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IMPACT OF BIOPESTICIDE *BEAUVERIA BASSIANA* (BALSAMO) VUILLEMIN ON BEHAVIOR OF TERMITE *ODONTOTERMES OBESUS* (R.) CASTE

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ABSTRACT

Termites are injurious to crops plantation, fruits, forest trees, grasses etc. Since termites mostly live in hidden quarters they are often difficult to be detected and approached. Due to long persistence of residual chemical insecticides in soil and their possible entry in food chain, recently there is more emphasis on characterization of biocontrol agents for control of termites that are safer and environment friendly. The behavioral response of an insect to a fungal pathogen will have a direct effect on the efficacy of the fungus as a biological control agent. In our study, we have applied various doses of entomopathogenic fungus *Beauveria bassiana* as an ecofriendly approach on various caste of termite *Odontotermes obesus* to see the behavioral changes. After undergoing fungal application, the termite castes depicted many behavioral changes. The observed abnormal behaviors were reduction in speed, disorientation, lethargic behavior, distorted behavior and moribundity. After exposure to different concentrations of *Beauveria bassiana* (Balsamo), the primary reproductives (both male and female) of termite also revealed the abnormal behavior.

KEY WORDS : *Beauveria bassiana*, Lethargic behavior, *Odontotermes obesus*, Moribundity.

INTRODUCTION

Termites have been reported worldwide as one of the most important group of insect pests that cause significant and serious damages to crops, structures and buildings (Su and Scheffrahn, 2000). Around US$ 22 billion are spent annually for termite control and repairing the damages (Su, 2003). In Japan, several hundred million dollars were spent annually for the prevention and control of termites (Kubota *et al.,* 2006). Surprisingly, that is much more higher than the cost of repairing the total damages caused by the natural disasters such as fires, earthquakes, tornadoes (Hedges, 1992). In Uganda, termite cause great loss to crops and trees ranging from 50 to 100 percent (Sekamatte, 2001). Termites are responsible for plant mortality about 5-50 percent and pod damage about 46 percent in groundnut (Rajagopal, 2002). The most damage to sugarcane is done by genera Amitermes, Pseudacanthotermes, Macrotermes, Odontotermes, Microtermes and Ancistrotermes. Yield losses of 18 percent were reported in Sudan, and 5-10percent in central Africa. In Nigeria plant germination failure of up to 28 percent has been reported. The most common damage to sugarcane is the destruction of the setts (planting materials).

An estimated total of four-thousand termite species, 2,800 species have been described so far. Out of all described species of termites, only a few hundred cause economic damage. Amongst them, around 50 species are considered to be serious pests of agriculture and other commodities (Pearce, 1997). It is considered, high compared to other social insects (Sands, 1977b). Termites are injurious to crops plantation, fruits, forest trees, grasses etc. The vegetation is generally attacked when it is not in a vigorous state of growth and its vitality is low either due to internal growth factors or due to environmental factors such as drought or poor soil fertility. In all cases it is the worker caste which does the maximum damage. Since termites mostly live in hidden quarters they are often difficult to be detected and approached. But once reached by any insecticide, they surrender easily to the poison.

The major principal ecological and physiological factors which influence the abundance, distribution and dispersal of termites are rainfall, vegetation, soil type, humidity and temperature. Amongst this soil type is the important factor in influencing abundance and distribution of termites. Since termites are soil-dwellers and carry various activities in hidden-quarters without being detected. In India, termites pose a serious threat to agricultural, horticultural crops, forestry trees, and wooden structures.

Termite control in agricultural crops is usually done by adopting suitable cultural practices or by preventing termite infestations through application of physical and chemical insecticidal barriers. Chemical insecticides mainly Malathion, Phorate, Chloropyriphos, Endosulfan etc. are used to control termites. Different pathogens including bacteria, fungi, protozoa, viruses, and nematodes have been found to kill the termites under laboratory as well as under field conditions. These pathogens could be applied as bio-termiticides.

One possible option for alternative termite control is biological control, using entomopathogenic fungi (Milner and Staples, 1995). Entomopathogenic fungi enter their hosts by direct penetration in the cuticle that functions as a barrier against most microbial attack. Consequently, fungal entomopathogens have a particularly high potential for biological control for sap-sucking insects that are difficult to combat with synthetic insecticides (Kang *et al*., 1999). In the case of social insects like termites, a small amount of inoculum might spread throughout a termite nest before being detected by the insects, resulting in an epizootic (Jones *et al*., 1996). Social activities like grooming and food sharing could additionally help to disperse the inoculum of the fungi in the complete colonies (Kramm *et al*., 1982).

In our study,we have applied various doses of entomopathogenic fungus *Beauveria bassiana* on various caste of termite *Odontotermes obesus* to see the behavioral changes in termites.

MATERIALS AND METHODS

Termite collection

Underground nests of *Odontotermes obesus* (R.) located at crop fields were opened up, termites consisting of workers, soldiers and nymphs together with nest material were collected in a tray and brought to the laboratory. The termite along with the nesting mound soil were put in plastic containers and were observed in the laboratory for 1-2 months. The rearing container was covered with lids. The size of the containers ranged from 0.03 - 0.07 m^2. Water was added to the containers every 3-7 days to maintain moisture. These termites were kept in petri dishes containing wet filter paper for 24 h before inoculation.

Culture of *Beauveria bassiana* (Balsamo) Vuillemin

Beauveria bassiana (Balsamo) was collected from Maharana Pratap University of Agriculture and Technology, Udaipur Rajasthan and subcultured on Sabouraud Dextrose Agar (SDA) media, in the laboratory.

Inoculation

A spore suspension was prepared by harvesting conidia from the SDA plates just before application. Conidial count was done using Neubauer Improved Haemocytometer under a microscope for conidia/ ml of dilution and recorded separately. Thirty termites of each caste were placed on different petri plates that contained 1 ml conidial suspension of *B. bassiana* at different concentration of 4.5 $x10^8$, 4.5 $x10^7$, 4.5 $x10^6$ and 4.5 $x10^5$ conidia/ml on Whatman's filter paper disc (9cm X 1mm) and 1 ml of control suspension solution which contained sterile water. The termites were exposed to conidial suspension for 24 hours. The treated termites caste were then transferred in sterile glass Petri dishes (10 cm in diameter), lined with sterile moistened Whatman No. 1 filter paper. A wood piece was placed in the petri plate for food. Both experimental and control petri dishes were kept at 28°C ± 2°C and relative humidity 75± 5 percent in BOD in the dark.

RESULTS

After undergoing fungal application, the termite castes depicted many behavioral changes. The observed abnormal behaviors were classified into three major types i.e., disorientation, lethargic behavior, and moribundity. Progression to more advanced behavioral stages was however steady, irrespective of the particular symptom exhibited in the previous stages.

Disoriented state: The first sign of behavioral changes in which termites caste started altered directions frequently i.e. disorientation, sluggish movement and shaking from side to side when walking.

Almost 40-50 percent of termite workers, soldiers and nymphs became disoriented after 24 hours of exposure to *Beauveria bassiana* (Balsamo) Vuillemin at highest concentration (4.5X 10^8 conidia/ml) when compared with control. No abnormal behavior i.e., disorientation was observed amongst termite caste in the control experiment.

In all treatments, introduction of termites into the treated arena resulted in an early burst of activities (mostly exploratory). Termites exposed to untreated filter paper (control) did not exhibit any unusual symptoms or behavior.

After the introduction into the test arenas, termites of all caste in both *Beauveria bassiana* (Balsamo) Vuillemin treated and untreated arenas walked around the arena and kept moving around; termites irregularly bit on the filter paper and after sometimes termites of all caste treated with different concentration of *Beauveria bassiana* (Balsamo) Vuillemin became uncoordinated in their movements. *B. bassiana* treatments were also characterized by assemblage of treated castes in particular area, a behavior that was not observed among controls. (Plate 1; Fig.-A&C) Loss of appetite was also an early behavioral symptom. Treated insects turn upside down and start kicking (Plate 1; Fig. D)

Uncoordinated Stage: In some caste after exposure to fungal conidia, the termites circled around the same spot, walked in reverse, fell on their backs and kicked their feet. Whole-body movements accompanied kicking behavior.

Moribundity: After 48 hours of treatment, the termite workers were unable to move to a small distance showing lethargic condition. This stage was followed by one characterized by very lethargic movements that persisted until moribundity. At the advanced stage of lethargy, termites walked in slow-motion with raised fore-part (head and thorax). There was a progressive reduction in walking speed until termites stopped moving (became moribund). Lethargic up and down and side to side movements of the head and thorax were also observed.

The antennae of treated termite workers were distorted (i.e. folded backwards). During the latter stages of intoxication, the tip of the antennae became distorted or bent. During the early stages of ataxia in solider and alates, the antennae were completely distorted. The antennae sometimes became folded beneath the mouthparts and termites appeared to be engaged in autogrooming. At 36 hrs of post inoculation, some of the infected termite castes were already in the moribund stage. The cadavers were removed from test areana (Plate 1; Fig. B).

They either remained on their feet or no movement. Some of the workers fell on their back until death. After 72 hours of treatment the kicking was weaker in soldiers and workers and not accompanied with the strong whole-body movements as observed in earlier time period (48 hrs.) associated with the uncoordinated stages.

Treated castes were clustered together, groomed, and engaged in more social interactions than control ones. The workers, soldiers and nymph released the stomodeal fluid (liquid excrement) visible on the white filter paper as yellow sticky spots.

Behavioral changes in primary reproductives (Plate-1)

After exposure to different concentrations of *Beauveria bassiana* (Balsamo), the primary reproductives (both male and female) of termite revealed abnormal behavior. The dealates in treated arena moved uncoordinatedly and dealates did not show normal tandem behavior which present in control dealates (Plate 1; Fig. E&F). After the exposure time when treated and untreated dealates were transferred to new petri plates containing the mixture of soil and sawdust, the control group showed normal tandem behavior and within an hour, starts to make royal chamber whereas the treated dealates of *Beauveria bassiana* (Balsamo) Vuillemin when released into soil, first dug the soil for royal chambers but after sometimes most of dealates were ultimately emerged on the soil surface and moved in a disoriented manner till death. There was no mating and courtship in treated ones.

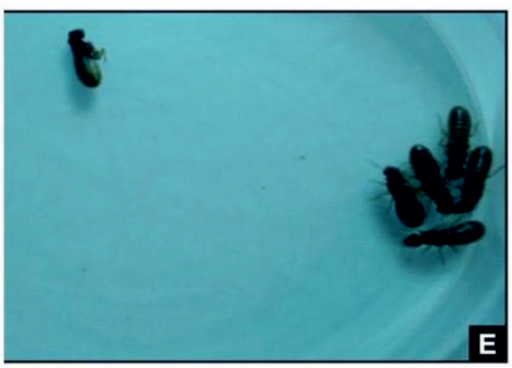
At the advanced stage of lethargy, termites walked in slow-motion with raised fore-part (head and thorax) and assumed the take-off posture of a plane. There was a progressive reduction in walking speed until termites stopped moving (became moribund). As the disease progressed there was a

Assembled termite soldiers in an area showing allogrooming after exposure.

Dead soldiers were removed from test area by other soldiers (helps in spreading of conidia).

Treated workers assembled and allogrooming

Dealates fall on back and start kicking.



Treated dealates assembled in an area and allogrooming starts

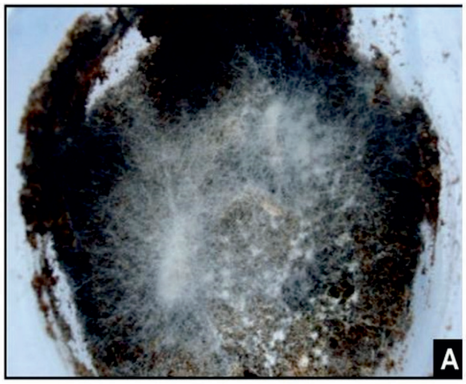
Treated dealates were move up to the surface and no mating

Plate 1. Behavioral abnormalities in termite of *Odontotermes obesus* (R.) after treatment with *Beauveria bassiana* (Balsamo) Vuillemin

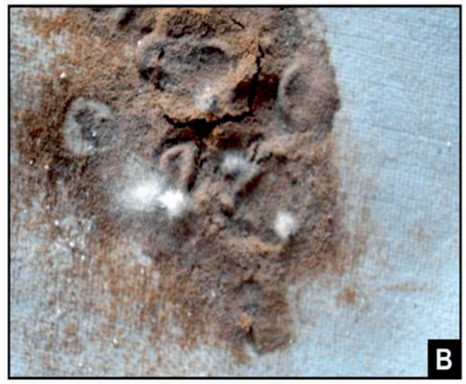
gradual loss of function resulting in paralysis Moribundity, the penultimate stage, was characterized by the inability of termites to cover a distance of at least their body length. Lethargic up and down and side to side movement of the head and thorax were also observed.

Death of the diseased insect and postmortem behavior of fungus (Plate: 2)

The time taken by a fungal pathogen *Beauveria bassiana* (Balsamo) Vuillemin to kill all termite caste commonly occurred between 2 to 8 days after exposure to the conidia. The time taken to kill the



Workers cadaver on the termiteria soil with profusely grown mycelia.



Soldiers cadaver on the termiteria soil showing a dense cluster of mycelia

Petri plate containing dead termite workers on filter paper, fungus is growing out of the infected cadavers

Petri plate containing dead termite workers on filter paper, fungus is growing out of the infected cadavers

Plate 2. Termite cadavers on filter paper and termiteria soil

insect depended upon the conidial concentrations of *Beauveria bassiana* (Balsamo). Higher the concentration greater the mortality occurred. The nymph and dealates required less time at same concentration when compared to workers and soldiers.

The fungus continued to grow and spread in a filamentous manner on the cadavers that confirm the death of the treated insect was due to *Beauveria bassiana* (Balsamo). The fungus colonizes the whole body and the fungus spread through all tissues as the insect lost their vital resistance (Plate 2; Fig. A, B&C). The cadavers were placed on filter paper or soil moistened with water in a separate petriplate in the B.O.D. at 27± 2^0 C and 75± 5 percent relative humidity to see the mycosis effect of the fungus on the insects (Plate 2; Fig. D).

After 4 to 5 days of termite's death the hyphae emerged through the whole body, usually through the articulating membranes. At high humidity the spore production occurred on mummified insects and conidia were released in the environment to infect other insects through auto infectivity.

DISCUSSION

The dynamic behavior of termites also favors infection under field conditions, as workers spend much time moving with soldiers in the nests and galleries. Although some behavioral characteristics of termites help in the removal of fungal spores from the body of contaminated individuals to result in random and low-level infections, grooming is not always effective in removing all fungal conidia and can, indeed, cause further cross-contamination.

For potential termite bait, high activity at a low concentration, low variability in termite responses and delayed toxicity are desirable characteristics. Based on these criteria, B. bassiana strains showed better promise. The ability to proliferate and maintain virulence when grown in the habitat of the host is also highly desirable (Ignoffo, 1992).

Dissemination strategies for pathogenic fungi are an important consideration. Social interaction, dark and damp habitats and a high relative humidity within a termite colony provide an ideal microenvironment for the germination of fungal spores on infected insects and also their spread to healthy members of the population. The dynamic behavior of termites also favors infection under field conditions, as workers spend much time moving with soldiers in the nests and galleries.

Singha *et al.* (2010) with the help of scanning electron microscopy, studied the minutiae details of morphological changes in the cuticles and cuticular sensilla present in various locations of the termite worker body treated with entomopathogenic fungus. The observations suggest that the ventral cuticle of the abdomen have been totally distorted along with the deformation in sensilla trichoidea. Fungal colonies were also clearly visible throughout the ventral portion of the body, which suggest that fungal growth can cause serious damage to the pest disturbing its major physiological activities resulting in its death.

This was supported by our observations on the behavioral response of the insect to the exposed fungus species, i.e. *Beauveria bassiana* (Balsamo) Vuillemin. The abnormalities in the cuticle and sensory systems of the insects indicate that the fungus *B. bassiana* changes the physiology of the insect leading to unusual behavior like sluggishness in movement, decreased feeding, shrinkage of the body surface, color change, brittleness of the appendages, and ultimately death.

Blanford *et al.* (2012) observed that a large impact of entomopathogenic fungus on feeding propensity, causing >50 percent pre-lethal reductions in feeding rate. There was clear correlation between fungal virulence and feeding reduction with virulence explaining nearly 70 percent of the variation in feeding reduction.

Several other studies also have reported reduced feeding propensity in mosquito vectors following fungal infection (Howard *et al.*, 2010; Blanford *et al.*, 2011). This is potentially a very important pre- or sub-lethal effect as there can be no transmission without feeding, even if the insects are alive.

It has been suggested previously that feeding reductions are due to resource competition within the host and/or mechanical damage of host tissue as the fungal hyphae proliferate (Hussain *et al.*, 2009). In phytophagous insects one of the feed-back mechanisms implicated in decreasing motivation to feed involves a concentration gradient of glucose between the gut and haemolymph (Chapman, 1998).

Fungal pathogens are known to release a range of toxins and secondary metabolites during infection that compete for energy reserves and cause general disruption of host tissues (Hajek and St Leger, 1994). Studies on the pre-lethal effects of fungal infection in locusts demonstrate that infected locusts feed less and are less able to sustain flight compared with uninfected controls (Arthurs and Thomas, 2001) and fungus-secreted "energy scavenging" enzymes suggested to play a role (Zhao *et al.*, 2006). The results presented here add a new dimension to such pre or sub-lethal effects, indicating an impact of fungal infection on insect sensory capability.

For this purpose a sublethal effect was considered to be significant in longevity, fecundity or any type of deformity, relative to that measured in control. The termite caste showed lesser movements as compared to control. After death, the insect became hard and stiff. At highest concentration $4.5X10^8$ conidia/mL mycelial growths on dead insect started 1 day after death, but at lower concentrations it took 2 - 4 days to grow. The slides prepared from this fungal growth confirmed *B. bassiana* infection. In addition to mortality, *B. bassiana* also induced the deleterious effects on termite caste survived the fungal infection.

The present findings indicated that lower concentrations produced more sublethal effects as compared to higher concentration. Vargas *et al.* (1995) and Fransen (1987) also documented similar sublethal effects in *T. vaporariorum*, when was applied on *A. aleyrodis* before pupation. The molting process is highly dependent on nutrients for the formation of new cuticle. So, nutrient imbalance in the hemolymph due to fungal infection has the potential to interfere with any of the steps in this process.

Kaur *et al.* (2011) observed that when *B.bassiana* was applied on larvae of *S. litura* there was significant decrease in larval period due to infection as compared to control. The life span of females emerging from treated larvae was half that of the control females. In addition to this, inhibitory effects were also manifested as reduced reproductive potential. The eggs descended from treated larvae showed significant decrease in hatchability. *B. bassiana* also induced pupal and adult deformities. A significantly higher number of deformed adults were observed at lower concentrations as compared to the highest concentration.

The present findings conclude that *B.bassiana* not only produce lethal effects but also adversely affect the various caste of termite. Reduction in longevity ultimately affects population build up in the next generation. Although *B.bassiana* have a wide application in integrated pest management strategies to reduce the load of chemical insecticides, but detailed studies need to be carried out on interaction between crops, pests, natural enemies and entomopathogenic fungus. Further to potentiate the efficacy of parasitoid and predators released after application of this pathogen, the behavioral changes like mobility, displacement and dispersal capacity of insect pests need to be determined at large scale. This is because the significantly reduced mobility and capacity to escape of a prey species could favor the functional response of predators and parasitoids and thus increasing their effectiveness as biological control agent.

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