides like spinosad also varied among the commodities and grain types (Athanassiou et al, 2008 b; Chintzoglou et al., 2008). This variable efficacy generally correlates with the adherence ratio of DE particles on different grain types (Korunic, 1997). Kavallieratos et al. (2010) investigated the effect of spinosad and DE adherence ratios on three wheat varieties and, resultantly, on their insecticidal efficiency against *S. oryzae*, *R. dominica* and *T. confusum*. The results revealed that the adherence ratios of the test compounds were >90%, but higher adult mortality rates were observed in the varieties Athos and Sifnos compared with Pontos.

In our studies, the highest numbers of dead adults of all insect species analysed were found on treated wheat, and the least effective control was observed on maize kernels. These outcomes agree with previous finding by other researchers who reported lower efficacy of DEs on maize than on wheat and rice (Subramanyam and Roesli, 2000; Athanassiou et al., 2003, 2005). The even surface of maize kernels may reduce the likelihood of DE adherence and the retention on the maize grain, hereby maybe directly influencing the insecticidal efficacy of DE. Kavallieratos et al. (2005) already proposed that reduced retention of DE particles on maize kernels as compared with other grain commodities may be one of the basic reasons for these diverging results among grains. Furthermore, the reduced effectiveness of DE on maize could be due to the high contents of oil in its pericarp gradually decreasing the lipid absorption ability of DE particles from the insect cuticle (Vayias et al., 2006).

In studies conducted to investigate the insecticidal effect of DE in combination with spinosad, Chintzoglou et al. (2008) found a variable effect of combined treatments towards commodities and insect species. As in the DE alone treatments, the grain type also significantly affected the performance of *B. bassiana* alone as well as combined treatments with DEs. Previously, Athanassiou et al. (2008 a) also found the combined effectiveness of the fungus *Metarhizium anisopliae* and DE to be higher on wheat than on maize. Michalaki et al. (2006) proved differential effects of the treatments with the fungus *M. anisopliae* and SilicoSec on wheat and maize. Padin et al. (2002) had already confirmed that the grain type affects the insecticidal activity of *B. bassiana*. The results of our bioassays are comparable with those of Athanassiou et al. (2008 a) where the mortality of *S. oryzae* and *R. dominica* was generally lower on maize than on wheat mixed with the fungus *M. anisopliae* and DE. The specific conidia-kernel lipids/oils interaction might be the possible reason for the reduced efficacy of *B. bassiana* in maize kernels as well, in addition to the lower tendency of maize kernels to stick with the DE particles (Athanassiou and Kavallieratos, 2005).

Although various researchers have studied the effect of grain types on the efficacy of different insecticides from different chemical groups (Arthur, 2002; Haung and Subramanyam, 2007; Kavallieratos et al., 2009), no data is available on the insecticidal activity of Imidacloprid in this regard. Vayias et al. (2009) tested the effect of spinosad against three species of stored-product beetles on wheat, maize, rice and barley. They found that mortality levels for *T. confusum* and *S. oryae* were generally reduced on maize compared on the other tested commodities. On the contrary, the efficacy of Abamectin was higher on maize than on wheat grains against all species tested, if dose rates higher than 0.5 ppm were applied (Kavallieratos et al., 2009). Similar findings have also been reported by Arthur (2002) and Haung and Subramanyam (2007). The results of the current study disagree with these previous findings, as Imidacloprid was more effective on wheat compared on maize in all of its concentrations.

The fact that the effectiveness of Imidacloprid varied among the tested grain commodities could be rendered to the differential physical or chemical features of the grains, differences in insect behavioural responses after receiving the treatment from treated grains of a particular type, or a blend of all of these mentioned aspects. The maize kernels are larger in size and heavier as compared to wheat grains or rice kernels, a fact which may have affected the efficiency of the tested chemical, as all test insect species were less vulnerable on maize than on wheat or rice. Apparently, the kernel size has a significant role in determining the effectiveness of the insecticides because the toxicant is distributed more evenly on small kernels as compared to larg sized ones (Haung and Subramanyam, 2007) so that insects may be able to easily keep away from the treated surfaces of the larger sized kernels and, as a result, avoid to get in touch with the toxic residues (Athanassiou et al., 2003). In any case, this difference in the efficacy levels of Imidacloprid should be considered cautiously when applied on different grain commodities not tested so far.

## 4.6 Effect of storage period and residual efficacy of the test grain protectants

The effectiveness of all test compounds (DE, *B. bassiana* and Imidacloprid) decreased with extending storage period. However, *B. bassiana* and DE alone proved to result in much more stable control effects as compared with Imidacloprid alone at the end of the storage period of six months. In the case of entomopathogenic fungi, Athanassiou et al. (2008 a) described a gradual decrease in the effect of *Metarhizium aniospliae* against *R. dominica* and *Sitophilus oryzae* over six months storage period. The conidial viability also decreases over time as suggested by Moore et al. (2000). This fact could also be the reason for declining efficacy of the tested fungus in our experiments. The recycle-ability of the fungal pathogens in the dead insects (Thomas et al., 1995) could be the best explanation for the long-term effectiveness of the *B. bassiana* as compared with any other control measure tested in the present bioassays.

DEs are extremely stable compounds (Kaufhold et al., 2008); they are inert and do not react to produce toxic residues on the treated substrates (Korunic, 1998). This was supported by our study as well, as DE provided a successful control of all tested pest insect species and maintained it controlling power throughout the entire storage period. However, some reduction of the performance of DE with extended storage period has been described by Stathers et al. (2002) and Athanassiou et al. (2005). This slight decrease of its efficacy could be due to the prevailing environmental conditions. Particularly, the relative humidity level might cause this efficacy decline because DE remains effective as grain protectant as long as it remains dry (Subramanyam and Roesli, 2000). However, when DE is kept for a long period of time, its particles may absorb moisture from the surrounding air, a process which ultimately is reducing its effectiveness (Fields and Korunic, 2000; Arthur, 2002). As a second possible reason, DE particles also may absorb grain oil, which subsequently is affecting the water lipid absorption potential of the DE particles (McGaughey, 1972; Nielsen, 1998).

Imidacloprid produced the highest mortality rates for all pest insect species analysed up to 120 days of storage. However, its effectiveness was rapidly declining after 120 days, and Imidacloprid caused lower mortality rates than the other treatments in the last bioassay after 180 days. The results clearly indicate a lower stability of Imidacloprid than obtained for the biological control measures (DE and entomopathogenic fungi). This is also mirrored in their combined treatments effects.

The residual effectiveness of any control measure refers to its availability on the treated substrate for a long period of time. Therefore, the occurrence of residues sometimes is considered as an advantageous feature of any given grain protectant for the prolonged protection of stored products. In our case, the sharply declined insecticidal effectiveness of Imidacloprid can be explained by the characteristic of certain neonicotinoid insecticides to degrade rapidly (Soliman, 2011). Dealing with insecticides belonging to another group of neurotoxins, Athanassiou et al. (2003) investigated the insecticidal and long-term effects of three pyrethroids (Deltamethrin, beta-Cyfluthrin and alpha-Cypermethrin) against *S. oryzae* on stored wheat. They also observed a strong reduction of efficacy with time: the highest mortality rates (>90%) observed after 32 days declined dramatically to less than 50% after 171 days of storage. Our results also agree with Wakil et al. (2012) showing that the insecticidal efficacy of Thiamethoxam quickly diminished after 90 days of storage; and less than 5% adults of *R. dominica* were killed by the treatment at the end of the experiment after 270 days.

# 4.7 Susceptibility of test insect species

Among all tested pest insect species, *T. castaneum* was the most resistant and *L. paeta* was the most susceptible species to all treatments. The order of species susceptibility, from least to most susceptible, of the four exposed species on the treated

grains was *T. castaneum*, *R. dominica*, *C. ferrugineus* and *L. paeta*. Insects differ in their susceptibility to DEs due to various factors; surface to volume ratios, life stage (Mewis and Reichmuth, 1999), body setation, thickness of the cuticle (Bartlett, 1951), and behaviour. Consequently, different application rates are required for the effective control of the different species. Psocids, which are considered as an emerging threat to stored grains and grain processing facilities (Throne et al., 2006) are soft bodied insects. It might be this feature, which makes them more vulnerable to DEs, thus allowing their effective control (Korunic, 1998). On the other hand, *T. castaneum* (Korunic and Ormesher, 1999; Rigaux et al., 2001; Athanassiou et al., 2005; Athanassiou and Korunic, 2007) and *R. dominica* (Fields and Korunic, 2000; Vardeman et al., 2006) are considered among the most DE-tolerant insect species. This fact has also been supported by our experiments.

The low susceptibility of *Tribolium* species has been observed in various studies. For instance, Athanassiou et al. (2004 b) observed that, even after 21 days of exposure to DEs, a considerable proportion of *T. confusum* adults was still alive if compared with *S. oryzae*. Consequently, an even longer exposure is needed to obtain a complete control. The mixture of three DE formulations (Insecto, Protect-It and PyriSec) caused 100% mortality of *S. oryzae* and *R. dominica* in some combinations, but mortality did not exceed 67% for *T. confusum* in any of these cases (Athanassiou et al., 2007). In another study, Athanassiou and Korunic (2007) used two new DE formulations (DEA-P/WP and DEBBM-P/WP). While mortality rates for all tested insect species were 100% on wheat mixed with 75 ppm, this effect was only achieved for *T. castaneum* at dose rate higher than 100 ppm. Evaluating the insecticidal effectiveness of DEBBM against three pest insesct species of stored commodities, Athanassiou et al. (2009 b) reported *C. ferrugineus* as the most susceptible species, while *T. confusum* was the most resistant one.

The adults of *T. castaneum* were also not very susceptible to fungi, since a substantial number of the treated individuals were alive after the given exposure periods in all bioassays. The addition of Protect-It increased the effect of *B. bassiana* in the highest fungal concentration and after exposure for 14 or more days. However, a complete suppression could only be achieved for wheat. Nevertheless, larvae of *Tribolium* species are more susceptible to DE or fungus treatments as compared with the

adult stage (Michalaki et al., 2007). Therefore, even dose rate of fungi not being enough for an acceptable level of protection against *T. castaneum* adults may gradually decrease the red flour beetle population through increased larval mortality.

Similarly, *Tribolium* species also have been reported to be more tolerant to different classes of insecticides, for instance pyrethroids (Athanassiou et al., 2004 a), Abamectin (Kavallieratos et al., 2009), Spinosad (Subramanyam et al., 2003; Nayak et al., 2005; Athanassiou et al., 2010 a) and the combination of Spinosad with DE (Kavallieratos et al., 2010) etc. This enhanced tolerance was also observed in our tests with Imidacloprid, and this effect was even noted at the highest dose rate tested in our experiments. Consequently, application rates lower than 5.0 ppm should be avoided because they may be ineffective at this concentration against some other pest species as well. However, 5.0 ppm gained a good control of *C. ferrugineus* and *L. paeta*. Therefore, the application of 5.0 ppm of Imidacloprid to grains provokes a satisfactory protection against primary insects (damage sound/whole grains), and thus may obstruct the development of secondary insects (feeding on the processed products or damaged/broken grains) like *T. castaneum*.

## 4.8 Susceptibility of test insect populations/strains

The different strains of test insect species belonging to different geographical regions differed in their vulnerability or tolerance levels to the tested insecticides. Generally, the field populations are found to be less susceptible to the applied chemicals as compared with the laboratory strains (Subramanyam and Hagstrum, 1995). In our trials, the varying mortality levels of *T. castaneum*, *C. ferrugineus*, *L. paeta* and *R. dominica* populations might be attributed to their status of being field strains used in our bioassays. The assumed higher genetic diversity of field strains might explain their decreased susceptibility to the applied test chemicals. The effect of the geographical site on the vulnerability level of *T. confusum* to Spinosad dust has also been reported by Athanassiou et al. (2008 b). They observed considerable differences in the susceptibility levels of the beetles and larvae of six populations of *T. confusum* from Europe. Kavallieratos et al. (2007) recorded similar differences of the effects of DEs against several *T. confusum* populations collected from several parts of the world. Similar findings have been reported

by Kljajic and Peric (2009) for the performance of Malathion and Deltamethrin against various pest beetle populations of *Sitophilus granarius* (Curculionidae: Coleoptera).

However, our study is the first one which analysed the variable efficacy levels of *B. bassiana*, DE and Imidacloprid among the different strains of four important stored grain pest insect species belonging to different geographical locations of Punjab (Pakistan). The results exhibited a significant difference among the populations, and the population from Multan district appeared to be more tolerant to all test compounds as compared with the three other populations of the four insect species.

The development of stored grain insect pest resistance to various classes of insecticides has been reported by several researchers in Pakistan (Hamid and Ahmad, 1988; Hasan et al., 1996; Hussain et al., 1996 a, b; Alam et al., 1999). However, variable tolerance levels of these insect species to Imidacloprid have to be considered, since Imidacloprid is not listed as a grain protectant in Pakistan. Consequently, insect populations with cross-resistance to other nerve toxic insecticides are also likely to survive Imidacloprid. On the other hand for DE, the different mobility rates of different populations can lead to pick-up more or less particles on their bodies (Rigaux et al., 2001), a factor ultimately affecting the DE efficacy. Therefore, the applied substances have to be selected cautiously because their efficacy may vary considerably among the districts. As so far no records are available about the response of different populations of stored grain insect pests to fungal efficacy, the differential effect of *B. bassiana* against all tested populations of each tested insect species requires additional experimental work to be done in this regards.

# **4.9** The effect on progeny production

The avoidance of progeny production is the primary objective of any stored grain protection strategy. In the current study, the progeny emergence for all insect species analysed was controlled completely for all bioassays when DE and Imidacloprid were applied together, particularly on wheat. Similarly, a complete suppression of offspring occurred at the higher dose combinations of *B. bassiana* with DE in the second bioassay. In the residual efficacy assessment of all three test compounds during the third bioassay, the least progeny production was observed for the combined treatment with DE and
Imidcloprid for all test insect species. However, the rate of progeny emergence tended to increase along the storage period. The efficacy of progeny production in DE, *B. bassiana* and Imidacloprid treated units was correlated with the insecticidal effect of each treatment against adults, and the treated units showing the lowest progeny production also had the highest mortality rates for all test insect species. This could be due to reduced numbers of eggs laid in treated units suffering from high adult mortality rates. The rate of emerging adults might also be further reduced when get in touch with the particles of DE or Imidacloprid residues attached to the egg shells.

#### 4.10 Field efficacy of the test grain protectants

The findings of our field trials revealed that DE, Imidacloprid and entomopathogenic fungi can be used effectively against *T. castaneum*, *C. ferrugineus*, *L. paeta* and *R. dominica* on wheat stored at farms situated in different districts of Punjab, Pakistan. However, their effectiveness is closely related with the local insect population, the length of the storage period and the climatic conditions prevailing in an area. In earlier studies, Stathers et al. (2002) conducted some trials for the efficacy assessment of Protect-It and Dryacide against various stored grain pest insects on small-scale farms in Zimbabwe. The tested DE formulations provided the protection of maize, sorghum and cowpeas for a storage period of 280 days. Cherry et al. (2007) used *B. bassiana* with lemon grass oil and a synthetic pesticide mixture for the management of the bean beetle *Callosobruchus maculatus* (Curculionidae: Coleoptera) in stored cowpea in Benin, West Africa. Stathers (2003) conducted small scale trials to evaluate the integrated treatments of DE with sublethal concentrations of pyrethroids, plant extracts or soil bacterial metabolites against *Prostephanus truncatus* (Bostrichidae: Coleoptera) in sub-Saharan Africa.

Our study is an addition to the existing list of field trials where the field application of certain novel approaches like DE, entomopathogenic fungi and Imidacloprid against several stored grain insect pests has been carried out. In these trials, the high percentages of seed damage in untreated as compared with the treated grains are in accordance with the findings by Stathers et al. (2002) and Cherry et al. (2007), who reported similar observations. The increased rates of damaged grains along the progress

of the storage period could be attributed to the increasing rates of insect survival with time during these trials.

## 4.11 Implications for agriculture and storage

The present study may be considered novel on the basis of using local fungal isolate for evaluating its efficacy against major stored grain insects of Pakistan not reported earlier. This is the first time that Imidacloprid has been tested as grain protectant in combine treatment with entomopathogenic fungi and DE. The variable tolerance levels among indigenous populations of *T. castaneum*, *C. ferrugineus*, *L. paeta* and *R. dominica* to test grain protectants were detected affecting the development of the resistance. Imidacloprid is not yet registered in Pakistan as grain protectant, and current work reveals that it has the potential to play a highly important role in the stored-product Integrated Pest Management programs in Pakistan. The baseline data presented here for different insect pest species can be used to observe the changes in vulnerability of these insect species in Pakistan to DE, entomopathogenic fungi and Imidacloprid when they will be registered and widely applied.

Present study is also unique in the sense that the field application of DE, entomopathogenic fingi and a new chemical insecticide (Imidacloprid) is being carried out for the first time in Pakistan. The findings of these field trials are of practical importance, particularly for local farmers, as the excellent performance of these novel control methods under the local climatic conditions of Pakistan may offer an alternative approach for the long-term safety of stored produce at such rural farms. Further studies on biological insecticides performed under field conditions are needed to address the consequences of the practical execution of the strategies. These studies have to provide safer, easily acceptable and applicable as well as cost effective alternatives to conventional grain protectants to the local stakeholders.

## 5 Summary

A big challenge for agriculture in the 21<sup>st</sup> century is the provision of food safety to fast growing world's population, which not only demands the well utilisation of the available agricultural resources but also to develop new advancements in the mass production of food crops. Wheat is the third largest food crop of the world and Pakistan is the eighth largest wheat producing country globally. Rice is the second most important staple food of Pakistan after wheat, grown in all provinces of the country. Maize is the world's top ranking food crop followed by wheat and rice. The harvested produts have to be stored in different types of storage structures on small or large scale for food as well as seed purpose. In Pakistan, the harvested grains are stored for the whole year till the introduction of fresh produce in order to ensure the regular food supply throughout the year. However, it is this extended storage period making the commodity more vulnerable to insect attacks.

Rhyzopertha dominica (Coleoptera: Bostrychidae), Cryptolestes ferrugineus (Coleoptera: Laemophloeidae), Tribolium castaneum (Coleoptera: Tenebrionidae) and Liposcelis spp. (Psocoptera: Liposcelididae) are the major and most damaging insect pests of stored products all around the world. Various management strategies have been adopted for stored grain insect pests mostly relying upon the use of a broad spectrum of insecticides, but the injudicious use of these chemicals raised various environmental and human health related issues, which necessitate the safe use of the prevailing control measures and evaluation of new and alternative control methods. The application of new chemical insecticides, microbial insecticides (particularly entomopathogenic fungi) and the use of inert dusts (diatomaceous earths) is believed amongst the potential alternatives to generally used insecticides in stored grain insect management system. In the current investigations, laboratory bioassays conducted to evaluate the effects of combining Imidacloprid (new chemistry insecticide) with and without Protect-It (diatomaceous earth formulation) against R. dominica, L. paeta, C. ferrugineus and T. castaneum, on three different grain commodities (i.e. wheat, maize and rice) revealed differences in adult mortality levels among grains and insect species tested. Individually, Imidacloprid was more effective as compared with Protect-It alone and the highest numbers of dead adults were recorded in wheat. The insecticidal efficacy of B. bassiana with Protect-It and

DEBBM was also assessed against all test insect species under laboratory conditions. The findings of these studies revealed that the more extended exposure period and the higher combined application rate of *B. bassiana* and DEs provided the highest mortality of the test insect species. The progeny emergence of each insect species was also greatly suppressed where the highest dose rates of the combined treatments were applied. The residual efficacy of all three control measures Imidacloprid, *B. bassiana* and DEBBM formulation was also evaluated against all test insect species. The bioassays were carried out after grain treatments and monthly for 6 months. The results indicated that the adult mortality of each test insect species was decreased within the six month storage period, and the integarted application of the test grain protectants enhanced the mortality rates than their alone treatments. The maximum mortality was noted in the combined treatment of DEBBM with Imidacloprid.

At the end, the effectiveness of *B. bassiana*, DEBBM and Imidacloprid applied alone as well as in combinations, against all above mentioned test insect species was also evaluated under field conditions in trials conducted in four districts of Punjab, Pakistan. For each district, a significant difference was observed between treatments, while the combined treatments gave better control of test species as compared with them alone. The least number of surviving adults and minimum percentage of grain damage was observed for the DEBBM and Imidacloprid combination, but DEBBM with *B. bassiana* provided the best long-term protection as compared with the remaining treatments.

# 6 Zusammenfassung

Eine große Herausforderung der Landwirtschaft des 21. Jahrhunderts ist die Garantierung von Nahrungsmittelsicherheit für die wachsende Weltbevölkerung, welche nicht nur die gute Nutzung der vorhandenen landwirtschaftlichen Ressourcen erfordert sondern auch weitere Fortschritte in der Massenproduktion von Lebensmitteln. Weizen ist das drittwichtigste Lebensmittel weltweit und Pakistan ist der achtgrößte Weizenproduzent der Welt. Reis ist das zweitwichtigste eingelagerte Lebensmittel Pakistans nach Weizen, und wird in allen Provinzen des Landes angebaut. Mais hält den ersten Platz der weltweit angebauten Nahrungsmittel, gefolgt von Weizen und Reis. Die geernteten Produkte müssen in unterschiedlichen Typen von Lagereinrichtungen unterschiedlicher Größen für den Zweck des Konsums oder als Samen eingelagert werden. In Pakistan werden die geernteten Getreide über das gesamte Jahr bis zur Einbringung der neuen Ernte gelagert, um die Ernährung der Bevölkerung ganzjährig sicher zu stellen. Jedoch macht diese längerfristige Einlagerung die entsprechenden Produkte anfällig für die Angriffe von Insekten.

Rhyzopertha dominica (Coleoptera: Bostrychidae), Cryptolestes ferrugineus (Coleoptera: Laemophloeidae), Tribolium castaneum (Coleoptera: Tenebrionidae) und Liposcelis spp. (Psocoptera: Liposcelididae) sind weltweit die wichtigsten und zerstörerischsten Schadinsekten gelagerten Getreides. Unterschiedliche Management strategien wurden auf Schadinsekten eingelagerten Getreides angepasst, die meisten von diesen basierend auf einem breiten Spektrum von Insektiziden, aber der unüberlegte Gebrauch dieser Chemikalien brachte verschiedene Umwelt- und menschliche Gesundheitsprobleme hervor, welche den sicheren Einsatz der vorherrschenden Kontrollmethoden erforderte und die Entwicklung neuer und alternativer Kontrollmethoden verlangt. Die Anwendung von neuen chemischen Insektiziden, mikrobiellen Insektiziden (speziell entomopathogene Pilze) und der Einsatz inerter Stäube (Kieselerden) werden als viel versprechende Alternativen zu den traditionellen Insektiziden im Insektenmanagement eingelagerten Getreides gesehen. In den hier präsentierten Untersuchungen zeigen Labor-Bioassays, welche zur Evaluierung des Effektes des kombinierten Einsatzes von Imidacloprid (ein neues chemisches Insektizid) mit und ohne Protect-It (eine Kieselerdeformulierung) gegen R. dominica, L. paeta, C.

ferrugineus und T. castaneum auf drei unterschiedlichen Getreidearten (diese sind Weizen, Mais und Reis) durchgeführt wurden, dass Unterschiede der Adultenmortalitäten zwischen den Getreide- und Insektenarten existieren. In der alleinigen Anwendung war Imidacloprid effektiver als Protect-It, und die höchsten Zahlen toten Adulti wurden für Weizen nachgewiesen. Die insektizide Effizienz von Beauveria bassiana mit Protect-It und DEBBM wurde auch unter Laborbedingungen gegen alle Insektenarten getestet. Die Ergebnisse zeigten, dass je länger der Anwendungszeitraum und je höher die kombinierten Konzentrationen von B. bassiana und Kieselerdeformulierungen, umso höher waren auch die Mortalitätsraten der getesteten Insektenarten. Die Emergenz von Nachwuchs aller Insektenarten wurde auch stark unterdrückt, wenn die höchsten Konzentrationen der synergistisch wirkenden Behandlungsmethoden angewandt wurden. Die sich ergebende Effizienz der Kontrollmethoden mit Imidacloprid, B. bassiana und DEBBM wurde auch gegen alle getesteten Insektenarten untersucht. Die Bioassays wurden nach der Behandlung und jeweils monatlich über einen Zeitraum von sechs Monaten durchgeführt. Die Ergebnisse zeigten, dass die Adultenmortalität für alle untersuchten Insektenarten über die Lagerungsperiode von sechs Monaten abnahm und dass die kombinierten Anwendungen die Mortalitätsraten im Vergleich zu den Einzelbehandlungen erhöhten. Die maximale Mortalität wurde für die Kombination von DEBBM mit Imidacloprid erhalten.

Abschließend wurde die Effektivität von *B. bassiana*, DEBBM und Imidacloprid, alleine und in Kombination, unter Feldbedingungen in Untersuchungen in vier Distrikten des Punjab, Pakistan, auf alle oben erwähnten Insektenarten evaluiert. Für jeden Distrikt ergaben sich signifikante Unterschiede zwischen den Behandlungen, jedoch zeigten die kombinierten Behandlungen durchwegs bessere Schädlingskontrollen als die alleinigen Anwendungen. Die geringste Anzahl überlebender Adulti und die minimale Schädigung der Getreidekörner wurde für die Kombination von DEBBM und Imidacloprid beobachtet, jedoch erbrachte DEBBM mit *B. bassiana* den besten Langzeitschutz im Vergleich mit den anderen Behandlungsmethoden. Die Ergebnisse dieser Studie zeigen, dass Kieselerden, Imidacloprid und *B. bassiana* effektiv gegen Schadinsekten von eingelagerten Getreiden eingesetzt werden können, dass jedoch ihre Effizienz stark abhängig ist von der jeweiligen Konzentration, den Einwirkzeiten, der Getreideart, der

Lagerungsdauer und den jeweiligen Zielinsektenarten. Folglich können diese Testsubstanzen erfolgreich in integrierten Schädlingskontroll-Management-Programmen nach der Ernte eingesetzt werden und können als Getreideschutzmittel registriert werden.

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Appendix 1 Corrected mortality (%) of *C. ferrugineus* in three commodities treated with one dose rate of Protect-It (DE: 150 mg/kg), three dose rates of Imidacloprid (Imid: 1.25, 2.5 and 5.0 mg/kg) applied alone or in combination (each repeat is an average of four vials in each treatment)

Treatments	Rep	Comm	odities											
	-	Maize				Rice				Wheat				
		24 h	48 h	96 h	7 d	24 h	48 h	96 h	7 d	24 h	48 h	96 h	7 d	
DE	R1	2.7	16.5	26.6	47.3	9.5	24.0	35.9	59.2	21.9	26.6	33.9	63.5	
	R2	14.8	19.7	31.0	48.4	18.9	29.7	46.9	52.7	12.8	36.7	48.4	50.0	
	R3	11.2	27.0	40.6	52.7	11.2	19.9	32.4	47.3	17.2	28.6	44.1	60.6	
Imid 1	R1	17.6	17.6	43.6	44.0	20.5	32.1	35.9	64.1	29.7	34.9	52.6	59.9	
	R2	13.3	23.9	31.0	52.7	24.0	22.4	49.5	51.6	26.0	38.8	46.4	62.2	
	R3	17.9	31.6	38.0	58.0	15.8	33.2	44.1	63.3	20.3	29.7	39.4	71.3	
Imid 2	R1	26.1	38.8	47.3	65.8	29.0	47.4	51.6	76.6	28.1	40.1	56.8	83.9	
	R2	21.4	32.4	41.8	74.5	31.1	37.0	48.4	73.9	37.2	45.2	48.4	80.3	
	R3	21.9	37.8	55.7	62.2	23.0	39.3	56.9	70.7	29.7	50.5	58.0	70.7	
Imid 3	R1	26.1	41.0	63.3	74.5	39.5	45.4	67.9	91.8	40.6	59.4	74.5	92.2	
	R2	23.0	45.2	48.9	84.2	33.2	49.0	60.9	81.4	34.2	51.6	58.9	87.8	
	R3	35.7	50.0	56.8	87.8	30.1	56.1	64.4	85.6	37.0	49.0	68.6	96.3	
DE + Imid 1	R1	26.1	28.2	38.8	72.3	26.5	36.2	53.8	76.6	22.4	42.7	44.3	82.8	
	R2	16.8	33.0	48.9	63.6	29.1	35.9	51.6	71.3	37.2	35.6	50.5	75.0	
	R3	21.9	39.8	46.4	68.6	21.4	43.9	48.4	68.6	27.6	48.4	56.4	70.7	
DE + Imid 2	R1	35.6	45.7	58.5	90.8	43.0	63.8	62.5	85.3	46.4	69.3	73.4	97.4	
	R2	29.1	51.6	63.6	87.5	30.1	48.4	73.4	96.3	39.3	59.0	75.5	88.8	
	R3	32.1	42.3	67.2	79.8	35.2	54.1	66.5	92.6	35.9	65.1	67.6	100.0	
DE + Imid 3	R1	35.6	61.2	80.3	100.0	41.5	71.9	88.6	100.0	52.6	73.4	84.4	100.0	
	R2	41.8	68.6	81.0	83.2	47.4	66.1	79.7	92.0	42.3	67.0	97.4	100.0	
	R3	31.1	57.7	74.5	87.8	35.2	69.9	81.4	100.0	46.9	79.7	92.0	100.0	

Appendix 2 Corrected mortality (%) of *R. dominica* in three commodities treated with one dose rate of Protect-It (DE: 150 mg/kg), three dose rates of Imidacloprid (Imid: 1.25, 2.5 and 5.0 mg/kg) applied alone or in combination (each repeat is an average of four vials in each treatment)

Treatments	Rep	Comn	nodities										
		Maize				Rice				Whea	t		
		24 h	48 h	96 h	7 d	24 h	48 h	96 h	7 d	24 h	48 h	96 h	7 d
DE	R1	10.7	8.9	28.6	38.0	8.5	12.0	30.2	45.3	14.1	29.7	29.7	52.1
	R2	3.1	18.7	33.2	51.0	15.3	27.6	24.0	55.2	10.7	25.0	41.1	59.9
	R3	8.5	12.8	19.3	43.9	10.5	16.0	37.2	47.3	17.7	18.6	35.1	48.4
Imid 1	R1	6.6	21.4	35.9	40.1	19.5	27.6	40.1	48.4	25.5	20.3	34.4	65.4
	R2	11.7	18.9	27.0	53.6	20.9	23.5	37.0	56.8	16.8	32.1	44.3	59.4
	R3	19.0	11.7	30.7	51.5	16.5	15.4	31.4	52.7	21.9	26.1	39.9	47.9
Imid 2	R1	14.8	23.4	32.8	55.7	22.5	38.0	40.1	71.4	24.5	42.2	47.4	64.4
	R2	25.5	29.1	39.3	63.0	18.9	32.1	46.4	65.6	33.7	35.7	39.9	80.7
	R3	14.5	36.2	42.7	67.9	28.5	33.5	49.5	68.6	26.6	34.6	54.8	75.0
Imid 3	R1	20.9	37.0	59.9	71.9	31.0	40.1	59.4	81.8	39.1	50.5	55.7	73.9
	R2	25.0	33.2	55.6	75.5	23.0	40.3	51.6	74.5	35.2	50.5	68.2	91.1
	R3	29.5	45.4	46.9	66.3	34.5	49.5	64.4	77.1	28.6	43.1	60.1	81.4
DE + Imid 1	R1	17.9	23.4	45.3	50.5	22.5	29.7	47.4	58.9	31.8	29.7	43.2	67.0
	R2	18.9	37.2	41.8	59.4	18.9	39.3	40.1	71.9	21.9	39.3	52.6	61.5
	R3	15.0	30.1	35.9	58.7	31.0	28.2	47.3	62.8	26.0	39.9	48.4	72.9
DE + Imid 2	R1	24.0	30.2	55.7	78.6	27.5	51.6	50.5	92.2	42.7	50.5	64.1	85.6
	R2	27.6	45.4	58.2	79.7	29.1	50.5	58.9	81.8	39.8	58.7	57.8	96.9
	R3	36.5	36.2	43.2	88.8	37.0	42.0	62.8	85.6	30.7	48.4	68.6	91.0
DE + Imid 3	R1	37.2	48.4	73.4	79.7	42.5	67.2	76.0	92.2	45.3	67.2	90.1	100.0
	R2	25.0	61.7	66.3	88.0	31.1	62.8	82.3	98.4	39.3	61.7	79.7	94.3
	R3	32.5	52.6	59.4	94.9	38.5	56.9	68.6	86.7	51.6	73.4	77.1	100.0

Appendix 3 Corrected mortality (%) of *T. castaneum* in three commodities treated with one dose rate of Protect-It (DE: 150 mg/kg), three dose rates of Imidacloprid (Imid: 1.25, 2.5 and 5.0 mg/kg) applied alone or in combination (each repeat is an average of four vials in each treatment)

Treatments	Rep	Comm	odities										
	-	Maize				Rice				Wheat	t		
		24 h	<b>48 h</b>	96 h	7 d	24 h	48 h	96 h	7 d	24 h	48 h	96 h	7 d
DE	R1	0.5	14.1	18.6	28.7	3.1	5.7	26.0	35.1	12.0	16.0	26.6	41.1
	R2	8.7	8.0	7.4	39.7	8.7	17.3	20.9	50.0	7.1	21.4	33.5	48.4
	R3	1.5	5.2	22.4	37.0	3.5	10.7	14.1	41.1	5.9	9.2	16.3	51.6
Imid 1	R1	5.2	7.8	20.7	33.5	9.4	14.1	27.6	45.2	10.9	22.9	31.5	54.4
	R2	3.6	16.5	28.7	37.5	15.3	13.8	34.2	46.4	21.4	29.7	28.2	45.2
	R3	13.3	15.1	20.3	43.2	9.5	23.5	24.5	40.1	13.8	17.9	37.5	48.4
Imid 2	R1	10.9	19.8	31.4	60.1	8.9	17.7	39.1	58.0	23.4	28.7	38.6	60.6
	R2	17.3	12.2	25.0	51.1	12.8	27.6	29.1	65.8	17.3	26.6	34.6	69.7
	R3	9.2	23.4	38.0	48.4	21.0	24.0	32.3	57.3	16.5	28.8	44.0	63.6
Imid 3	R1	14.1	31.8	37.8	54.3	15.6	28.1	44.8	71.8	21.4	44.1	41.8	82.8
	R2	11.2	22.9	28.2	70.1	25.0	41.3	41.8	63.8	30.1	38.5	47.3	75.0
	R3	23.5	27.6	42.2	63.0	21.0	37.2	33.9	69.3	23.9	40.8	54.9	67.9
DE + Imid 1	R1	14.1	28.1	30.3	50.0	18.2	25.5	39.1	47.9	13.5	27.1	38.0	58.9
	R2	6.6	18.6	23.9	38.0	11.2	32.1	35.7	52.6	16.8	38.0	44.1	54.8
	R3	10.7	17.7	36.5	48.4	10.5	23.0	27.6	59.9	20.7	32.1	47.3	55.4
DE + Imid 2	R1	21.4	21.9	45.2	72.9	20.3	43.2	48.4	79.8	24.5	48.4	52.7	76.1
	R2	15.3	25.0	32.4	63.6	15.3	31.6	54.6	82.7	29.6	43.2	60.1	86.7
	R3	18.9	31.8	40.6	77.6	27.5	36.2	41.1	69.3	30.9	37.5	49.5	90.2
DE + Imid 3	R1	26.6	43.2	53.7	84.6	29.7	52.6	63.5	79.8	32.8	54.8	63.6	85.6
	R2	17.3	37.2	41.5	79.9	21.9	39.3	52.0	92.3	41.3	59.9	61.2	91.0
	R3	23.0	31.8	47.4	74.0	27.0	43.9	59.4	85.9	28.2	48.4	71.2	96.7

Appendix 4 Corrected mortality (%) of *L. paeta* in three commodities treated with one dose rate of Protect-It (DE: 150 mg/kg), three dose rates of Imidacloprid (Imid: 1.25, 2.5 and 5.0 mg/kg) applied alone or in combination (each repeat is an average of four vials in each treatment)

Treatments	Rep	Comn	odities										
		Maize				Rice				Whea	t		
		24 h	48 h	96 h	7 d	24 h	48 h	96 h	7 d	24 h	48 h	96 h	7 d
DE	R1	14.4	23.9	38.8	52.7	19.5	27.0	49.5	74.5	31.6	44.3	59.9	67.0
	R2	19.4	33.9	51.6	62.2	19.9	31.8	51.6	62.8	19.9	35.1	50.5	69.0
	R3	21.4	25.0	45.3	69.3	24.0	39.3	43.6	67.9	24.0	38.5	49.5	80.2
Imid 1	R1	26.1	39.4	47.3	67.9	33.0	41.8	52.6	89.7	33.2	40.6	61.5	92.0
	R2	25.0	38.0	43.1	75.5	27.6	44.8	63.3	75.5	27.6	50.5	50.5	78.8
	R3	21.4	31.1	56.8	79.7	21.4	35.2	48.4	82.1	35.9	38.0	65.1	90.6
Imid 2	R1	26.1	37.8	64.9	85.9	42.5	53.6	69.3	85.9	41.3	52.6	72.4	100.0
	R2	35.2	48.4	58.5	90.4	35.2	48.4	77.7	91.0	35.2	60.6	72.3	98.4
	R3	33.2	43.9	72.4	79.7	31.8	43.9	67.6	96.7	44.3	43.2	78.6	90.1
Imid 3	R1	41.5	55.9	84.0	91.8	42.5	57.7	80.2	91.8	41.3	69.8	92.7	100.0
	R2	39.3	53.1	71.8	88.3	39.3	65.6	87.8	100.0	53.6	63.3	90.8	100.0
	R3	33.7	62.8	78.6	96.4	44.3	62.8	84.6	100.0	44.3	62.0	97.4	100.0
DE + Imid 1	R1	19.7	43.1	65.4	81.0	32.5	45.4	65.1	92.9	31.1	64.1	84.4	100.0
	R2	31.6	53.1	73.4	97.3	34.2	50.5	77.1	100.0	34.2	50.0	72.3	100.0
	R3	28.1	41.8	63.0	87.0	26.6	58.2	73.9	86.4	41.1	57.8	78.1	100.0
DE + Imid 2	R1	44.1	62.2	79.8	100.0	47.0	63.8	85.9	100.0	46.4	80.7	100.0	100.0
	R2	43.4	67.2	87.8	86.2	43.4	67.2	90.4	100.0	58.2	67.6	92.4	100.0
	R3	37.2	58.2	76.6	100.0	51.6	74.5	83.5	100.0	49.5	71.4	100.0	100.0
DE + Imid 3	R1	46.3	79.3	94.1	100.0	49.0	80.1	98.4	100.0	66.3	90.1	100.0	100.0
	R2	53.6	77.6	86.7	100.0	51.5	77.6	96.3	100.0	51.5	78.2	100.0	100.0
	R3	42.3	72.4	81.8	100.0	56.8	86.2	87.8	100.0	56.8	83.9	100.0	100.0

Appendix 5 Mean number of *C. ferrugineus*, *R. dominica*, *T. castaneum* and *L. paeta* adult progeny in three commodities treated with one dose rate of Protect-It (DE: 150 mg/kg), three dose rates of Imidacloprid (Imid: 1.25, 2.5 and 5.0 mg/kg) applied alone or in combination (each repeat is an average of four vials in each treatment)

Treatments	Rep	Insect s	species										
		C. ferru	ıgineus		L. paeto	ı		R. dom	inica		T. casta	ineum	
		Maize	Rice	Wheat	Maize	Rice	Wheat	Maize	Rice	Wheat	Maize	Rice	Wheat
DE	R1	16.2	16.2	5.5	11.5	5.5	3.2	16.5	16.7	17.2	29.5	31.2	25.2
	R2	15.2	10.2	7.2	8.5	6.0	4.2	24.7	18.2	15.5	37.2	27.7	21.7
	R3	11.2	9.7	11.5	13.2	10.2	2.2	23.5	22.7	12.7	32.5	25.5	18.2
Imid 1	R1	12.2	5.0	7.2	6.7	2.2	0.0	15.7	16.7	6.7	28.2	21.7	20.7
	R2	16.5	7.2	5.7	4.7	4.0	3.7	21.2	11.7	12.7	24.2	18.2	12.2
	R3	8.5	9.5	3.2	8.2	6.2	0.0	18.2	10.7	8.2	30.2	24.7	16.7
Imid 2	R1	5.2	5.0	2.2	1.7	0.0	0.0	13.2	8.2	7.7	20.0	18.2	8.2
	R2	10.2	6.2	4.0	3.2	4.7	0.0	9.5	5.2	2.2	17.2	13.2	15.7
	R3	8.7	8.5	3.5	4.5	0.0	0.0	14.0	9.2	3.2	24.2	11.7	9.2
Imid 3	R1	5.2	2.2	0.0	5.2	0.0	0.0	6.2	3.2	5.2	12.2	12.7	5.2
	R2	7.0	4.0	3.5	0.0	0.0	0.0	8.2	0.5	2.2	17.2	7.7	12.5
	R3	3.2	6.2	0.0	0.0	0.0	0.0	10.0	6.5	0.2	19.7	9.7	7.5
DE + Imid 1	R1	11.7	5.7	3.7	0.0	0.0	0.0	23.2	16.5	11.0	24.5	18.2	17.2
	R2	9.2	6.2	2.2	0.0	0.0	0.0	16.5	12.2	14.5	18.2	14.7	10.5
	R3	6.7	9.7	6.2	0.0	0.0	0.0	17.5	14.0	9.0	20.2	19.2	12.7
DE + Imid 2	R1	3.7	4.0	0.0	0.0	0.0	0.0	8.2	8.2	0.0	16.2	7.2	1.2
	R2	0.0	0.5	0.0	0.0	0.0	0.0	12.2	4.0	4.5	12.7	11.2	5.2
	R3	5.2	2.7	0.0	0.0	0.0	0.0	10.2	6.2	2.2	10.5	6.2	6.2
DE + Imid 3	R1	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.0	0.0	4.7	3.2	0.0
	R2	4.7	0.0	0.0	0.0	0.0	0.0	3.2	3.2	0.0	6.5	7.2	1.2
	R3	2.0	0.0	0.0	0.0	0.0	124.2	7.2	0.0	0.0	10.5	0.2	2.5
Control	R1	72.5	80.2	85.2	93.2	167.2	132.2	87.2	93.2	102.2	46.2	62.2	83.2
	R2	67.2	74.2	84.2	96.0	155.2	159.7	70.2	78.2	80.2	59.2	69.5	64.7
	R3	62.0	66.2	79.2	103.2	146.2	3.2	77.2	74.7	91.7	63.2	70.5	76.2

Appendix 6 Corrected mortality (%) of *C. ferrugineus* in three commodities (maize, rice, wheat) treated with one dose rate of Protect-It (150 mg/kg), DEBBM (50 mg/kg), two dose rates of *B. bassiana* (Bb1: 1.5 x 10<sup>8</sup>; Bb2: 1.5 x 10<sup>10</sup> conidia kg<sup>-1</sup>) applied alone or in combination (each repeat is an average of four vials in each treatment)

Treatments	Rep	Comm	odities							
	-	Maize			Rice			Wheat	t	
		7 d	14 d	21 d	7 d	14 d	21 d	7 d	14 d	21 d
Bb1	R1	21.9	52.0	60.7	32.8	42.2	58.9	37.5	52.7	73.4
	R2	36.5	43.5	64.5	40.5	57.5	70.5	31.1	63.8	68.4
	R3	24.5	34.2	46.2	23.9	48.4	60.1	41.0	46.3	62.8
Bb2	R1	46.4	60.2	68.4	55.7	69.3	81.8	71.2	71.2	78.8
	R2	53.0	71.5	70.5	67.5	62.5	73.5	61.7	67.9	76.5
	R3	57.6	54.9	79.3	50.0	70.7	79.3	64.4	81.4	87.8
Protect-It	R1	42.3	64.3	72.4	43.2	63.0	81.8	48.9	59.2	72.3
	R2	46.5	52.5	59.5	55.0	70.5	71.0	63.8	68.4	73.0
	R3	53.8	56.0	64.1	55.9	53.7	64.4	55.9	70.7	83.5
DEBBM	R1	58.2	74.0	84.2	63.0	83.9	92.2	71.2	81.5	94.6
	R2	72.5	82.5	73.5	76.5	74.5	77.5	80.1	74.5	82.1
	R3	60.3	63.6	77.7	68.6	71.3	84.0	64.9	85.6	86.2
Bb1+Protect-It	R1	38.3	58.7	61.7	52.6	59.4	58.9	40.8	53.3	77.2
	R2	42.5	51.5	72.5	40.5	62.5	66.5	50.0	67.9	68.9
	R3	46.7	45.1	53.3	43.1	50.5	76.1	56.4	62.2	71.3
Bb2+Protect-It	R1	68.4	77.0	87.2	81.8	96.4	86.5	83.2	91.8	100.0
	R2	61.0	82.5	81.0	74.5	87.0	100.0	69.9	100.0	100.0
	R3	72.8	88.6	94.0	66.5	79.3	96.3	76.1	84.6	100.0
Bb1+DEBBM	R1	50.5	65.8	80.1	60.9	67.2	82.8	71.2	81.5	87.5
	R2	56.5	75.5	74.5	56.5	65.5	72.5	57.7	67.9	75.0
	R3	62.5	57.6	66.3	66.5	75.5	77.7	64.4	73.4	78.2
Bb2+DEBBM	R1	74.6	91.3	100.0	71.4	99.0	100.0	86.4	52.7	73.4
	R2	68.5	87.0	100.0	84.5	91.0	100.0	74.0	63.8	68.4
	R3	68.5	96.7	89.7	64.9	100.0	100.0	78.2	46.3	62.8

Appendix 7 Corrected mortality (%) of *R. dominica* in three commodities (maize, rice, wheat) treated with one dose rate of Protect-It (150 mg/kg), DEBBM (50 mg/kg), two dose rates of *B. bassiana* (Bb1:  $1.5 \times 10^8$ ; Bb2:  $1.5 \times 10^{10}$  conidia kg<sup>-1</sup>) applied alone or in combination (each repeat is an average of four vials in each treatment)

Treatments	Rep	Comm	odities							
	-	Maize			Rice			Wheat	ţ	
		7 d	14 d	21 d	7 d	14 d	21 d	7 d	14 d	21 d
Bb1	R1	26.5	31.5	40.5	20.7	33.7	57.1	38.8	53.7	49.5
	R2	16.0	39.9	43.6	36.5	53.0	48.5	34.9	44.3	63.0
	R3	27.6	34.4	48.4	26.1	45.7	51.6	23.3	43.9	56.7
Bb2	R1	47.0	61.0	66.5	49.5	56.0	81.0	52.7	75.5	75.0
	R2	49.5	67.0	56.9	58.5	64.5	73.5	56.8	69.3	69.3
	R3	39.1	56.8	72.4	48.4	70.7	66.5	62.8	60.6	85.6
Protect-It	R1	41.0	55.0	52.5	40.2	50.5	57.1	50.0	56.4	72.9
	R2	43.1	46.3	56.4	53.5	47.0	70.5	44.3	66.1	69.3
	R3	31.8	43.2	63.0	37.2	55.9	60.1	53.9	50.0	60.6
DEBBM	R1	54.5	63.0	83.5	52.7	78.8	85.3	69.1	81.9	89.9
	R2	42.0	67.0	69.1	65.0	74.5	73.0	57.8	77.6	78.6
	R3	56.8	71.4	76.6	50.5	67.0	79.8	61.7	70.6	83.9
Bb1+Protect-It	R1	37.0	39.5	47.5	33.2	40.2	57.1	42.0	49.5	54.3
	R2	38.8	48.4	60.1	32.5	56.5	64.5	46.9	46.4	65.6
	R3	25.5	42.7	49.5	41.0	45.2	51.6	31.7	60.6	67.2
Bb2+Protect-It	R1	54.5	74.5	87.5	58.2	70.1	83.7	61.2	92.0	100.0
	R2	58.5	81.4	79.8	74.5	85.0	100.0	69.3	74.5	96.4
	R3	65.6	69.8	94.3	63.3	79.3	92.6	74.4	80.6	92.8
Bb1+DEBBM	R1	51.5	65.0	74.5	46.2	65.8	81.0	49.5	54.3	87.8
	R2	35.6	49.5	68.6	56.5	61.0	74.5	53.6	63.0	74.0
	R3	45.3	57.8	62.0	43.1	54.3	64.4	61.1	67.2	76.1
Bb2+DEBBM	R1	75.1	85.5	95.0	70.1	85.3	100.0	83.5	94.1	100.0
	R2	66.5	78.2	100.0	80.5	82.5	100.0	71.4	85.9	100.0
	R3	60.9	90.1	85.9	64.9	94.1	92.0	75.0	100.0	100.0
Appendix 8 Corrected mortality (%) of *T. castaneum* in three commodities (maize, rice, wheat) treated with one dose rate of Protect-It (150 mg/kg), DEBBM (50 mg/kg), two dose rates of *B. bassiana* (Bb1: 1.5 x 10<sup>8</sup>; Bb2: 1.5 x 10<sup>10</sup> conidia kg<sup>-1</sup>) applied alone or in combination (each repeat is an average of four vials in each treatment)

Treatments	Rep	Comm	odities							
	-	Maize			Rice			Wheat	t	
		7 d	14 d	21 d	7 d	14 d	21 d	7 d	14 d	21 d
Bb1	R1	19.8	27.6	36.7	24.5	32.3	39.3	17.9	33.7	52.8
	R2	16.8	25.5	42.0	17.2	27.0	45.2	34.9	43.1	45.2
	R3	11.7	35.3	30.7	26.1	35.3	46.2	23.3	35.9	46.2
Bb2	R1	42.7	56.8	58.5	50.5	55.2	59.7	48.9	56.6	73.9
	R2	31.1	50.5	53.7	33.9	60.5	71.8	53.6	68.6	68.6
	R3	40.3	50.5	68.2	39.4	50.5	62.0	38.9	52.6	62.0
Protect-It	R1	38.5	48.4	53.7	43.0	48.4	59.7	48.9	56.6	53.9
	R2	25.5	43.9	58.0	34.4	45.0	62.2	44.3	45.2	68.6
	R3	33.2	36.4	49.5	34.6	51.1	47.3	33.9	52.6	61.4
DEBBM	R1	50.5	54.7	75.0	48.5	53.1	80.1	48.9	60.7	85.6
	R2	39.3	64.3	64.9	57.3	68.5	69.1	53.1	68.6	72.9
	R3	45.9	46.7	62.0	41.5	61.4	76.6	58.3	63.0	76.6
Bb1+Protect-It	R1	19.3	34.4	38.8	25.0	34.4	59.7	38.0	51.5	56.1
	R2	32.1	31.6	53.7	33.9	46.5	45.2	34.4	45.2	64.9
	R3	27.0	37.5	47.4	34.6	37.5	46.2	31.7	40.1	48.4
Bb2+Protect-It	R1	52.6	66.1	75.0	63.0	68.2	80.1	61.4	68.9	92.2
	R2	39.3	74.0	69.7	46.4	74.5	87.8	53.1	72.9	89.9
	R3	50.0	57.1	82.8	53.7	61.4	82.1	54.4	77.6	79.3
Bb1+DEBBM	R1	30.7	45.3	53.7	46.5	46.9	74.0	44.0	56.6	78.3
	R2	33.7	49.5	64.9	32.8	58.5	64.9	53.6	62.2	64.9
	R3	39.3	51.1	58.9	41.5	51.1	57.1	38.9	50.5	76.6
Bb2+DEBBM	R1	60.9	69.8	94.7	72.5	69.8	94.9	67.9	88.3	100.0
	R2	50.5	79.1	81.4	57.8	76.5	100.0	65.1	75.5	100.0
	R3	60.2	64.7	86.5	62.2	83.2	87.5	72.2	82.3	100.0

Appendix 9 Corrected mortality (%) of *L. paeta* in three commodities (maize, rice, wheat) treated with one dose rate of Protect-It (150 mg/kg), DEBBM (50 mg/kg), two dose rates of *B. bassiana* (Bb1: 1.5 x 10<sup>8</sup>; Bb2: 1.5 x 10<sup>10</sup> conidia kg<sup>-1</sup>) applied alone or in combination (each repeat is an average of four vials in each treatment)

Treatments	Rep	Comm	odities							
	-	Maize			Rice			Wheat		
		7 d	14 d	21 d	7 d	14 d	21 d	7 d	14 d	21 d
Bb1	R1	40.5	59.5	62.5	30.2	48.4	71.4	40.1	54.7	74.5
	R2	32.3	49.5	71.4	40.5	61.5	61.5	45.2	66.5	62.8
	R3	24.5	41.8	53.3	41.0	60.1	66.5	44.5	65.5	76.5
Bb2	R1	59.0	76.5	73.0	60.9	85.9	91.1	72.4	78.6	89.1
	R2	71.4	81.8	74.0	75.0	77.0	77.0	78.2	81.9	84.0
	R3	60.3	67.9	83.2	68.6	81.4	81.4	66.5	93.5	93.0
Protect-It	R1	61.0	65.0	78.5	51.6	69.3	71.4	67.2	73.4	81.8
	R2	46.4	69.3	71.4	63.0	64.5	80.5	60.1	67.0	75.5
	R3	50.5	52.7	63.6	55.9	71.3	75.5	57.0	79.0	87.0
DEBBM	R1	66.5	78.5	87.0	71.4	92.2	86.5	71.9	84.4	94.8
	R2	75.5	85.9	80.2	82.5	79.5	98.5	83.5	95.2	100.0
	R3	60.3	70.1	91.8	72.9	81.4	89.9	80.5	88.5	89.0
Bb1+Protect-It	R1	47.0	65.0	65.0	52.6	63.0	67.2	53.1	59.9	84.4
	R2	40.6	51.6	75.5	46.5	54.5	80.5	47.9	70.7	78.2
	R3	51.1	47.3	67.9	55.9	67.0	75.5	62.5	64.5	73.0
Bb2+Protect-It	R1	72.5	79.5	100.0	83.9	100.0	100.0	85.9	100.0	100.0
	R2	79.7	85.9	100.0	74.5	82.5	100.0	73.4	100.0	100.0
	R3	68.5	92.9	85.9	71.8	100.0	100.0	82.5	100.0	100.0
Bb1+DEBBM	R1	51.0	66.5	84.5	60.9	73.4	88.5	74.0	85.9	88.0
	R2	54.7	74.5	75.5	56.5	69.5	80.5	58.5	73.4	79.8
	R3	62.5	60.3	69.0	70.7	79.3	75.5	69.5	78.5	84.5
Bb2+DEBBM	R1	79.5	91.5	100.0	90.1	100.0	100.0	87.0	100.0	100.0
	R2	84.4	100.0	100.0	84.5	100.0	100.0	81.9	100.0	100.0
	R3	71.2	97.3	100.0	79.3	100.0	100.0	93.0	100.0	100.0

Appendix 10 Mean number of *C. ferrugineus*, *R. dominica*, *T. castaneum* and *L. paeta* adult progeny in three commodities (maize, rice, wheat) treated with one dose rate of Protect-It (150 mg/kg), DEBBM (50 mg/kg), two dose rates of *B. bassiana* (Bb1:  $1.5 \times 10^8$ ; Bb2:  $1.5 \times 10^{10}$  conidia kg<sup>-1</sup>) applied alone or in combination (each repeat is an average of four vials in each treatment)

Treatments	Rep	Insect s	pecies										
		C. ferru	gineus		L. paeto	ı		R. dom	inica		T. casta	neum	
		Maize	Rice	Wheat	Maize	Rice	Wheat	Maize	Rice	Wheat	Maize	Rice	Wheat
Bb1	R1	24.2	22.5	18.5	18.5	16.2	5.5	31.5	33.7	26.2	44.5	38.25	31.2
	R2	19.2	16.2	10.5	13.5	10.7	12.2	35.5	22.2	22.5	53.2	47.5	39.5
	R3	20.2	18.7	14.5	17.2	12.2	7.5	36.25	26.5	20.5	46.5	40.5	36.5
Bb2	R1	12.2	5.5	2.5	6.7	2.2	1.7	25.5	21.2	14.2	28.2	21.75	21.7
	R2	16.5	7.2	5.7	4.7	4.0	4.5	20.5	12.2	9.5	36.5	26.25	14.5
	R3	8.5	9.5	7.2	9.7	6.2	0.0	16.2	16.7	12.7	32.5	24.75	18.2
Protect-It	R1	20.2	16.7	7.2	8.7	9.7	7.2	28.2	25.5	20.7	33.5	28.5	30.5
	R2	14.2	10.2	15.2	7.7	4.7	3.5	25.2	20.2	12.2	41.2	36.5	21.5
	R3	18.2	12.7	9.2	11.2	7.2	4.7	21.2	18.2	16.7	36.7	33.25	25.7
DEBBM	R1	11.7	8.2	0.0	3.2	0.0	0.0	22.2	14.2	8.2	21.5	18.75	14.5
	R2	9.2	3.2	4.2	9.2	5.7	0.0	12.2	9.2	4.7	28.5	14.5	9.5
	R3	6.7	6.2	5.5	4.7	1.5	0.0	16.7	10.2	9.2	25.7	15.25	12.7
Bb1+Protect-It	R1	20.2	18.2	15.2	14.5	10.2	9.5	31.2	28.2	16.0	38.5	30.75	33.5
	R2	16.5	10.5	9.2	10.2	5.7	4.7	27.2	25.2	18.2	47.5	39.5	24.2
	R3	18.7	14.5	12.7	9.7	8.5	7.2	27.5	21.7	22.7	40.5	36.5	26.5
Bb2+Protect-It	R1	5.2	1.2	0.0	4.7	0.0	0.0	14.2	5.2	1.2	24.5	17.5	6.5
	R2	7.2	4.2	0.0	0.0	0.0	0.0	9.7	12.5	5.2	18.2	10.2	12.5
	R3	9.2	5.2	0.0	1.7	0.0	0.0	10.2	7.5	6.2	20.2	12.7	8.7
Bb1+DEBBM	R1	17.5	6.7	8.7	10.2	8.2	0.0	26.7	20.7	16.5	31.2	33.5	25.7
	R2	10.5	7.5	3.5	6.5	4.5	6.2	20.5	12.2	12.2	35.5	22.2	20.2
	R3	14.5	10.5	6.5	8.5	3.7	2.7	19.2	16.7	14.0	36.0	26.5	18.2
Bb2+DEBBM	R1	0.0	0.0	0.0	0.0	0.0	0.0	9.2	0.0	0.0	17.2	8.7	1.2
	R2	1.2	0.0	0.0	0.0	0.0	0.0	3.7	4.2	0.0	10.2	10.2	5.2
	R3	2.5	0.0	0.0	0.0	0.0	0.0	4.5	2.5	0.0	12.7	5.2	6.2
Control	R1	94.2	107.2	121.5	112.2	170.2	167.7	97.2	107.2	116.2	76.5	81.2	101.5
	R2	106.2	100.5	114.5	132.2	165.7	149.7	88.2	117.2	133.2	68.2	93.7	93.5
	R3	95.2	95.5	109.7	124.2	153.7	157.2	101.2	102.5	125.2	73.5	80.5	88.2

Appendix 11	Germination rate of <i>B. bassiana</i> conidia on SDA mixed with Imidacloprid
	(Imid1: 1.25, Imid2: 2.5, Imid3: 5.0, Imid4: 7.5 and Imid5: 10 ppm) on
	SDA media (each repeat is an average of four plates in each treatment)

Treatments	Replications	%age of germinated conidia
Imid 1	R1	96
	R2	99
	R3	90
	R4	99
	R5	93
Imid 2	R1	95
	R2	93
	R3	84
	R4	97
	R5	91
Imid 3	R1	93
	R2	81
	R3	94
	R4	98
	R5	90
Imid 4	R1	78
	R2	86
	R3	82
	R4	76
	R5	91
Imid 5	R1	73
	R2	80
	R3	79
	R4	70
	R5	82
Control	R1	94
	R2	99
	R3	100
	R4	97
	R5	95

Appendix 12 Corrected mortality (%) of *C. ferrugineus* adults exposed for seven and 14 days on wheat treated with one dose rate of DEBBM (50 mg/kg), Imidacloprid (Imid: 5.0 mg/kg), two dose rates of *B. bassiana* (Bb1: 2.8 x 10<sup>8</sup>; Bb2: 2.8 x 10<sup>10</sup> conidia kg<sup>-1</sup>) and their respective combinations in seven bioassays conducted from 0 to 180 days post grain treatment (each repeat is an average of four vials in each treatment)

Treatments	Rep	Bioass	says												
	_	1 <sup>st</sup> (0 c	<b>d</b> )	2 <sup>nd</sup> (30	<b>d</b> )	3 <sup>rd</sup> (6	0 d)	4 <sup>th</sup> (90	<b>d</b> )	5 <sup>th</sup> (12	20 d)	6 <sup>th</sup> (1:	50 d)	7 <sup>th</sup> (18	80 d)
		7 d	14 d	7 d	14 d	7 d	14 d	7 d	14 d	7 d	14 d	7 d	14 d	7 d	14 d
DEBBM	R1	62.5	83.2	69.3	80.3	52.5	68.2	56.5	76.6	58.7	73.4	46.9	69.8	43.4	60.7
	R2	74.5	75.5	54.7	78.1	63.8	79.7	49.5	70.7	51.0	62.0	45.5	59.2	50.5	64.1
	R3	67.6	79.3	65.4	70.7	57.8	72.9	63.0	66.5	42.4	69.7	52.1	63.3	40.1	67.2
Imid	R1	84.2	100.0	79.7	96.3	80.5	85.9	71.5	63.0	55.6	49.5	47.4	54.7	33.2	41.3
	R2	91.3	94.3	91.1	89.1	75.0	84.4	54.9	72.3	50.5	61.4	42.5	46.2	42.5	46.4
	R3	85.6	100.0	81.4	100.0	71.4	95.2	65.5	67.0	41.8	58.0	32.4	44.1	30.2	39.1
Bb1	R1	46.2	69.0	44.3	61.2	46.5	62.0	42.5	58.9	33.2	51.6	26.6	38.5	31.1	41.3
	R2	41.3	60.9	38.0	55.7	33.7	53.1	33.7	47.3	29.7	50.5	35.5	37.5	22.5	29.7
	R3	42.0	63.3	42.0	63.3	36.5	58.0	32.5	50.0	35.3	41.0	22.3	47.3	25.5	38.0
Bb2	R1	70.1	92.9	71.4	89.9	71.5	91.1	66.5	74.0	69.9	76.6	54.7	65.1	63.8	63.8
	R2	68.9	90.1	77.6	84.9	67.9	81.8	73.4	83.7	64.1	78.8	68.5	78.8	53.5	69.3
	R3	77.1	84.0	75.0	86.7	75.5	80.3	65.0	78.2	59.2	69.7	60.1	72.9	54.7	71.4
DEBBM+Imid	R1	100.0	100.0	100.0	100.0	90.5	100.0	84.5	86.5	62.8	74.5	69.8	63.5	59.7	63.8
	R2	100.0	100.0	99.0	100.0	96.9	91.1	78.8	81.0	67.2	67.9	62.5	72.3	50.5	67.2
	R3	100.0	100.0	93.1	100.0	84.4	100.0	74.5	92.0	71.2	77.7	58.0	68.6	53.6	57.3
DEBBM+Bb1	R1	52.7	69.0	50.5	70.7	53.0	61.5	38.5	58.9	37.2	46.4	34.4	46.4	33.2	41.3
	R2	51.5	60.9	48.4	69.3	48.0	56.8	45.1	64.7	51.0	61.4	45.5	50.5	38.5	51.0
	R3	58.0	75.5	58.0	60.1	42.2	69.1	47.5	53.7	38.6	58.0	38.8	41.5	25.5	44.3
DEBBM+Bb2	R1	81.0	100.0	84.9	100.0	82.5	86.5	73.5	79.7	67.9	75.5	76.6	83.9	63.8	74.0
	R2	77.0	97.4	79.7	93.2	73.0	97.4	75.5	89.7	82.8	83.2	66.5	73.4	66.5	78.6
	R3	88.3	100.0	72.9	87.7	79.7	91.0	80.5	83.5	70.1	87.8	70.7	77.1	75.5	70.3
Imid+Bb1	R1	69.0	86.4	65.6	75.0	52.5	81.8	59.0	57.8	45.4	51.6	40.1	44.3	33.2	43.9
	R2	66.3	82.8	60.9	88.5	61.7	78.1	48.4	67.9	39.1	44.6	40.5	51.1	40.5	33.9
	R3	78.2	94.1	72.9	81.4	57.8	68.6	49.5	64.9	48.9	58.0	30.9	38.8	29.7	38.0
Imid+Bb2	R1	92.9	100.0	100.0	100.0	80.5	100.0	77.0	88.0	69.9	81.8	61.5	77.6	63.8	76.0
	R2	88.3	100.0	92.2	99.5	88.8	92.7	79.9	96.2	79.7	92.9	67.0	70.1	66.5	67.7
	R3	98.9	100.0	83.5	93.1	84.4	86.7	88.5	84.6	76.6	79.3	70.7	75.0	53.1	71.4
Control	R1	0.0	8.0	4.0	6.0	0.0	4.0	0.0	4.0	2.0	8.0	4.0	4.0	2.0	2.0
	R2	2.0	4.0	4.0	4.0	2.0	4.0	8.0	8.0	4.0	4.0	0.0	8.0	0.0	4.0
	R3	6.0	6.0	6.0	6.0	4.0	6.0	0.0	6.0	8.0	8.0	6.0	6.0	4.0	4.0

Appendix 13 Corrected mortality (%) of *R. dominica* adults exposed for seven and 14 days on wheat treated with one dose rate of DEBBM (50 mg/kg), Imidacloprid (Imid: 5.0 mg/kg), two dose rates of *B. bassiana* (Bb1: 2.8 x 10<sup>8</sup>; Bb2: 2.8 x 10<sup>10</sup> conidia kg<sup>-1</sup>) and their respective combinations in seven bioassays conducted from 0 to 180 days post grain treatment (each repeat is an average of four vials in each treatment)

Treatments	Rep	Bioas	says												
	_	1 <sup>st</sup> (0	d)	2 <sup>nd</sup> (30	) d)	3 <sup>rd</sup> (6	0 d)	4 <sup>th</sup> (90	) d)	5 <sup>th</sup> (1)	20 d)	6 <sup>th</sup> (1:	50 d)	7 <sup>th</sup> (18	30 d)
		7 d	14 d	7 d	14 d	7 d	14 d	7 d	14 d	7 d	14 d	7 d	14 d	7 d	14 d
DEBBM	R1	51.6	72.8	48.5	79.7	47.4	66.8	58.2	74.5	51.5	58.9	49.5	65.8	41.0	53.8
	R2	60.9	69.8	53.7	69.3	53.6	65.6	51.6	57.1	42.2	61.4	37.2	53.3	37.2	63.5
	R3	58.0	78.8	61.7	65.4	58.5	75.0	45.2	64.4	46.4	70.7	43.2	64.8	46.4	52.1
Imid	R1	81.4	99.5	74.5	94.3	74.5	89.7	55.6	70.3	50.0	54.7	28.1	44.4	34.6	44.0
	R2	76.6	90.1	81.4	88.0	61.5	77.6	58.0	57.1	33.9	57.1	37.2	48.9	23.0	33.9
	R3	88.3	100.0	79.1	96.3	78.2	86.7	67.0	60.1	44.3	43.1	40.1	40.3	27.6	37.8
Bb1	R1	28.2	59.2	39.8	53.1	39.3	46.7	33.2	52.6	27.6	43.2	24.5	32.1	19.7	24.5
	R2	37.0	52.6	39.9	57.8	41.1	55.2	26.1	39.7	35.9	31.0	21.4	39.7	29.1	31.8
	R3	43.1	47.3	33.2	45.2	26.1	44.1	38.8	39.4	21.9	47.3	29.7	35.2	16.1	37.8
Bb2	R1	65.4	83.2	66.8	93.2	64.3	76.5	70.9	70.3	56.6	78.6	51.6	74.0	41.0	57.1
	R2	58.9	79.7	58.0	79.7	57.3	91.1	55.9	76.6	63.0	66.3	56.6	59.8	51.5	60.9
	R3	71.3	89.7	76.5	86.7	73.9	80.3	63.3	79.3	53.1	70.7	49.5	64.8	52.6	69.1
DEBBM+Imid	R1	86.2	100.0	86.2	101.0	82.7	94.6	70.9	89.1	58.2	54.7	51.6	55.6	38.8	48.9
	R2	92.4	100.0	96.8	100.0	90.1	100.0	81.4	76.6	67.2	59.2	58.7	64.7	49.5	57.3
	R3	98.4	100.0	82.1	93.1	79.3	87.8	64.9	81.4	53.1	70.7	49.5	52.6	51.6	47.3
DEBBM+Bb1	R1	49.5	61.4	37.2	52.6	37.2	60.3	45.4	51.0	38.3	43.2	26.0	42.3	28.2	46.2
	R2	40.6	54.7	41.0	61.5	42.2	54.7	36.7	45.1	25.5	52.7	33.2	48.9	25.0	44.3
	R3	41.0	67.9	48.0	58.0	45.7	47.9	30.3	56.9	38.0	47.3	35.9	39.3	19.3	37.8
DEBBM+Bb2	R1	69.1	85.3	69.9	94.8	65.8	96.2	70.9	74.5	71.9	75.5	61.5	67.9	64.4	74.5
	R2	80.2	100.0	81.4	84.9	79.7	80.2	79.3	77.7	65.6	83.2	58.2	70.1	60.7	68.2
	R3	72.9	94.6	78.1	88.3	73.9	86.7	64.9	83.5	58.9	70.7	70.3	80.1	52.6	69.1
Imid+Bb1	R1	56.4	79.3	51.5	79.7	43.4	64.1	49.5	57.8	45.9	44.3	26.0	36.2	32.4	27.7
	R2	63.5	76.0	63.3	68.2	58.9	60.9	51.6	47.3	38.0	40.2	33.2	41.8	20.9	31.8
	R3	66.5	83.2	56.6	72.9	49.5	71.8	38.8	66.5	31.8	52.1	38.0	37.8	27.6	39.9
Imid+Bb2	R1	96.3	100.0	90.3	100.0	71.9	100.0	74.5	84.9	63.8	90.1	55.7	74.0	45.2	59.2
	R2	83.9	100.0	81.4	88.0	84.9	90.1	83.5	79.3	65.1	75.5	65.8	60.3	56.1	63.0
	R3	88.3	100.0	79.1	98.9	78.2	84.6	67.6	94.1	78.6	79.3	52.6	66.8	50.5	71.3
Control	R1	6.0	8.0	2.0	4.0	2.0	8.0	2.0	4.0	2.0	8.0	4.0	2.0	6.0	8.0
	R2	4.0	4.0	6.0	4.0	4.0	4.0	6.0	8.0	4.0	4.0	2.0	8.0	2.0	4.0
	R3	6.0	8.0	2.0	6.0	6.0	6.0	6.0	6.0	4.0	8.0	4.0	2.0	4.0	6.0

Appendix 14 Corrected mortality (%) of *T. castaneum* adults exposed for seven and 14 days on wheat treated with one dose rate of DEBBM (50 mg/kg), Imidacloprid (Imid: 5.0 mg/kg), two dose rates of *B. bassiana* (Bb1: 2.8 x 10<sup>8</sup>; Bb2: 2.8 x 10<sup>10</sup> conidia kg<sup>-1</sup>) and their respective combinations in seven bioassays conducted from 0 to 180 days post grain treatment (each repeat is an average of four vials in each treatment)

Treatments	Rep	Bioas	says												
	-	1 <sup>st</sup> (0	d)	2 <sup>nd</sup> (30	) d)	3 <sup>rd</sup> (6	<b>(0 d</b> )	4 <sup>th</sup> (90	) d)	5 <sup>th</sup> (1)	20 d)	6 <sup>th</sup> (1	50 d)	7 <sup>th</sup> (18	80 d)
		7 d	14 d	7 d	14 d	7 d	14 d	7 d	14 d	7 d	14 d	7 d	14 d	7 d	14 d
DEBBM	R1	35.3	72.8	44.3	77.6	44.3	66.1	45.4	69.7	38.3	55.7	26.1	49.5	25.5	48.9
	R2	53.6	63.5	54.7	69.3	38.0	63.0	35.3	57.1	27.6	62.0	37.2	53.3	33.2	57.3
	R3	44.1	68.6	41.5	64.4	48.4	69.1	32.4	60.1	40.1	52.7	40.1	58.0	21.4	48.4
Imid	R1	72.8	92.9	71.4	79.7	69.3	80.6	48.0	49.5	35.2	40.6	22.9	28.1	16.1	20.7
	R2	70.4	90.1	79.7	84.9	57.1	79.7	37.5	45.1	28.6	32.1	20.9	37.5	20.9	28.1
	R3	77.1	81.4	78.2	86.7	62.2	69.1	30.3	58.5	24.5	43.6	27.6	32.4	14.1	34.9
Bb1	R1	34.2	40.8	35.9	49.0	27.6	36.1	23.0	30.3	18.9	40.1	22.9	35.9	15.6	17.9
	R2	30.1	38.0	40.1	53.6	35.3	51.0	31.0	39.7	25.5	29.3	18.9	27.2	8.7	28.1
	R3	25.0	47.3	26.1	38.8	31.4	44.1	23.9	36.7	21.4	28.2	10.9	22.3	14.1	30.7
Bb2	R1	52.7	70.7	66.1	81.8	56.8	72.2	58.2	64.4	51.5	58.9	45.7	65.1	44.8	53.8
	R2	65.8	67.2	58.9	69.3	64.1	70.3	50.5	74.5	42.2	63.6	37.2	53.3	35.2	60.9
	R3	58.0	75.5	70.7	78.4	58.0	75.5	45.2	63.3	46.4	70.7	43.2	63.3	38.0	51.6
DEBBM+Imid	R1	85.9	100.0	78.6	101.0	79.7	80.6	70.9	69.7	55.6	57.8	48.4	54.7	31.8	39.7
	R2	92.6	100.0	90.1	94.3	76.6	92.7	57.1	78.8	52.6	63.6	41.8	44.0	37.2	51.0
	R3	83.5	100.0	81.4	93.1	71.8	88.3	63.3	81.4	45.3	52.7	43.2	44.1	28.6	40.1
DEBBM+Bb1	R1	26.6	55.4	35.9	52.6	27.6	45.6	25.5	47.3	18.9	42.7	25.0	30.7	14.1	22.3
	R2	38.3	52.6	42.2	61.5	35.3	55.2	33.2	39.7	28.6	33.7	21.4	39.7	20.9	29.7
	R3	41.0	46.3	31.4	58.0	38.8	44.1	27.1	36.7	21.9	30.9	15.6	32.4	10.9	39.1
DEBBM+Bb2	R1	64.7	83.2	69.3	93.2	73.4	76.0	55.6	69.7	55.6	78.6	50.5	67.2	42.2	55.4
	R2	62.8	79.7	74.5	84.9	59.8	88.0	61.4	72.8	60.9	66.3	56.6	72.3	51.5	68.2
	R3	72.9	89.9	65.4	86.7	62.2	80.3	56.9	81.4	50.5	70.7	49.5	63.3	52.6	69.8
Imid+Bb1	R1	51.1	65.8	35.9	54.7	34.4	56.1	33.2	47.3	25.0	43.2	25.0	34.9	18.2	24.5
	R2	61.7	63.0	42.2	63.0	35.3	53.1	24.5	42.4	31.8	36.4	21.4	39.7	25.5	31.8
	R3	47.3	68.6	45.7	66.5	39.9	47.9	36.7	53.7	21.9	47.3	17.7	35.1	14.1	39.1
Imid+Bb2	R1	81.0	95.1	69.3	94.3	71.4	89.4	65.8	73.9	56.6	80.2	50.5	69.8	42.2	59.2
	R2	77.0	99.0	83.9	88.0	67.9	77.6	71.2	77.7	65.1	68.5	41.3	53.3	37.2	65.1
	R3	88.3	100.0	79.3	96.3	75.0	86.7	58.5	85.6	53.6	71.8	49.5	63.3	48.4	50.5
Control	R1	8.0	8.0	4.0	4.0	4.0	10.0	2.0	6.0	2.0	8.0	6.0	4.0	4.0	8.0
	R2	2.0	4.0	4.0	4.0	8.0	4.0	8.0	8.0	4.0	4.0	2.0	8.0	2.0	4.0
	R3	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	4.0	8.0	4.0	6.0	4.0	4.0

Appendix 15 Corrected mortality (%) of *L. paeta* adults exposed for seven and 14 days on wheat treated with one dose rate of DEBBM (50 mg/kg), Imidacloprid (Imid: 5.0 mg/kg), two dose rates of *B. bassiana* (Bb1: 2.8 x 10<sup>8</sup>; Bb2: 2.8 x 10<sup>10</sup> conidia kg<sup>-1</sup>) and their respective combinations in seven bioassays conducted from 0 to 180 days post grain treatment (each repeat is an average of four vials in each treatment)

Treatments	Rep	Bioass	says												
	_	1 <sup>st</sup> (0 c	<b>d</b> )	2 <sup>nd</sup> (30	<b>d</b> )	3 <sup>rd</sup> (60	) d)	4 <sup>th</sup> (90	) d)	5 <sup>th</sup> (12	20 d)	6 <sup>th</sup> (1:	50 d)	7 <sup>th</sup> (18	80 d)
		7 d	14 d	7 d	14 d	7 d	14 d	7 d	14 d	7 d	14 d	7 d	14 d	7 d	14 d
DEBBM	R1	82.7	83.5	70.3	89.9	72.4	91.1	73.0	74.0	56.5	76.1	59.0	64.3	59.7	61.2
	R2	74.5	92.2	71.4	79.7	68.9	81.8	57.6	84.4	67.7	79.7	61.7	72.3	47.4	67.2
	R3	69.1	100.0	77.7	86.7	75.5	77.1	65.5	78.2	59.2	69.0	47.9	68.6	53.6	71.4
Imid	R1	100.0	100.0	83.9	100.0	80.1	89.1	65.8	69.3	59.5	69.1	51.0	60.2	52.0	52.7
	R2	91.3	100.0	94.3	98.4	78.1	84.4	73.4	79.7	50.5	63.0	44.4	49.5	41.3	46.4
	R3	100.0	100.0	100.0	100.0	85.9	95.2	65.0	75.0	62.0	57.1	54.8	63.3	44.8	55.2
Bb1	R1	53.6	70.7	52.6	79.3	48.5	59.9	43.9	58.9	39.0	45.2	29.5	47.4	31.1	38.8
	R2	47.4	80.2	42.2	73.4	37.8	67.2	33.7	66.1	32.3	60.9	31.1	53.3	35.7	51.0
	R3	56.4	77.1	48.4	62.2	40.6	69.1	37.0	53.7	37.5	57.1	34.6	44.1	22.4	46.9
Bb2	R1	82.1	100.0	75.5	98.4	72.4	92.7	69.9	77.6	65.0	76.1	72.5	73.0	62.2	70.7
	R2	79.1	89.1	85.9	93.2	82.1	88.5	72.3	85.9	70.3	79.7	67.9	81.0	68.4	78.6
	R3	88.3	100.0	81.9	86.2	78.1	80.3	80.5	83.5	72.3	85.9	60.1	75.0	53.1	67.2
DEBBM+Imid	R1	100.0	100.0	100.0	100.0	86.2	100.0	78.6	88.0	64.5	73.9	72.5	66.3	59.7	59.0
	R2	100.0	100.0	100.0	100.0	96.9	100.0	83.2	96.4	82.8	83.9	61.7	75.5	65.8	64.1
	R3	100.0	100.0	100.0	100.0	100.0	100.0	88.5	84.6	70.1	77.2	58.0	68.6	49.5	70.3
DEBBM+Bb1	R1	60.2	87.8	57.3	73.4	51.5	80.2	60.2	64.1	56.5	67.6	55.0	50.5	43.4	52.1
	R2	73.0	81.8	60.9	78.6	61.7	74.0	51.1	73.4	51.0	60.9	48.0	50.5	37.2	51.0
	R3	62.2	77.1	65.4	83.5	57.8	68.6	51.5	67.0	47.3	55.4	42.0	58.0	46.4	44.3
DEBBM+Bb2	R1	90.3	100.0	88.0	100.0	80.1	86.5	76.5	82.8	70.5	81.4	80.5	88.3	76.5	73.9
	R2	88.3	100.0	84.9	99.5	88.8	97.4	79.9	92.7	82.8	93.2	68.4	76.6	67.9	80.2
	R3	96.3	100.0	94.1	93.1	81.8	91.0	88.5	83.5	76.6	78.8	70.7	81.9	73.4	86.5
Imid+Bb1	R1	85.2	96.3	73.4	92.0	69.9	84.4	69.9	76.0	59.5	52.7	45.0	52.6	52.0	42.0
	R2	91.3	87.0	82.8	85.9	64.3	78.1	57.6	71.4	51.0	65.6	45.9	56.0	39.3	53.1
	R3	85.6	100.0	79.3	81.4	76.6	72.9	62.5	64.9	47.3	57.1	52.1	45.7	44.8	46.9
Imid+Bb2	R1	100.0	100.0	100.0	100.0	90.3	100.0	79.1	90.1	75.5	84.6	78.5	84.2	66.3	79.8
	R2	100.0	100.0	92.2	100.0	88.8	92.7	83.2	100.0	84.4	94.8	64.8	75.0	74.0	70.3
	R3	100.0	100.0	100.0	100.0	80.2	86.7	91.5	84.0	78.8	82.1	70.7	78.2	61.5	73.4
Control	R1	2.0	6.0	4.0	6.0	2.0	4.0	2.0	4.0	0.0	6.0	0.0	2.0	2.0	6.0
	R2	2.0	4.0	4.0	4.0	2.0	4.0	8.0	4.0	4.0	4.0	2.0	8.0	2.0	4.0
	R3	6.0	6.0	6.0	6.0	4.0	6.0	0.0	6.0	8.0	8.0	6.0	6.0	4.0	4.0

Appendix 16 Average number of *C. ferrugineus* offspring (per replicate) produced in wheat treated with one dose rate of DEBBM (50 mg/kg), Imidacloprid (Imid: 5.0 mg/kg), two dose rates of *B. bassiana* (Bb1:  $2.8 \times 10^8$ ; Bb2:  $2.8 \times 10^{10}$  conidia kg<sup>-1</sup>) and their respective combinations in seven bioassays conducted from 0 to 180 days post grain treatment (each repeat is an average of four vials in each treatment)

Treatments	Rep	Bioassays						
		1 <sup>st</sup> (0 d)	$2^{nd}$ (30 d)	3 <sup>rd</sup> (60 d)	4 <sup>th</sup> (90 d)	5 <sup>th</sup> (120 d)	6 <sup>th</sup> (150 d)	7 <sup>th</sup> (180 d)
DEBBM	R1	7.5	11.5	19.2	16.7	20.5	27.2	32.7
	R2	5.3	7.2	12.2	19.5	21.5	24.2	27.2
	R3	10.8	13.5	15.2	21.2	24.7	29.7	30.5
Imid	R1	0.0	3.5	6.5	19.5	29.5	30.2	37.5
	R2	0.0	2.0	10.5	21.5	26.7	28.5	35.7
	R3	0.0	5.2	7.2.0	23.5	23.5	34.2	41.0
Bb1	R1	11.8	16.2	19.2	27.5	31.5	40.2	43.2
	R2	15.8	11.7	22.5	35.7	37.2	43.7	49.5
	R3	9.8	18.5	26.5	32.7	35.5	37.7	45.2
Bb2	R1	6.0	7.7	7.2	10.5	20.5	22.5	23.2
	R2	5.5	5.2	9.5	13.5	18.2	24.2	24.2
	R3	2.3	10.5	13.7	16.7	13.5	18.5	19.7
DEBBM+Imid	R1	0.0	0.0	0.0	8.2	21.5	27.2	32.5
	R2	0.0	0.0	3.7	3.5	24.5	22.5	36.5
	R3	0.0	0.0	0.0	4.5	18.5	29.7	29.2
DEBBM+Bb1	R1	10.3	11.7	14.5	27.7	28.0	34.2	41.2
	R2	8.8	15.2	17.5	24.5	32.5	32.5	37.0
	R3	6.3	9.2	20.2	31.0	26.7	36.5	39.5
DEBBM+Bb2	R1	0.0	6.2	7.5	6.5.0	13.2	16.7	15.75
	R2	0.0	5.2	5.2	10.7	11.2	12.7	12.7
	R3	0.0	3.2	3.7	7.5.0	8.2	10.2	18.2
Imid+Bb1	R1	3.5	10.7	11.5	20.2	30.2	31.2	37.2
	R2	6.3	7.0	14.7	23.7	34.7	39.2	42.0
	R3	7.5	8.7	16.7	26.7	27.5	37.7	41.5
Imid+Bb2	R1	0.0	0.0	17.5	3.5.0	7.5.0	13.2	22.7
	R2	0.0	0.0	23.2	6.2	5.7	19.2	20.2
	R3	0.0	0.0	19.0	4.5	3.2	16.5	16.7
Control	R1	74.3	89.0	87.5	97.2	89.5	86.2	91.2
	R2	78.8	80.2	82.5	99.5	94.0	91.5	95.2
	R3	82.5	84.2	91.2	92.5	91.2	89.5	93.2

Bioassays Treatments Rep  $2^{nd}$  (30 d) 3<sup>rd</sup> (60 d)  $4^{\text{th}} (90 \text{ d})$ 5<sup>th</sup> (120 d) 6<sup>th</sup> (150 d) 7<sup>th</sup> (180 d)  $1^{st} (0 d)$ DEBBM R1 16.2 22.2 25.2 32.7 20.2 27.5 32.7 R2 10.2 14.7 16.5 17.2 23.5 33.5 33.5 25.2 18.2 18.5 27.5 R3 12.2 21.2 29.7 R1 3.5 13.5 30.2 Imid 0.0 28.5 33.7 46.7 2.2 12.5 26.2 39.2 R2 0.0 21.5 45.7 R3 6.7 8.2 31.2 36.2 26.7 0.0 41.2 26.2 32.7 48.5 Bb1 21.7 36.7 36.2 55.2 R1 33.2 34.2 R2 17.5 29.7 40.7 43.7 53.7 R3 28.5 38.5 50.2 19.5 23.5 38.5 49.2 19.2 Bb2 R1 7.2 11.2 18.7 20.5 22.7 32.7 5.5 18.2 21.2 7.2 R2 12.7 25.7 26.5 R3 13.2 15.2 21.2 24.7 28.2 10.5 30.2 DEBBM+Imid 9.2 R1 0.0 0.0 29.5 27.25 32.5 0.0 2.5 10.2 26.7 30.2 R2 0.0 0.0 36.5 6.7 6.7 24.5 29.7 R3 0.0 0.0 37.5 21.2 28.7 37.2 DEBBM+Bb1 R1 16.2 18.2 35.5 41.2 21.2 26.7 35.7 35.2 41.5 45.5 R2 13.2 32.7 31.2 R3 18.0 24.2 28.5 37.5 39.5 DEBBM+Bb2 R1 2.5 7.2 11.2 11.2 14.7 16.7 16.5 5.2 8.7 13.7 11.2 R2 1.5 12.7 22.2 7.5 R3 5.7 10.0 16.7 16.7 19.2 18.2 Imid+Bb1 R1 12.7 20.2 30.2 36.2 45.5 46.5 11.7 R2 6.2 15.2 22.5 24.2 34.7 42.2 45.0 R3 9.2 25.7 31.5 40.5 40.2 10.5 44.2 Imid+Bb2 R1 1.5.0 7.2 5.2 7.5.0 0.0 21.7 24.5 R2 5.5 8.2 9.2 0.0 1.2 23.5 21.5 R3 3.7 6.2 0.0 4.2 11.5 16.5 28.2 R1 84.2 94. 89.7 99.2 Control 87.5 90.5 96.7 R2 78.2 84.5 79.0 89.7 84.0 91.5 95.2 R3 82.5 90.2 93.2 86.7 87.5 85.7 93.7

Appendix 17 Average number of *R. dominica* offspring (per replicate) produced in wheat treated with one dose rate of DEBBM (50 mg/kg), Imidacloprid (Imid: 5.0 mg/kg), two dose rates of *B. bassiana* (Bb1: 2.8 x  $10^8$ ; Bb2: 2.8 x  $10^{10}$  conidia kg<sup>-1</sup>) and their respective combinations in seven bioassays conducted from 0 to 180 days post grain treatment (each repeat is an average of four vials in each treatment)

Bioassays Treatments Rep  $2^{nd}$  (30 d) 3<sup>rd</sup> (60 d)  $4^{th}$  (90 d) 5<sup>th</sup> (120 d) 6<sup>th</sup> (150 d) 7<sup>th</sup> (180 d)  $1^{st} (0 d)$ DEBBM R1 19.5 27.2 35.7 44.2 22.7 30.2 33.2 R2 21.5 21.7 23.7 31.2 33.5 42.5 14.2 28.7 25.7 40.2 R3 22.5 17.2 23.7 39.5 R1 45.2 42.2 Imid 7.5.0 4.0 15.7 31.2 46.7 12.5 41.7 R2 5.25 9.5 25.5 41.7 45.7 R3 6.2 28.7 39.7 37.2 10.7 3.5.0 49.7 28.7 32.7 49.7 Bb1 25.2 36.7 53.2 56.5 R1 37.7 42.2 R2 28.7 32.7 46.2 51.5 58.7 R3 31.7 48.7 20.2 25.7 38.5 45.5 62.2 20.2 Bb2 R1 17.5 11.7 25.2 26.7 34.7 34.7 14.2 18.2 22.2 18.2 R2 11.7 32.7 36.7 R3 12.5 14.5 18.2 21.2 24.7 29.7 39.5 DEBBM+Imid R1 0.0 6.2 13.2 29.5 30.7 43.5 0.0 2.2 4.2 10.2 R2 0.0 26.7 35.7 36.5 4.7 31.2 R3 2.5 7.75 32.7 38.5 0.0 32. 33.2 49.2 DEBBM+Bb1 R1 18.2 26.7 48.5 47.2 29.2 26.5 39.7 42.5 43.2 54.5 R2 24.5 28.5 40.5 R3 20.7 24.2 34.5 50.2 51.5 DEBBM+Bb2 R1 12.5 15.2 17.5 18.7 14.7 18.7 26.7 13.0 13.7 22.5 R2 6.2 10.2 24.5 22.2 20.5 R3 10.5 12.2 11.7 16.7 20.5 28.5 Imid+Bb1 R1 29.2 30.2 36.2 47.2 48.7 17.2 20.2 R2 15.2 23.7 36.5 41.7 45.0 14.5 45.0 R3 18.2 25.7 31.5 39.2 41.7 18.0 44.2 Imid+Bb2 R1 2.5 10.2 12.0 18.7 26.7 0.0 26.7 R2 1.5 7.2 9.5.0 0.0 11.2 23.5 30.2 R3 5.5 5.2 14.7 28.5 0.0 7.0 28.5 R1 73.5 65.7 78.7 77.7 88.2 Control 60.7 86.7 R2 75.5 71.2 74.7 82.5 81.2 84.2 68.7 68.2 72.2 83.2 R3 65.7 67.7 74.5 83.2

Appendix 18 Average number of *T. castaneum* offspring (per replicate) produced in wheat treated with one dose rate of DEBBM (50 mg/kg), Imidacloprid (Imid: 5.0 mg/kg), two dose rates of *B. bassiana* (Bb1: 2.8 x  $10^8$ ; Bb2: 2.8 x  $10^{10}$  conidia kg<sup>-1</sup>) and their respective combinations in seven bioassays conducted from 0 to 180 days post grain treatment (each repeat is an average of four vials in each treatment)

an average of four vials in each treatment) Bioassays Treatments Rep  $2^{nd}$  (30 d) 3<sup>rd</sup> (60 d) 4<sup>th</sup> (90 d) 5<sup>th</sup> (120 d) 6<sup>th</sup> (150 d) 7<sup>th</sup> (180 d)  $1^{st} (0 d)$ DEBBM R1 3.5 7.5 7.2 10.5 22.5 19.0 21.2 R2 2.0 5.5 9.5 14.7 20.2 24.2 26.0 8.5 16.5 3.7 R3 5.7 11.0 19.7 27.2 Imid R1 6.2 18.0 29.2 0.0 0.0 15.7 26.7 4.7 16.2 20.5 25.5 R2 0.0 0.0 31.5 R3 0.0 2.7 12.5 22.2 28.7 21.5 0.0 12.5 14.5 21.2 27.5 Bb1 10.2 34.2 40.5 R1 17.5 23.5 31.2 R2 8.7 10.0 32.7 35.2 22.7 26.5 R3 6.5 8.0 26.7 36.5 39.5 7.2 Bb2 R1 6.5.0 10.0 15.7 13.7 0.0 0.0 5.25 5.2 15.2 R2 0.0 10.5 12.7 20.2 R3 1.5 3.75 7.5.0 12.2 18.2 0.0 17.7 DEBBM+Imid R1 0.0 0.0 5.2 15.7 17.7 0.0 21.5 2.2 0.0 0.0 21.2 R2 0.0 11.2 26.7 0.0 3.0 18.5 20.2 R3 0.0 0.0 22.5 12.7 17.2 25.2 DEBBM+Bb1 R1 2.7 8.0 24.5 36.5 4.2 5.2 16.2 21.2 21.0 31.0 29.7 R2 20.5 R3 6.2 9.2 11.5 23.5 26.7 31.2 0.0 0.0 5.5.0 DEBBM+Bb2 R1 0.0 2.7 3.2 11.0 5.2 5.7 8.5 R2 0.0 0.0 6.7 12.2 1.7 4.2 7.2 R3 0.0 7.2 7.5.0 0.0 Imid+Bb1 R1 4.0 11.5 18.0 28.7 37.2 3.5 28.7 R2 6.2 14.7 16.2 23.5 31.5 33.2 0.0 R3 3.2 8.5 14.2 25.2 33.2 40.2 0.0 Imid+Bb2 R1 0.0 0.0 2.0 4.2 7.0 13.2 0.0 R2 3.2 4.0 5.5 0.0 0.0 11.5 10.2 R3 1.2 2.5 8.5. 0.0 0.0 0.0 14.7 R1 87.5 148.2 150.5 142.7 Control 142.2 133.2 146.2 R2 133.0 127.7 82.7 151.2 142.5 133.2 142.5 R3 136.5 131.2 90.2 153.2 146.2 140.7 140.2

Appendix 19 Average number of *L. paeta* offspring (per replicate) produced in wheat treated with one dose rate of DEBBM (50 mg/kg), Imidacloprid (Imid: 5.0 mg/kg), two dose rates of *B. bassiana* (Bb1: 2.8 x  $10^8$ ; Bb2: 2.8 x  $10^{10}$  conidia kg<sup>-1</sup>) and their respective combinations in seven bioassays conducted from 0 to 180 days post grain treatment (each repeat is an average of four vials in each treatment)

Treatments	Rep	Distri	cts														
	_	Multa	n			Faisal	abad			Chini	ot			Sahiw	al		
		С	L	R	Т	С	L	R	Т	С	L	R	Т	С	L	R	Т
DE	R1	17.2	12.2	9.2	12.5	8.7	19.7	10.5	11.2	18.2	18.25	16.7	14.2	17.2	16.2	11.2	10.2
	R2	16.5	16.7	11.2	10.7	11.5	16.5	5.2	14.5	13.2	19.25	11.2	13.2	13.2	20.0	12.2	13.2
	R3	15.5	13.2	14.2	8.2	10.2	15.7	7.2	10.2	16.7	14.25	14.2	12.5	15.5	18.2	15.5	15.5
Imid	R1	19.7	26.2	18.5	22.2	19.2	18.2	18.2	20.2	20.7	21.25	18.5	15.2	19.5	27.7	13.2	14.2
	R2	23.7	25.5	13.2	17.2	16.7	15.2	14.7	24.2	16.7	26.50	16.7	18.2	22.2	25.2	15.2	16.2
	R3	21.5	24.5	19.5	19.2	14.2	22.2	12.2	22.2	22.2	20.25	20.2	20.2	21.7	27.5	19.0	18.5
Bb	R1	19.5	20.2	12.7	14.2	15.2	20.5	11.2	19.2	15.5	20.25	14.2	14.2	21.0	27.7	14.7	11.2
	R2	22.5	14.2	15.2	12.2	17.2	22.7	16.5	15.2	13.2	19.25	17.2	10.2	22.2	23.7	20.2	19.5
	R3	17.5	17.2	8.5	10.5	19.2	18.0	15.7	18.5	18.5	15.25	11.7	15.2	24.2	25.7	17.5	15.2
DE+Imid	R1	36.2	24.7	20.2	26.2	19.2	31.2	12.0	28.7	29.7	30.50	29.7	29.7	27.7	25.0	19.2	29.7
	R2	34.5	27.7	24.0	22.2	18.0	25.5	16.2	27.2	37.2	37.25	37.2	34.2	22.7	21.2	17.0	27.5
	R3	38.7	30.7	25.7	24.7	17.7	25.7	14.7	30.2	34.2	34.25	34.2	33.2	28.7	24.0	14.0	24.7
DE+Bb	R1	31.2	31.2	11.2	22.7	17.2	29.7	19.5	29.2	21.2	30.50	17.2	21.2	32.2	27.2	20.2	21.2
	R2	28.2	27.2	16.0	18.5	18.2	33.7	16.2	24.7	25.2	26.25	22.2	17.2	30.7	24.7	18.5	18.7
	R3	26.7	30.2	14.2	16.5	21.2	27.5	14.2	27.2	20.5	27.50	24.2	20.2	34.2	23.5	24.0	16.2
Imid+Bb	R1	32.2	22.7	12.2	23.2	20.2	27.5	19.2	21.2	28.5	31.25	29.7	22.2	32.2	24.2	22.2	30.2
	R2	28.7	27.5	15.2	25.2	18.0	21.5	14.7	26.2	26.2	28.75	26.2	25.2	28.7	19.7	18.0	27.5
	R3	29.7	21.7	17.7	20.5	18.0	26.7	17.2	23.7	30.5	30.25	25.2	28.7	27.7	20.5	18.2	25.7
Control	R1	3.2	3.2	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	0.0	3.2	3.2	0.0	3.2
	R2	0.0	0.0	3.2	0.0	0.0	0.0	0.0	2.5	0.0	0.00	0.0	0.0	0.0	0.0	0.0	0.0
	R3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	2.50	1.0	1.0	1.0	1.0	0.0	1.0

Appendix 20 Average number of dead adults (C: *C. ferrugineus*; R: *R. dominica*; T: *T. castaneum* and L: *L. paeta*) recorded after the first month of storage in different districts on wheat treated with DEBBM (DE: 150 mg/kg), *B. bassiana* (Bb: 3 x 10<sup>10</sup> conidia kg<sup>-1</sup>), Imidacloprid (Imid: 5.0 mg/kg) and their respective combinations

Treatments	Rep	Distri	cts														
		Multa	n			Faisal	abad			Chini	ot			Sahiw	al		
		С	L	R	Т	С	L	R	Т	С	L	R	Т	С	L	R	Т
DE	R1	22.2	14.2	6.2	10.2	12.5	10.2	9.2	11.2	12.7	18.2	10.5	11.2	19.2	17.2	11.7	13.7
	R2	20.2	16.0	11.2	8.2	14.0	13.2	11.5	14.0	17.7	24.2	15.2	15.2	16.0	16.0	10.2	9.2
	R3	16.5	12.2	9.5	12.0	11.2	8.2	6.5	15.5	15.2	22.5	12.2	13.2	20.2	13.7	15.2	10.7
Imid	R1	25.2	18.2	19.7	17.7	26.2	26.2	25.2	25.2	27.7	26.2	29.7	27.7	30.2	25.7	25.5	26.2
	R2	22.5	16.5	17.2	21.2	24.7	23.2	22.2	23.2	26.7	34.2	26.2	26.2	24.5	26.7	21.2	23.2
	R3	26.7	22.0	21.2	20.2	22.0	28.7	20.2	22.0	31.7	30.7	23.2	23.7	28.2	25.7	28.2	20.2
Bb	R1	23.7	22.5	18.7	14.2	17.2	19.7	15.2	17.2	26.2	26.7	18.2	17.2	27.2	26.7	20.2	22.5
	R2	27.7	20.2	21.2	11.2	21.0	22.2	20.2	21.0	23.7	23.7	23.7	22.2	25.7	27.2	25.2	19.2
	R3	25.7	24.0	16.2	16.2	22.2	23.5	21.2	14.2	21.7	21.7	20.2	20.2	24.0	24.0	23.5	20.2
DE+Imid	R1	29.7	34.7	25.2	28.5	24.5	24.5	24.5	24.5	27.5	27.5	27.5	27.5	32.2	25.2	25.2	25.2
	R2	27.5	35.7	20.2	25.2	21.5	21.5	21.5	21.5	28.2	28.2	28.2	28.2	28.2	29.7	28.2	28.2
	R3	24.5	38.2	20.7	24.7	26.7	19.2	19.2	25.5	30.2	25.2	20.7	32.2	27.7	23.7	20.7	20.7
DE+Bb	R1	33.5	26.7	20.5	20.5	20.0	18.2	13.5	20.5	24.7	25.7	18.2	20.2	32.2	26.7	23.5	20.2
	R2	30.7	25.7	17.2	16.7	18.7	15.5	11.2	22.2	22.5	22.5	23.2	22.5	25.2	24.2	18.7	17.2
	R3	27.7	29.7	21.2	21.2	17.7	13.0	13.0	24.2	24.0	24.5	24.0	18.2	28.5	21.7	20.0	20.0
Imid+Bb	R1	23.7	29.7	22.0	23.7	21.2	20.0	20.0	31.2	31.0	31.0	31.0	30.0	32.0	33.5	32.0	32.0
	R2	28.7	24.7	18.7	25.5	21.7	21.7	17.7	28.2	35.2	29.0	28.5	27.2	31.2	32.7	31.2	31.2
	R3	24.7	28.7	20.0	26.75	21.0	22.0	20.2	32.2	30.5	33.0	32.2	29.5	35.0	37.5	35.0	33.0
Control	R1	1.00	1.7	1.7	1.0	1.5	1.2	0.0	3.7	3.2	3.2	3.2	3.2	3.2	3.2	0.0	3.2
	R2	2.75	2.7	3.0	2.0	1.2	1.2	1.2	1.5	1.5	2.7	2.7	2.7	2.7	2.7	2.7	2.7
	R3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Appendix 21 Average number of dead adults (C: *C. ferrugineus*; R: *R. dominica*; T: *T. castaneum* and L: *L. paeta*) recorded after the second month of storage in different districts on wheat treated with DEBBM (DE: 150 mg/kg), *B. bassiana* (Bb: 3 x 10<sup>10</sup> conidia kg<sup>-1</sup>), Imidacloprid (Imid: 5.0 mg/kg) and their respective combinations

Appendix 22 Average number of dead adults (C: *C. ferrugineus*; R: *R. dominica*; T: *T. castaneum* and L: *L. paeta*) recorded after the third month of storage in different districts on wheat treated with DEBBM (DE: 150 mg/kg), *B. bassiana* (Bb: 3 x 10<sup>10</sup> conidia kg<sup>-1</sup>), Imidacloprid (Imid: 5.0 mg/kg) and their respective combinations

Treatments	Кер	Distri	cts														
		Multa	n			Faisal	abad			Chini	ot			Sahiw	al		
		С	L	R	Т	С	L	R	Т	С	L	R	Т	С	L	R	Т
DE	R1	13.2	12.2	11.2	8.2	15.2	16.2	11.2	16.2	12.0	17.2	12.5	15.2	20.2	21.2	15.7	12.5
	R2	16.7	15.2	14.5	13.2	17.2	18.0	14.2	14.2	17.5	15.2	13.75	16.2	15.2	17.2	17.5	15.2
	R3	17.2	17.2	17.0	10.2	19.2	20.5	19.2	10.2	14.2	19.7	17.75	12.2	18.5	19.2	20.5	19.2
Imid	R1	22.7	19.5	17.2	18.2	18.7	20.5	18.7	21.2	24.2	27.2	16.5	18.2	23.2	22.2	24.5	17.2
	R2	21.2	23.7	14.2	15.2	22.2	19.2	16.7	24.2	22.7	29.5	18.25	16.2	20.0	22.5	20.0	21.5
	R3	19.0	21.5	18.2	14.2	21.2	24.7	22.2	19.2	19.5	32.5	21.25	21.2	24.5	25.2	21.2	19.0
Bb	R1	21.2	23.2	13.2	15.2	15.7	15.2	14.2	15.2	18.5	22.2	16.25	16.5	27.7	18.7	13.2	13.5
	R2	25.7	18.7	16.0	17.2	18.5	17.5	17.2	18.7	14.2	18.7	18.25	18.2	23.7	16.0	16.0	16.0
	R3	23.2	20.5	11.25	12.7	14.5	18.2	13.2	19.2	19.2	20.7	12.25	21.2	24.5	19.0	17.7	12.2
DE+Imid	R1	35.2	28.5	26.0	24.5	22.0	26.5	22.0	23.2	31.2	32.2	22.5	22.5	26.0	26.0	26.0	26.0
	R2	31.7	26.7	24.0	20.2	20.5	29.5	20.5	27.7	27.7	34.2	25.5	24.7	25.5	25.5	25.5	25.5
	R3	29.7	25.2	23.2	22.0	23.7	25.7	23.0	25.2	32.7	31.5	21.25	20.7	29.5	29.5	29.5	29.5
DE+Bb	R1	31.2	31.2	17.2	26.2	23.7	33.7	14.2	24.7	29.7	29.5	16.75	30.2	30.5	22.0	19.2	17.2
	R2	28.5	28.2	13.2	22.2	20.2	29.7	18.7	27.7	32.7	20.2	21.25	28.2	33.0	23.5	23.2	21.2
	R3	33.7	26.7	15.2	24.2	19.7	31.5	16.5	30.2	28.2	25.5	15.25	26.2	29.5	19.0	17.2	16.5
Imid+Bb	R1	32.2	27.0.	16.5	24.5	26.2	27.2	13.7	29.2	25.7	25.2	15.25	25.2	26.5	28.5	16.5	16.5
	R2	31.2	21.2	12.5	29.2	24.5	24.0	18.0	34.2	25.7	21.5	18.75	30.2	28.7	29.7	17.2	15.7
	R3	29.2	28.7	18.2	25.2	28.7	23.5	16.5	31.2	31.2	23.7	17.25	27.2	31.2	32.2	21.7	20.2
Control	R1	1.2	2.2	2.25	2.2	3.0	2.0	2.2	2.0	2.2	0.0	2.25	2.2	2.2	2.2	1.0	2.2
	R2	3.0	3.0	3.0	3.0	0.0	4.0	4.0	4.0	5.2	5.2	5.25	5.2	5.2	5.2	4.0	5.2
	R3	1.0	1.2	1.2	1.0	1.7	1.2	0.0	1.0	1.2	1.2	1.25	1.2	1.2	1.2	0.0	1.2

Treatments	Rep	Distric	rts														
	Trop	Multa	n			Faisal	abad			Chinic	ot			Sahiw	al		
		С	L	R	Т	С	L	R	Т	С	L	R	Т	С	L	R	Т
DE	R1	12.2	13.7	12.2	11.2	15.2	19.2	10.2	9.2	19.7	21.7	12.2	13.2	14.2	16.5	12.2	12.2
	R2	14.7	14.2	7.5	7.2	12.2	16.7	13.7	11.5	13.5	16.2	16.2	14.2	15.7	20.5	14.5	14.5
	R3	16.7	16.2	10.0	9.5	10.2	18.5	8.7	8.7	17.2	18.7	18.7	18.7	18.7	18.7	19.5	16.7
Imid	R1	23.2	20.2	12.2	8.2	18.2	26.7	16.2	13.2	23.7	22.2	15.2	13.2	21.7	26.7	14.7	13.2
	R2	22.2	18.2	15.7	11.7	15.7	25.5	12.2	11.2	19.7	21.5	17.2	15.7	17.7	23.2	17.7	16.2
	R3	20.7	16.2	11.2	13.2	13.2	24.5	13.7	7.5	20.7	18.7	19.2	12.7	15.7	25.7	19.7	12.7
Bb	R1	19.2	21.2	11.2	11.2	19.2	19.7	12.7	12.7	22.7	21.2	22.5	13.2	20.2	26.5	21.7	19.2
	R2	14.5	18.2	12.2	15.2	21.2	21.2	15.7	8.2	16.5	23.2	15.7	16.2	23.2	23.7	16.7	15.7
	R3	16.00	23.2	14.2	10.2	23.2	19.5	10.5	10.5	17.7	19.5	17.7	19.2	19.2	24.2	18.2	16.5
DE+Imid	R1	29.2	34.2	14.2	27.2	21.5	25.2	15.2	31.2	25.7	26.2	15.2	25.2	33.2	30.2	15.5	20.5
	R2	27.2	30.2	19.5	20.7	19.7	26.2	17.0	28.7	29.7	31.2	19.2	27.7	31.2	26.0	20.0	30.2
	R3	26.5	29.7	12.2	22.2	17.7	23.2	15.0	32.2	33.2	30.2	20.2	25.2	28.5	27.0	18.0	29.2
DE+Bb	R1	26.2	21.2	21.2	20.0	23.2	34.2	22.5	18.5	30.75	28.2	26.0	25.2	28.5	27.7	24.0	25.5
	R2	29.5	24.2	24.7	23.2	19.7	30.2	19.2	16.7	32.5	34.2	32.2	28.7	26.0	24.2	23.0	23.0
	R3	31.2	26.7	20.5	18.2	22.7	32.5	20.2	21.2	29.5	35.5	29.2	26.2	24.7	27.0	27.0	20.2
Imid+Bb	R1	29.7	31.2	15.0	22.7	17.2	26.7	15.2	21.2	23.5	25.2	18.7	30.2	29.7	32.7	20.2	17.2
	R2	27.7	29.7	14.2	21.2	22.7	22.7	14.0	28.2	20.7	26.5	16.5	22.2	27.7	34.2	18.2	18.2
	R3	24.2	33.2	11.5	25.7	18.7	24.5	12.7	23.5	20.7	20.7	20.7	24.2	30.5	36.5	16.7	16.5
Control	R1	0.0	1.2	2.2	1.0	0.0	3.25	2.7	4.7	2.2	1.5	1.2	3.2	3.2	2.0	0.0	3.2
	R2	5.2	4.2	5.2	5.2	4.5	4.5	4.7	6.2	6.2	5.5	6.2	6.2	5.2	5.2	5.2	5.2
	R3	1.5	3.7	3.7	2.2	2.5	2.5	1.5	2.2	3.7	3.7	3.7	3.7	3.7	3.7	1.7	3.7

Appendix 23 Average number of dead adults (C: *C. ferrugineus*; R: *R. dominica*; T: *T. castaneum* and L: *L. paeta*) recorded after the fourth month of storage in different districts on wheat treated with DEBBM (DE: 150 mg/kg), *B. bassiana* (Bb: 3 x 10<sup>10</sup> conidia kg<sup>-1</sup>), Imidacloprid (Imid: 5.0 mg/kg) and their respective combinations

Treatments	Rep	Distri	cts														
	-	Multa	n			Faisal	abad			Chini	ot			Sahiw	al		
		С	L	R	Т	С	L	R	Т	С	L	R	Т	С	L	R	Т
DE	R1	17.5	12.2	12.2	8.2	11.2	19.2	12.5	12.7	17.5	17.5	145	15.2	17.7	19.5	17.7	13.2
	R2	14.2	14.5	8.5	6.2	13.25	16.5	8.5	11.2	16.2	22.2	15.2	12.2	18.2	22.2	15.5	14.7
	R3	15.2	16.2	10.0	10.0	10.0	13.0	10.0	10.0	19.2	20.5	19.2	17.7	21.2	20.7	20.2	19.2
Imid	R1	24.7	20.7	8.5	7.2	19.7	27.2	15.5	10.2	16.2	17.2	15.2	15.7	21.2	20.2	20.2	13.2
	R2	20.2	15.7	11.2	10.7	16.7	22.5	18.2	14.5	17.2	21.2	17.2	10.7	17.7	17.2	19.2	17.2
	R3	21.5	14.7	13.2	12.7	12.7	24.2	12.5	11.5	20.2	19.5	14.2	12.2	18.7	22.2	14.7	12.2
Bb	R1	16.2	19.5	11.5	11.2	18.7	21.2	11.2	13.2	12.5	20.2	10.5	11.2	23.2	18.2	14.2	11.0
	R2	13.7	20.7	12.0	13.7	14.2	18.2	13.5	16.7	15.2	22.2	13.2	15.2	19.7	22.2	12.7	13.2
	R3	19.2	15.7	9.7	10.7	16.5	19.5	9.2	14.2	17.2	24.5	15.2	10.5	22.2	20.2	11.5	10.7
DE+Imid	R1	22.7	32.5	16.2	23.2	17.7	23.2	16.5	23.2	21.7	28.2	20.5	20.2	20.5	32.2	19.2	20.5
	R2	21.5	27.2	20.2	21.2	22.7	27.5	22.5	21.2	24.5	22.7	22.2	21.7	21.2	34.5	23.0	18.7
	R3	25.7	26.7	18.2	20.7	20.2	25.7	19.5	25.7	26.7	26.7	26.5	23.2	26.7	39.2	22.2	22.2
DE+Bb	R1	26.7	33.2	13.2	17.2	27.5	33.5	14.2	16.2	19.7	22.2	16.5	17.2	25.7	26.7	17.7	17.2
	R2	21.7	27.2	18.5	19.7	28.5	34.2	19.2	17.2	23.2	23.7	21.2	15.2	24.7	29.7	15.5	13.2
	R3	23.7	29.7	12.5	20.2	31.7	30.7	17.0	20.2	20.7	22.5	20.2	20.5	28.5	27.7	20.2	18.2
Imid+Bb	R1	22.7	33.2	16.7	15.2	22.2	25.7	17.0	16.2	24.7	26.5	23.2	21.2	25.7	28.2	20.2	18.2
	R2	27.7	28.5	19.5	19.2	19.5	22.2	12.2	19.7	27.2	28.2	25.5	23.2	21.2	23.2	22.5	23.2
	R3	26.5	29.7	22.2	12.7	17.2	20.5	14.5	21.2	30.2	29.5	28.2	26.2	25.7	25.2	25.2	20.2
Control	R1	6.5	2.7	4.50	5.7	5.7	3.2	3.7	5.7	3.5	3.2	4.5	4.5	4.5	4.5	5.0	4.5
	R2	0.0	3.7	7.5	0.0	0.0	4.2	6.2	9.2	7.5	6.5	7.2	7.5	7.5	7.5	0.0	7.5
	R3	4.0	4.0	4.0	4.2	4.2	4.0	2.2	4.0	4.2	4.2	2.2	5.2	4.0	3.2	4.0	4.2

Appendix 24 Average number of dead adults (C: *C. ferrugineus*; R: *R. dominica*; T: *T. castaneum* and L: *L. paeta*) recorded after the fifth month of storage in different districts on wheat treated with DEBBM (DE: 150 mg/kg), *B. bassiana* (Bb: 3 x 10<sup>10</sup> conidia kg<sup>-1</sup>), Imidacloprid (Imid: 5.0 mg/kg) and their respective combinations

Treatments	Rep	Distri	cts														
	_	Multa	n			Faisal	abad			Chini	ot			Sahiw	al		
		С	L	R	Т	С	L	R	Т	С	L	R	Т	С	L	R	Т
DE	R1	14.2	14.7	7.2	7.2	16.7	18.2	14.7	11.7	12.2	15.2	13.2	13.2	16.7	15.5	12.0	13.2
	R2	12.0	13.7	11.2	6.2	13.2	15.7	11.0	10.2	15.7	17.2	9.2	9.2	11.7	13.5	10.5	11.2
	R3	8.7	9.7	8.7	10.2	10.5	12.2	10.5	8.7	10.5	10.2	10.5	11.2	13.7	9.7	8.7	9.5
Imid	R1	21.2	17.2	7.5	6.7	13.5	21.2	10.5	11.2	24.2	26.2	22.2	14.7	19.7	20.5	12.2	16.2
	R2	16.5	14.5	8.0	9.2	16.7	24.2	15.2	8.2	22.7	21.7	17.2	15.2	14.7	15.7	14.5	11.2
	R3	18.7	10.2	11.2	10.2	10.2	19.2	12.5	9.2	18.2	22.2	20.2	18.2	15.5	18.5	17.2	13.2
Bb	R1	13.5	17.2	10.2	6.2	17.2	21.2	8.2	17.2	12.2	18.2	12.7	13.2	21.5	13.2	13.2	11.7
	R2	15.5	14.5	12.2	8.2	13.7	27.2	13.2	14.2	16.5	14.2	15.2	9.5	19.5	15.7	15.2	14.2
	R3	17.7	15.7	14.2	10.7	14.2	24.5	10.7	12.2	18.5	15.7	11.2	11.2	23.7	17.2	11.7	10.2
DE+Imid	R1	19.2	25.5	10.2	10.7	14.2	22.2	13.7	15.2	22.2	23.2	16.2	15.2	23.7	26.5	11.2	24.2
	R2	17.7	21.2	15.2	14.2	13.2	23.2	17.2	17.2	20.2	22.2	20.2	18.2	21.7	22.5	16.5	21.2
	R3	21.2	23.5	12.5	16.2	19.7	27.2	15.2	12.2	17.2	25.5	17.7	16.5	25.7	23.7	15.0	18.2
DE+Bb	R1	23.7	27.7	18.5	17.7	16.5	32.2	17.2	21.7	25.7	28.7	19.5	22.2	25.2	27.7	22.5	22.2
	R2	27.7	32.2	16.7	23.0	17.2	33.7	15.2	20.2	21.2	23.5	21.2	20.2	21.2	23.2	24.7	21.2
	R3	25.5	25.7	15.0	20.2	20.2	30.2	11.2	23.2	22.5	21.2	23.2	17.5	24.2	24.2	20.5	19.2
Imid+Bb	R1	22.7	30.5	13.2	15.7	26.7	23.2	17.2	19.2	26.2	22.5	19.2	19.2	27.7	27.7	15.5	18.2
	R2	21.2	28.2	12.7	18.5	24.2	25.2	14.5	16.2	21.7	23.7	17.2	17.5	23.7	31.7	17.5	13.7
	R3	24.5	25.5	16.5	21.2	28.2	21.2	19.7	15.2	25.7	19.2	21.2	16.2	26.7	29.2	20.5	17.5
Control	R1	5.7	4.5	5.7	5.5	3.5	1.7	5.7	5.5	3.2	3.2	5.7	5.7	4.2	4.2	3.5	5.2
	R2	0.0	2.5	8.7	2.0	2.2	6.5	2.5	9.2	8.2	7.2	3.2	8.2	8.5	8.5	1.5	9.2
	R3	6.5	6.5	6.5	6.2	6.5	3.5	7.2	9.5	6.2	5.2	7.2	6.2	6.2	6.2	5.2	6.2

Appendix 25 Average number of dead adults (C: *C. ferrugineus*; R: *R. dominica*; T: *T. castaneum* and L: *L. paeta*) recorded after the sixth month of storage in different districts on wheat treated with DEBBM (DE: 150 mg/kg), *B. bassiana* (Bb: 3 x 10<sup>10</sup> conidia kg<sup>-1</sup>), Imidacloprid (Imid: 5.0 mg/kg) and their respective combinations

Treatments	Rep	Distri	cts														
	-	Multa	n			Faisal	abad			Chini	ot			Sahiw	al		
		С	L	R	Т	С	L	R	Т	С	L	R	Т	С	L	R	Т
DE	R1	16.7	24.0	24.7	21.5	9.5	15.5	7.7	17.0	16.0	17.5	19.0	21.5	10.0	16.0	16.0	17.0
	R2	19.7	12.7	17.5	21.5	10.5	12.2	16.7	17.7	11.0	2.7	10.7	12.0	12.5	9.7	9.7	19.0
	R3	15.5	19.2	16.7	22.7	13.0	15.5	8.7	24.0	16.5	23.0	23.0	25.0	13.2	6.0	8.7	12.0
Imid	R1	8.2	8.5	9.5	16.0	2.7	3.7	3.7	17.0	1.5	3.2	4.0	7.0	2.7	0.0	9.0	8.0
	R2	6.5	10.2	10.7	17.0	3.2	4.7	5.2	11.2	11.5	1.7	11.5	12.0	2.5	7.0	4.7	3.7
	R3	6.0	12.2	8.0	12.5	11.2	4.2	13.2	11.2	4.5	6.5	6.5	6.5	8.4	3.2	0.2	6.7
Bb	R1	11.7	7.0	7.2	15.0	14.7	13.0	18.7	10.7	5.2	7.2	6.5	6.5	7.0	6.5	13.2	16.5
	R2	5.2	17.5	8.7	11.7	7.7	5.0	8.5	20.0	9.0	3.0	5.0	14.0	8.5	8.7	3.7	4.5
	R3	17.7	17	16.5	14.5	6.7	11.5	8.0	14.2	6.7	10.0	14.0	10.0	8.2	6.0	8.5	10.7
DE+Imid	R1	0.0	4.7	5.7	5.0	0.0	0.0	0.7	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	R2	0.0	0.0	0.0	5.5	0.0	0.0	1.7	0.0	0.0	0.0	0.0	3.0	0.0	0.0	0.0	0.0
	R3	0.0	0.5	3.0	4.0	0.0	0.0	0.2	5.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0
DE+Bb	R1	1.2	7.2	10.7	9.5	7.7	2.7	5.5	8.5	0.0	0.7	4.0	2.0	3.5	0.0	4.7	3.7
	R2	8.5	6.5	0.0	9.2	0.0	2.7	2.0	14.0	1.5	0.0	4.5	9.5	0.0	0.0	1.5	1.2
	R3	3.0	5.5	4.7	13.0	1.7	3.0	7.7	7.0	5.0	2.7	1.2	5.2	0.0	0.0	0.0	7.7
Imid+Bb	R1	4.2	5.0	4.2	4.0	1.7	0.0	2.7	11.2	2.7	0.0	1.5	9.0	0.0	0.0	0.0	2.0
	R2	0.0	2.2	2.7	4.5	0.0	2.0	3.2	2.5	0.0	0.0	0.0	1.0	0.0	0.0	0.0	1.0
	R3	1.7	2.0	3.2	12.7	2.0	0.0	2.7	3.5	0.0	0.0	5.0	1.5	0.0	0.0	0.0	2.5
Control	R1	35.7	41.5	37.7	39.0	26.7	26.7	26.7	36.7	34.2	34.2	34.2	35.7	35.2	33.5	25.0	32.5
	R2	34.0	41.2	30.7	34.0	30.2	28.2	30.2	37.7	36.7	31.7	31.7	32.2	40.5	40.5	28.0	39.5
	R3	35.7	39.7	35.0	35.0	32.2	24.7	24.7	44.2	30.7	34.7	29.2	29.2	42.7	38.7	22.0	36.2

Appendix 26 Average number of alive adults (C: *C. ferrugineus*; R: *R. dominica*; T: *T. castaneum* and L: *L. paeta*) recorded after the first month of storage in different districts on wheat treated with DEBBM (DE: 150 mg/kg), *B. bassiana* (Bb: 3 x 10<sup>10</sup> conidia kg<sup>-1</sup>), Imidacloprid (Imid: 5.0 mg/kg) and their respective combinations

Treatments	Rep	Distri	cts														
		Multa	n			Faisal	abad			Chini	ot			Sahiw	al		
		С	L	R	Т	С	L	R	Т	С	L	R	Т	С	L	R	Т
DE	R1	15.2	13.0	11.7	17.0	12.2	6.7	7.7	16.0	10.0	14.0	12.2	11.5	12.0	7.7	9.5	7.5
	R2	12.5	15.2	9.2	13.7	6.5	6.5	9.0	16.7	12.0	6.5	5.2	10.0	14.5	4.0	10.2	11.2
	R3	17.7	12.0	7.5	12.2	11.0	6.7	8.5	10.2	9.0	12.0	12.0	11.0	7.2	8.7	2.2	6.7
Imid	R1	7.5	6.5	10.2	14.5	3.7	3.7	4.7	4.7	3.5	5.0	1.5	3.5	3.2	4.2	4.5	3.7
	R2	7.7	7.2	7.7	9.2	3.2	4.7	5.7	13.0	9.7	2.2	10.2	10.2	3.2	1.5	4.0	2.0
	R3	3.0	4.2	6.5	9.2	11.5	4.7	13.2	11.5	2.5	3.5	11.0	10.5	4.2	3.7	4.2	12.2
Bb	R1	14.7	13.7	13.2	13.7	8.7	6.2	10.7	17.0	4.7	4.2	12.7	13.7	3.7	5.2	11.7	9.5
	R2	6.5	10.5	9.0	12.7	9.0	9.0	9.7	9.0	9.2	9.2	9.2	10.7	10.0	7.2	9.2	15.2
	R3	10.7	8.5	11.7	9.7	5.7	4.5	6.7	13.7	8.0	4.2	5.7	10.2	5.7	4.0	4.7	7.7
DE+Imid	R1	0.0	0.0	0.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	R2	0.0	0.0	1.2	2.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	R3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DE+Bb	R1	1.7	2.0	4.5	4.5	0.0	1.7	6.5	6.7	1.0	0.0	7.5	5.5	0.0	0.0	1.5	4.7
	R2	0.0	6.0	2.7	12.0	0.0	0.0	3.7	0.0	0.0	0.0	0.7	0.0	0.0	0.0	1.2	2.7
	R3	6.5	4.5	5.7	2.7	5.0	0.0	0.0	13.0	0.5	0.0	0.5	6.25	0.0	0.0	1.0	1.0
Imid+Bb	R1	1.5	0.0	0.0	8.5	0.0	0.0	0.0	3.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0
	R2	0.0	3.0	2.5	4.2	0.0	0.0	4.0	3.5	0.0	0.0	0.5	1.7	0.0	0.0	0.0	0.0
	R3	0.0	2.5	5.0	1.7	1.0	0.0	1.7	6.0	0.0	0.0	0.7	3.7	0.0	0.0	0.0	2.0
Control	R1	40.2	45.0	31.2	37.7	35.0	35.0	36.0	38.5	37.7	37.7	37.7	40.0	36.5	37	36.0	32.7
	R2	37.0	40.5	26.0	40.2	31.5	29.7	29.7	43.7	34.7	36.0	33.5	33.5	40.0	41	29.2	29.2
	R3	37.2	42.5	31.0	44.5	35.0	35.0	35.0	39.7	34.5	34.5	34.5	34.5	44.7	37.5	34.0	34.0

Appendix 27 Average number of alive adults (C: *C. ferrugineus*; R: *R. dominica*; T: *T. castaneum* and L: *L. paeta*) recorded after the second month of storage in different districts on wheat treated with DEBBM (DE: 150 mg/kg), *B. bassiana* (Bb: 3 x 10<sup>10</sup> conidia kg<sup>-1</sup>), Imidacloprid (Imid: 5.0 mg/kg) and their respective combinations

Treatments	Rep	Distri	cts														
	-	Multa	n			Faisal	abad			Chini	ot			Sahiw	al		
		С	L	R	Т	С	L	R	Т	С	L	R	Т	С	L	R	Т
DE	R1	15.0	15.0	22.7	15.0	18.7	17.7	22.7	16.0	12.0	8.2	11.5	17.0	7.5	4.75	10.2	12.7
	R2	14.0	17.2	14.2	17.2	13.2	10.7	14.5	16.5	9.2	11.5	13.0	10.5	13.5	11.5	11.2	13.5
	R3	10.0	11.2	14.0	17.5	11.7	10.5	11.7	15.5	9.5	10.5	12.5	18.0	15.2	12.25	11.5	12.2
Imid	R1	7.2	13.7	10.7	14.0	9.2	7.5	9.2	13.0	6.0	5.2	5.7	11.0	4.7	5.75	3.5	10.7
	R2	3.7	5.0	9.7	11.5	4.2	4.7	7.2	12.0	1.7	5.2	6.5	8.2	4.0	1.5	4.0	2.5
	R3	8.5	10.0	9.2	13.2	6.2	2.7	5.2	14.2	8.0	5.0	6.2	6.2	3.0	2.25	6.2	8.5
Bb	R1	12.5	11.5	6.7	16.7	7.0	4.7	5.7	19.0	3.2	8.2	4.5	13.7	2.7	1.25	6.7	6.5
	R2	5.7	13.5	8.0	12.7	5.5	6.5	6.7	11.2	8.5	4.0	4.5	14.0	8.0	5.0	5.0	5.0
	R3	15.2	11.2	13.7	15.2	10.5	6.7	11.7	17.0	7.0	5.5	14.0	7.5	10.7	6.0	7.2	12.7
DE+Imid	R1	0.0	0.7	0.0	0.7	0.0	0.0	0.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	R2	0.0	0.0	0.0	1.2	0.0	0.0	0.0	2.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0
	R3	0.7	2.2	5.5	7.7	0.5	0.0	1.2	1.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0
DE+Bb	R1	4.0	3.5	4.7	9.0	1.5	0.5	3.2	8.5	2.0	0.0	2.5	3.5	2.0	0.0	2.7	4.7
	R2	2.0	4.2	2.7	9.0	1.2	2.0	0.2	7.5	0.5	0.0	1.7	9.0	0.7	0.0	0.2	2.2
	R3	3.7	6.7	3.7	9.5	3.7	1.7	5.5	7.0	1.5	2.2	2.5	6.0	0.0	0.0	1.7	2.5
Imid+Bb	R1	0.0	0.0	0.0	8.7	0.0	0.0	2.2	5.0	0.0	0.0	0.0	3.5	0.0	0.0	0.0	0.0
	R2	1.0	4.5	5.5	2.0	4.0	0.5	0.0	7.0	2.7	0.0	0.0	2.0	0.0	0.0	1.5	3.0
	R3	6.2	2.5	2.7	10.5	2.0	0.0	3.5	7.0	0.0	0.0	4.2	7.5	1.2	0.0	0.7	2.2
Control	R1	36.2	41.0	36.7	43.5	33.7	43.2	43.0	40.2	39.2	43.5	38.7	39.7	38.0	37.7	37.2	41.0
	R2	37.2	45.5	31.0	37.7	40.2	36.2	36.2	41.2	38.0	32.5	40.0	38.5	37.5	39.5	38.0	36.7
	R3	41.5	45.2	33.7	41.7	37.5	38.0	39.2	45.2	39.0	38.5	37.5	39.0	43.5	35.25	36.0	44.5

Appendix 28 Average number of alive adults (C: *C. ferrugineus*; R: *R. dominica*; T: *T. castaneum* and L: *L. paeta*) recorded after the third month of storage in different districts on wheat treated with DEBBM (DE: 150 mg/kg), *B. bassiana* (Bb: 3 x 10<sup>10</sup> conidia kg<sup>-1</sup>), Imidacloprid (Imid: 5.0 mg/kg) and their respective combinations

Treatments	Rep	Distri	cts														
	-	Multa	n			Faisal	abad			Chini	ot			Sahiw	al		
		С	L	R	Т	С	L	R	Т	С	L	R	Т	С	L	R	Т
DE	R1	19.0	19.5	13.7	17.5	9.5	16.5	12.7	13.7	8.0	6.7	15.5	17.0	11.7	9.5	13.7	13.7
	R2	14.7	18.2	16.0	20.2	14.0	12.7	12.5	14.7	21.2	18.2	17.0	22.0	10.0	10.7	10.0	17.7
	R3	9.0	12.2	11.7	14.5	11.7	12.5	13.2	13.2	14.0	11.7	11.0	13.5	11.0	9.5	8.7	11.5
Imid	R1	11.0	10.2	14.7	15.7	3.7	7.0	5.7	8.7	3.2	4.7	11.7	13.7	5.2	0.2	12.2	13.7
	R2	8.2	9.0	6.2	9.0	8.0	5.7	7.7	8.7	13.0	3.0	5.5	7.0	1.5	10.0	1.5	3.0
	R3	12.0	15.2	14.7	14.5	10.7	13.2	10.2	16.5	4.5	7.7	6.0	12.5	8.5	5.0	4.5	11.5
Bb	R1	3.7	14.5	8.7	11.7	8.2	10.0	4.5	4.5	6.0	7.5	6.2	15.5	8.2	8.2	6.7	9.2
	R2	10.7	15.0	9.5	10.0	13.0	4.2	8.2	15.7	9.0	2.2	9.7	9.2	1.5	4.5	8.0	9.0
	R3	11.5	8.5	10.7	17.2	10.5	8.2	12.7	12.7	7.0	11.0	12.7	11.2	12.0	7.0	13.0	14.7
DE+Imid	R1	2.5	2.5	0.7	3.0	0.7	0.0	5.2	7.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0
	R2	2.5	3.0	1.7	9.0	0.0	2.2	0.0	2.5	2.0	0.0	2.0	3.5	0.0	0.0	1.2	4.0
	R3	0.0	2.7	4.0	4.2	3.0	0.0	0.0	4.0	1.0	0.0	0.0	4.5	0.0	0.0	0.0	0.0
DE+Bb	R1	6.0	4.0	10.0	12.2	5.0	3.0	5.7	9.7	0.0	2.5	4.7	5.5	1.7	1.5	6.2	4.7
	R2	6.2	6.0	1.5	2.5	5.0	2.2	5.5	8.0	1.7	0.0	2.0	5.5	0.0	0.0	3.0	3.0
	R3	0.5	4.5	6.7	12.5	0.0	2.0	2.5	1.5	8.2	2.2	8.5	11.5	2.2	0.0	0.0	6.7
Imid+Bb	R1	3.0	6.2	0.0	8.5	2.5	2.0	1.7	6.5	1.7	0.0	6.5	5.0	2.7	0.0	2.2	5.2
	R2	0.0	4.0	2.7	6.5	0.5	2.5	0.0	6.2	2.0	2.2	1.7	6.5	1.0	0.0	0.0	0.0
	R3	6.2	3.5	7.5	7.7	2.7	0.0	7.2	7.5	0.0	0.0	0.0	6.2	0.0	0.0	4.0	4.2
Control	R1	45.2	46.5	40.7	52.7	41.2	44.5	52.5	50.5	43.2	44.0	44.7	42.2	43.2	39.5	43.0	39.7
	R2	36.0	45.5	34.2	46.5	39.0	37.7	47.0	45.5	42.5	43.2	41.5	42.5	41.7	40.0	39.2	42.0
	R3	38.2	42.7	37.5	54.2	37.7	39.0	48.2	47.5	39.7	37.5	39.7	39.7	38.7	38.7	40.7	38.7

Appendix 29 Average number of alive adults (C: *C. ferrugineus*; R: *R. dominica*; T: *T. castaneum* and L: *L. paeta*) recorded after the fourth month of storage in different districts on wheat treated with DEBBM (DE: 150 mg/kg), *B. bassiana* (Bb: 3 x 10<sup>10</sup> conidia kg<sup>-1</sup>), Imidacloprid (Imid: 5.0 mg/kg) and their respective combinations

Treatments	Rep	Distri	cts														
	-	Multa	n			Faisal	abad			Chini	ot			Sahiw	al		
		С	L	R	Т	С	L	R	Т	С	L	R	Т	С	L	R	Т
DE	R1	18.7	23.0	17.0	17.7	13.5	12.5	10.5	20.5	12.7	14.7	14.5	17.0	11.5	9.7	11.5	16.0
	R2	19.5	18.0	19.7	17.2	14.5	13.0	19.2	16.5	18.5	14.5	21.5	24.5	16.5	12.5	19.2	20.0
	R3	16.2	18.2	15.0	14.2	12.0	18.0	12.0	22.7	19.0	17.2	19.0	20.5	16.0	16.5	17.0	18.0
Imid	R1	12.5	9.7	15.5	19.7	5.0	8.0	9.2	14.5	7.7	6.7	8.7	8.2	2.7	3.7	3.7	10.7
	R2	12.2	11.5	8.7	11.2	9.5	10.7	8.0	11.7	10.5	6.0	10.0	16.5	10.2	10.7	8.7	10.7
	R3	14.7	18.2	11.7	13.2	16.5	13.5	16.7	17.7	5.0	7.2	11.0	13.0	6.5	3.0	10.	13.0
Bb	R1	11.0	8.7	8.5	17.0	9.0	10.0	5.7	14.2	12.2	14.0	14.2	13.5	7.0	11.0	5.7	9.0
	R2	10.5	12.5	10.0	10.5	9.7	8.25	10.5	17.5	7.2	10.2	9.2	7.2	12.7	2.5	9.2	8.7
	R3	6.5	16.0	9.7	16.5	9.2	10.2	9.7	15.5	3.0	3.2	2.0	10.7	5.5	6.5	5.7	6.5
DE+Imid	R1	5.5	4.2	4.2	8.0	2.7	2.0	3.7	7.2	6.5	0.0	7.7	8.0	0.0	0.0	1.2	0.0
	R2	3.2	6.0	2.7	7.5	3.5	1.0	4.0	8.5	0.0	1.7	2.2	2.7	1.7	2.0	0.0	4.2
	R3	0.7	4.5	6.5	7.0	2.0	4.0	3.0	3.0	0.0	0.0	0.2	3.5	0.0	0.0	4.5	4.5
DE+Bb	R1	4.7	6.5	4.7	14.0	4.7	2.7	7.0	5.0	8.0	4.0	9.7	9.0	2.7	2.0	0.2	0.7
	R2	4.2	9.5	3.5	6.5	6.0	4.2	5.5	7.5	0.0	0.0	1.5	7.5	2.0	1.5	7.2	9.5
	R3	3.5	5.0	4.5	7.0	4.5	3.7	0.0	7.5	0.7	0.0	0.5	0.2	1.7	0.0	0.5	2.5
Imid+Bb	R1	4.0	4.2	12.0	9.0	3.0	4.2	0.0	11.5	5.5	3.7	7.0	9.0	4.5	2.0	10.0	12.0
	R2	2.0	5.2	3.7	3.0	1.0	3.5	4.2	6.5	1.0	0.0	2.7	5.0	2.0	0.0	0.7	0.0
	R3	6.7	7.0	4.5	8.0	5.5	4.0	8.2	1.5	4.0	4.7	6.0	8.0	1.0	1.5	1.5	6.5
Control	R1	36.5	38.5	46.5	48.0	34.0	44.5	51.5	49.5	47.7	48.0	48.7	49.7	42.7	44.7	46.0	46.5
	R2	46.2	43.5	41.0	58.2	45.2	40.5	54.5	51.5	41.2	42.2	41.5	41.2	39.0	38.7	48.5	41.0
	R3	37.2	42.5	41.2	51.5	40.0	38.7	50.5	48.7	40.5	41.0	43.5	42.0	40.7	41.0	39.7	39.2

Appendix 30 Average number of alive adults (C: *C. ferrugineus*; R: *R. dominica*; T: *T. castaneum* and L: *L. paeta*) recorded after the fifth month of storage in different districts on wheat treated with DEBBM (DE: 150 mg/kg), *B. bassiana* (Bb: 3 x 10<sup>10</sup> conidia kg<sup>-1</sup>), Imidacloprid (Imid: 5.0 mg/kg) and their respective combinations

Treatments	Rep	Distri	cts														
	-	Multa	n			Faisal	abad			Chini	ot			Sahiw	al		
		С	L	R	Т	С	L	R	Т	С	L	R	Т	С	L	R	Т
DE	R1	15.0	21.5	21.5	22.0	14.5	16.5	15.0	18.0	17.2	13.0	15.0	15.0	12.0	8.7	6.7	15.0
	R2	16.2	16.5	16.2	21.5	15.5	13.7	15.2	16.0	11.0	9.5	16.5	16.5	11.5	9.7	12.7	12.0
	R3	16.2	24.7	15.2	14.7	25.7	18.7	25.7	27.5	15.2	15.2	13.7	19.0	15.7	9.7	10.7	15.7
Imid	R1	13.0	13.2	16.5	17.2	10.5	14.0	13.5	12.7	10.0	8.0	12.0	19.5	7.7	7.2	15.2	11.2
	R2	14.2	12.0	12.7	18.0	5.7	7.0	7.2	14.2	14.7	15.7	20.2	22.2	8.0	7.0	8.2	20.0
	R3	16.5	13.2	9.2	14.7	16.7	18.5	14.5	17.7	14.2	10.2	12.2	14.2	9.2	6.2	7.5	11.5
Bb	R1	15.2	9.0	12.7	13.7	8.5	13.5	12.7	15.0	9.5	3.5	9.0	8.5	9.7	8.5	8.5	10.0
	R2	10.2	18.7	13.0	12.5	10.0	9.2	2.7	14.5	8.0	10.2	9.2	15.0	15	8.7	9.2	10.2
	R3	7.0	16.0	13.2	8.7	12.5	15.2	12.2	22.0	6.7	7.5	8.2	8.2	5.0	1.0	6.5	8.0
DE+Imid	R1	10.0	8.7	10.2	9.7	6.2	5.5	6.7	5.2	4.5	3.0	9.0	10.0	6.5	2.2	8.7	6.0
	R2	5.2	9.2	3.7	8.7	5.7	5.2	1.7	7.7	4.2	3.2	4.2	6.2	7.7	3.0	2.5	8.0
	R3	3.5	7.7	3.5	8.5	6.0	4.0	10.5	13.5	5.2	5.0	4.0	5.2	0.0	3.2	0.0	7.5
DE+Bb	R1	5.0	12.0	3.5	11.0	8.2	3.7	4.7	10.5	3.0	0.0	9.2	6.5	3.5	1.0	6.2	6.5
	R2	4.7	3.0	9.0	9.2	4.5	4.7	0.7	6.5	5.2	3.0	5.2	6.2	4.5	2.5	1.0	4.5
	R3	7.7	9.0	5.5	9.5	0.2	4.2	9.2	8.0	1.7	3.0	1.0	6.7	0.0	0.0	3.7	5.0
Imid+Bb	R1	6.0	8.2	7.7	13.0	5.0	6.5	3.7	5.5	6.5	0.0	3.2	3.2	4.5	4.5	7.0	4.2
	R2	12.0	7.5	4.2	12.7	12.2	1.2	11.7	10.0	7.7	3.5	10.0	9.7	3.7	2.0	0.0	3.7
	R3	2.2	12.2	5.5	5.5	5.5	4.2	3.5	8.0	2.0	5.2	3.2	8.2	4.0	1.5	4.75	7.7
Control	R1	37.7	47.7	47.7	50.7	37.7	46.0	57.7	58.0	43.7	43.2	40.7	40.7	41.0	49.0	41.7	40.0
	R2	48.5	44.2	42.2	58.2	43.2	39.2	58.5	56.7	44.5	45.5	49.5	44.5	44.5	44.5	51.2	43.5
	R3	38.7	42.2	48.7	52.0	36.2	40.2	57.7	55.7	47.0	43.5	48.0	49.0	40.0	40.0	41.0	40.0

Appendix 31 Average number of alive adults (C: *C. ferrugineus*; R: *R. dominica*; T: *T. castaneum* and L: *L. paeta*) recorded after the sixth month of storage in different districts on wheat treated with DEBBM (DE: 150 mg/kg), *B. bassiana* (Bb: 3 x 10<sup>10</sup> conidia kg<sup>-1</sup>), Imidacloprid (Imid: 5.0 mg/kg) and their respective combinations

Appendix 32 Damage to wheat grains treated with DEBBM (DE: 150 mg/kg), *B. bassiana* (Bb: 3 x 10<sup>10</sup> conidia kg<sup>-1</sup>), Imidacloprid (Imid: 5.0 mg/kg) and their respective combinations by *C. ferrugineus* during 180 days storage period in different districts

Treatments	Rep	Districts	8										
		Multan			Faisalal	oad		Chiniot			Sahiwal	l	
		<b>30 d</b>	120 d	180 d	30 d	120 d	180 d	30 d	120 d	180 d	30 d	120 d	180 d
DE	R1	316.2	398.2	527.2	175.5	302.5	345.7	252.2	257.2	302.5	134.2	252.2	223.7
	R2	289.7	342.2	456.2	231.2	342.2	407.2	197.2	310.2	352.2	94.2	197.2	315.2
	R3	240.2	278.2	387.2	211.2	216.2	315.2	118.2	182.7	248.7	153.7	157.2	187.2
Imid	R1	174.2	192.7	342.2	168.7	175.2	379.7	94.2	146.2	257.2	42.2	78.7	195.2
	R2	225.5	302.2	365.2	224.2	221.2	314.2	124.2	214.7	310.2	83.2	128.2	271.2
	R3	135.7	222.25	425.2	68.2	100.7	269.7	83.2	123.2	221.2	74.2	95.2	127.2
Bb	R1	215.2	228.	292.2	146.2	175.2	326.7	134.2	154.2	210.2	81.2	186.5	175.2
	R2	255.2	376.2	415.2	245.2	263.2	314.2	94.2	225.7	278.2	157.5	115.7	251.2
	R3	225.2	216.2	258.2	176.5	214.7	248.2	153.7	176.2	192.5	67.7	136.2	127.2
DE+Imid	R1	84.2	112.2	285.	25.2	74.2	226.2	0.0	28.7	210.2	0.0	0.0	84.7
	R2	138.2	168.7	315.2	67.2	94.2	273.2	0.0	0.0	253.2	0.0	0.0	215.2
	R3	97.2	142.2	242.2	52.2	101.5	218.2	0.0	57.75	147.2	0.0	0.0	128.7
DE+Bb	R1	108.2	189.2	168.2	58.7	94.2	134.2	81.2	132.5	98.7	25.2	72.2	76.2
	R2	145.2	268.2	223.2	127.2	132.2	183.2	104.5	87.5	175.2	86.5	83.2	147.2
	R3	207.2	135.7	289.	163.2	203.2	248.7	68.2	93.5	139.7	41.7	51.5	91.2
Imid+Bb	R1	93.2	138.2	257.2	58.7	94.2	217.2	14.2	64.2	123.7	0.0	0.0	89.7
	R2	143.2	238.2	315.2	111.2	148.2	263.2	68.2	101.2	210.5	0.0	25.7	149.2
	R3	128.2	158.2	204.2	83.2	114.2	192.2	58.2	87.7	145.2	0.0	56.5	123.2
Control	R1	497.2	524.2	598.	465.2	527.2	578.7	497.2	525.2	612.2	365.5	493.2	587.7
	R2	547.2	623.2	648.	548.2	578.2	636.2	386.2	593.2	675.2	328.2	525.5	614.2
	R3	453.2	479.7	668.5	425.7	624.2	619.2	415.2	445.2	625.7	418.2	437.2	524.2

Treatments	Rep	Districts											
		Multan			Faisalal	bad		Chiniot			Sahiwa	l	
		<b>30 d</b>	120 d	180 d	30 d	120 d	180 d	30 d	120 d	180 d	30 d	120 d	180 d
DE	R1	324.2	376.2	546.2	310.5	376.7	475.2	251.2	268.5	420.2	258.5	254.2	365.7
	R2	382.2	397.2	488.7	257.2	342.2	455.2	232.2	359.2	287.2	86.2	297.7	276.7
	R3	298.2	419.2	521.2	221.2	289.5	387.2	193.2	279.5	388.7	215.2	195.7	214.2
Imid	R1	215.2	287.7	447.2	175.5	291.2	433.7	243.2	175.5	268.5	134.5	134.2	275.7
	R2	255.2	396.2	419.2	231.2	324.2	387.5	197.2	286.5	359.5	94.5	110.2	256.2
	R3	225.2	225.25	431.2	211.2	243.2	338.7	118.2	198.5	279.5	153.7	176.5	197.7
Bb	R1	327.2	398.2	408.7	310.	312.2	365.2	252.2	251.2	241.2	186.5	193.5	235.2
	R2	312.2	342.2	342.2	257.2	345.2	345.2	210.2	347.2	322.2	86.5	225.2	256.5
	R3	276.2	278.2	376.2	186.2	258.2	288.2	159.2	196.7	264.2	215.2	144.7	172.5
DE+Imid	R1	110.2	138.2	366.2	58.7	168.7	351.2	37.2	69.2	234.2	0.0	74.2	174.2
	R2	173.2	238.25	351.2	116.2	196.2	345.2	85.2	136.5	297.7	0.0	34.5	221.2
	R3	137.7	158.2	324.2	134.2	68.2	264.5	58.2	98.5	178.2	0.0	54.5	136.2
DE+Bb	R1	185.2	215.2	215.2	156.2	175.5	265.5	134.2	146.2	145.5	128.2	138.2	131.5
	R2	245.2	302.2	369.2	202.2	231.2	186.5	94.2	214.7	226.5	76.5	76.5	92.2
	R3	192.2	254.2	263.2	135.2	211.2	229.5	153.7	93.5	165.2	87.5	87.2	153.7
Imid+Bb	R1	108.2	199.2	236.7	168.7	158.2	297.2	81.2	64.2	210.2	97.2	118.2	157.5
	R2	145.2	268.2	387.7	196.2	211.7	234.5	111.2	129.5	278.2	46.2	46.5	213.2
	R3	207.2	176.2	297.2	68.2	175.2	248.5	68.2	172.5	147.5	65.7	79.2	136.5
Control	R1	546.2	579.2	714.2	476.2	521.2	628.5	475.2	528.5	714.5	456.5	4362.	552.5
	R2	488.7	625.2	818.2	447.2	589.2	778.2	442.5	486.5	632.2	403.7	517.5	624.5
	R3	521.2	658.2	735.2	428.2	489.2	679.7	397.5	458.7	668.5	365.7	378.2	682.5

Appendix 33 Damage to wheat grains treated with DEBBM (DE: 150 mg/kg), *B. bassiana* (Bb:  $3 \times 10^{10}$  conidia kg<sup>-1</sup>), Imidacloprid (Imid: 5.0 mg/kg) and their respective combinations by *R. dominica* during 180 days storage period in different districts

Appendix 34	Damage to wheat grains treated with DEBBM (DE: 150 mg/kg), <i>B. bassiana</i> (Bb: 3 x 10 <sup>10</sup> conidia kg <sup>-1</sup> ), Imidacloprid
	(Imid: 5.0 mg/kg) and their respective combinations by T. castaneum during 180 days storage period in different
	districts

Treatments	Rep	Distric	ts											
		Multar	Multan			Faisalabad			Chiniot			Sahiwal		
		<b>30 d</b>	120 d	180 d	30 d	120 d	180 d	30 d	120 d	180 d	30 d	120 d	180 d	
DE	R1	456.7	473.2	546.2	294.7	518.2	568.5	352.2	498.2	521.25	228.2	342.2	445.7	
	R2	426.2	521.7	642.2	397.2	453.2	497.7	325.2	325.7	486.25	310.2	413.2	425.2	
	R3	392.	493.7	668.2	375.2	421.2	598.7	297.7	386.5	551.25	275.2	274.2	497.7	
Imid	R1	362.2	342.2	524.2	275.2	324.2	474.2	310.	302.5	432.25	169.7	284.7	378.7	
	R2	269.5	443.2	598.2	387.7	412.2	534.	257.2	342.2	416.25	246.7	334.7	425.2	
	R3	352.7	418.5	488.5	235.7	354.2	498.2	186.2	254.2	518.75	203.5	236.5	459.2	
Bb	R1	396.7	419.2	502.2	295.7	465.2	437.2	326.2	398.7	487.25	276.2	327.2	415.2	
	R2	364.5	432.7	581.2	413.2	424.5	513.5	297.7	342.2	325.75	196.2	362.2	365.2	
	R3	386.7	454.2	451.2	268.2	402.5	498.2	225.2	316.2	453.25	258.5	274.2	319.7	
DE+Imid	R1	261.7	254.2	479.2	197.2	245.2	389.2	168.7	175.2	498.25	105.2	127.2	376.5	
	R2	252.7	362.2	521.2	249.2	293.2	452.5	224.2	256.2	325.75	129.2	164.2	327.2	
	R3	217.5	294.5	417.2	120.2	214.2	412.5	68.2	123.2	372.25	92.5	146.2	319.7	
DE+Bb	R1	294.2	427.2	394.5	241.5	269.7	294.7	264.2	302.5	287.25	158.2	253.2	268.2	
	R2	328.7	342.7	462.7	323.5	338.5	415.2	217.7	342.2	407.25	224.2	225.2	224.2	
	R3	276.5	327.2	374.2	235.7	297.2	375.2	186.2	211.2	248.25	182.2	192.2	215.2	
Imid+Bb	R1	236.2	342.2	451.2	196.2	241.2	374.2	227.2	276.2	334.25	147.2	233.7	301.2	
	R2	324.7	319.2	521.2	262.5	258.2	446.2	202.5	257.2	388.75	193.5	205.2	316.7	
	R3	287.2	299.5	417.2	216.2	301.2	403.7	167.2	186.2	312.25	159.5	169.2	224.2	
Control	R1	587.2	696.2	838.2	537.2	704.2	786.2	469.75	636.2	803.25	458.2	493.2	614.2	
	R2	615.2	643.5	802.7	486.5	557.2	738.7	453.2	587.5	765.25	504.5	535.2	789.5	
	R3	643.7	723.2	925.2	572.5	659.5	816.2	506.2	569.2	678.75	427.2	549.2	736.5	

Treatments	Rep	Districts												
		Multan	Multan			Faisalabad			Chiniot			Sahiwal		
		30 d	120 d	180 d	30 d	120 d	180 d	30 d	120 d	180 d	30 d	120 d	180 d	
DE	R1	185.2	268.2	408.7	146.2	175.2	326.7	134.2	154.2	279.2	59.2	78.7	195.2	
	R2	245.2	379.2	351.2	245.2	297.2	314.2	94.2	212.2	297.7	84.2	194.7	271.2	
	R3	192.2	225.2	324.2	176.5	214.7	248.2	153.7	185.2	213.5	136.2	95.2	127.2	
Imid	R1	110.2	189.2	325.2	54.7	74.2	185.2	25.2	69.2	219.7	25.5	38.7	84.7	
	R2	158.7	268.2	415.2	128.2	196.7	372.8	91.2	136.2	297.7	61.2	65.2	215.2	
	R3	137.7	135.7	258.2	87.7	126.7	248.2	68.2	98.5	213.5	0.0	82.5	128.7	
Bb	R1	195.2	236.2	236.7	87.2	175.2	234.5	81.2	146.2	198.2	46.2	72.2	126.7	
	R2	225.5	259.2	387.7	127.2	256.2	273.25	124.2	196.2	135.2	84.2	153.2	215.2	
	R3	135.7	178.2	263.2	163.2	100.7	223.2	89.2	93.5	215.2	56.2	51.5	148.2	
DE+Imid	R1	42.2	54.2	257.2	0.0	26.2	218.2	0.0	0.0	115.2	0.0	0.0	75.5	
	R2	83.2	114.2	315.2	0.0	73.5	235.2	0.0	0.0	226.2	0.0	0.0	147.5	
	R3	74.2	86.2	204.2	0.0	47.2	150.2	0.0	0.0	165.2	0.0	0.0	82.2	
DE+Bb	R1	96.2	189.2	147.7	47.2	121.5	86.7	12.5	36.2	76.2	0.0	0.0	14.2	
	R2	152.2	159.2	263.2	91.2	107.2	189.7	51.2	96.2	125.2	0.0	0.0	78.7	
	R3	124.2	135.7	186.2	67.2	87.5	158.2	19.5	65.2	48.5	0.0	0.0	39.5	
Imid+Bb	R1	72.2	135.2	168.2	24.2	26.2	134.2	0.0	14.2	88.7	0.0	0.0	36.5	
	R2	103.2	176.2	223.2	17.2	97.2	183.2	0.0	72.2	175.2	0.0	0.0	147.5	
	R3	67.7	116.2	289.	52.5	58.7	248.7	0.0	47.2	119.7	0.0	0.0	82.2	
Control	R1	493.2	524.2	527.2	342.2	498.2	510.2	310.2	358.2	420.2	304.2	356.2	476.2	
	R2	386.2	426.2	487.7	296.2	325.7	456.2	294.2	321.2	534.2	254.2	395.2	348.2	
	R3	415.2	449.2	654.2	378.2	421.2	543.2	338.2	407.2	357.5	324.2	297.2	384.5	

Appendix 35 Damage to wheat grains treated with DEBBM (DE: 150 mg/kg), *B. bassiana* (Bb: 3 x 10<sup>10</sup> conidia kg<sup>-1</sup>), Imidacloprid (Imid: 5.0 mg/kg) and their respective combinations by *L. paeta* during 180 days storage period in different districts

## **Curriculum Vitae**

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