PATHOGENICITY OF THE ENTOMOPATHOGENIC FUNGUS, *PURPUREOCILLIUM LILACINUM* TR1 AGAINST AMBROSIA BEETLES, *XYLOSANDRUS GERMANUS* (BLANDFORD) AND *XYLEBORUS DISPAR* (FABRICIUS) (COLEOPTERA: CURCULIONIDAE: SCOLYTINAE)

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ABSTRACT: *Xylosandrus germanus* and *Xyleborus dispar* (Coleoptera: Curculionidae: Scolytinae) should be considered a high-risk quarantine pest. These ambrosia beetles are very polyphagous. It attacks many deciduous trees, probably all in its distribution range. Chemical control of them is very difficult and expensive due to its protected breeding sites and its resistance to many insecticides. In this study, it is searched the effects of entomopathogenic fungi, [*Purpureocillium lilacinum* TR1 (syn: *Paecilomyces lilacinus*)] on the BTBB and PBB adults in three conidial suspensions (10^6, $10^7$and 10^8cfu ml^{-1}) in laboratory conditions. The data for mortality was recorded after 2, 4, 6 and 8 days intervals. The mortality rate was found respectively after 8 days %94.72 for *Xylosandrus germanus* and %100 for *Xyleborus dispar* in 10^8cfu ml^{-1}. It will be appropriate that the results obtained from this first study performed in laboratory conditions should also to be tried in the field conditions for the control of ambrosia beetles, *Xylosandrus germanus* and *Xyleborus dispar*. It is hoped that the study will be helpful in the control strategies of this beetle species that will be put forward in a future time. It is seen that after having obtained hopeful results from this study, making similar works on the other beetle species are also necessary.

KEY WORDS: Entomopathogenic fungi, *Purpureocillium lilacinum*, *Xylosandrus germanus*, *Xyleborus dispar*, Ambrosia beetles

Hazelnut is one of the most important agricultural products of Turkey with a production area of 650 thousand hectares, an average production of 600 thousand tons and a foreign exchange inflow of approximately 700 million dollars (Anonymous, 2002).

The Central and Eastern Black Sea Region constitutes approximately 65% of Turkey's hazelnut production and also is the most important agricultural product of the Black Sea Region. There are many factors in Turkey that reduce yield in hazelnut fields, the most important being pests. Hazelnut pests have a direct or indirect effect on quality and yield. Although about 150 insect species have been detected in hazelnut plantations in Turkey, 10-15 of them have caused economic damage in varying regions and years (Işık et al., 1987). According to many studies, it is reported that the nut weevil (*Curculio nucum* L.) is the most important pest threatening hazelnut trees in Turkey. However, according to recent studies and observations, bark beetles, which reduce the yield by drying crops and have a high

population in hazelnut fields, are a significant pest group (Ak, 2004; Ak et al., 2004).

In recent years, bark beetles, which are reported to be harmful to drupe and pome trees and forests in Turkey, have been found to cause significant damage especially in hazelnut fields in the coastal and mid regions (Ak et al., 2005).

Ambrosia beetles, *Xylosandrus germanus* (Blandford) (Coleoptera: Scolytidae) (black timber bark beetle or Alnus Ambrosia beetle) is a strongly invasive species. It is capable of flight over distances of at least 2 km (Grégoire et al., 2001) and appears to be able to spread over much longer distances. Initial rates of spread in the USA and Europe suggest several tens of kilometres per year (Henin & Versteirt, 2004). It can also be spread long distances as the result of the transport of infested wood by humans (LaBonte et al., 2005). The impact of *X. germanus* can often be severe, and in Europe, it has become one of the dominant species in forests. Within about 50 years following its introduction to Europe, *X. germanus* has become one of the dominant scolytids in many European forests, on both deciduous and coniferous trees. In the USA, *X. germanus* has become an important pest of black walnut (*Juglans nigra*) (Weber, 1979, 1983a, 1984, 1985) and chestnut (*Castanea mollissima*) (Oliver & Mannion, 2001). Weber & McPherson (1983b) list over 200 host species belonging to 51 plant families. The species is not strongly size-selective, and breeds both in small branches and in large logs and stumps. There may be some preference for stems of less than 10 cm diameter (Henin & Versteirt, 2004). In Japan, *X. germanus* also attacks the roots of tea (Kaneko et al., 1965). The female feeds on the ambrosia fungus, *Ambrosiella hartigii*, which she has introduced into the gallery system before oviposition begins. The eggs are laid loosely in the gallery over some days, and the larvae feed on the ambrosia fungus on the walls of the gallery.

Xyleborus dispar (Fabricius) (Coleoptera: Scolytidae) (pear blight beetle) should be considered a high-risk quarantine pest. A very wide range of host plants have been recorded for many species of *Xyleborus* and related genera. *X. dispar* is usually a secondary species, it may become a primary species attacking healthy trees, especially in areas where it is an exotic species (Kühnholz et al., 2003). Such a change in habits considerably increases its potential for causing economic damage to crop and forest trees.

In Europe, *X. dispar* is widespread from Spain to the Urals and from Italy to Finland (Stark, 1952; Balachowsky, 1963; Postner, 1974; Schedl, 1981; Pfeffer, 1995). In Asia, it is known from the Middle East through Siberia to Sakhalin Island and north-eastern China. In Africa, it is present only in the North in Mediterranean countries (Stark, 1952; Balachowsky, 1963; Postner, 1974; Schedl, 1981; Pfeffer, 1995; Yanovskii, 1999). In North America, where it was accidentally introduced from Europe before 1817 (Wood, 1977). *X. dispar* now occurs in eastern North America west to the Great Lakes states and south to South Carolina, western Canada, the Pacific Northwest states and California (Linsley & MacLeod, 1942; Bright 1968; Wood, 1982; Kovach & Gorsuch, 1985; Hobson & Bright, 1994). The distribution in North America suggest two introductions, one in the east and one in the west.

A number of species of *Xyleborus* and related genera with similar habits to *X. dispar* have become important pests of tree crops, ornamental and native trees in tropical, subtropical and warm temperate zone areas where they have been introduced.

X. dispar is very polyphagous. It attacks many deciduous trees, probably all in its distribution range (Schvester, 1954; Balachowsky, 1963; Bright, 1968; Schedl, 1981; Wood, 1982; Wood & Bright, 1992; Pfeffer, 1995).

Favoured species are fruit trees, such as apple, apricot, peach, nectarine, pear, cherry, plum, hazel (Mathers, 1940; Linsley & MacLeod, 1942; Vasseur & Schvester, 1948; Schvester, 1954; Balachowsky, 1963; Postner, 1974; Viggiani, 1979; Chepurnaya & Myalova, 1981; Mani & Schwaller, 1983; Kovach & Gorsuch, 1985; Furniss & Johnson, 1987; Hesjedal & Edland, 1988; Schick & Thines, 1988; Juillard-Condat & Perrau, 1989; Schröder, 1996; Lagowska & Winiarska, 1997; Morone & Scortichini, 1998). of forest trees, maple, oak (Postner, 1974), birch, poplar, alder (Balachowsky, 1963), chestnut (Schvester, 1954) and Chinese chestnut (Tsankov & Ganchev, 1988) are mostly attacked. Damage on urban hawthorn trees has also been reported (Nachtigall, 1993).

The economic damage caused by *X. dispar* is not easy to estimate. The beetle preferentially attacks stressed trees. Such trees might often have a chance of recovery without beetle attack.

No method of biological control of *X. dispar* exists at present. The natural enemies listed do not provide effective control, and augmentation is not likely to produce much improvement. Canganella et al. (1994) suggest that the bacteria (*Pseudomonas chlororaphis* and *Bacillus subtilis*) they found on the insect and in the galleries may represent the starting point for future research.

Chemical control of *X. dispar* is very difficult and expensive due to its protected breeding sites and its resistance to many insecticides. Therefore, sprays are only applied in exceptional cases. Compounds used previously, such as DDT, lindane and endosulfan (Schvester, 1954; Roediger, 1956; Mani & Schwaller, 1983; Juillard-Condat & Perrau, 1989), may no longer be used. Compounds registered at present, such as carbaryl, organophosphates and pyrethroids (Viggiani, 1979; Juillard-Condat & Perrau, 1989; Schröder, 1996) often give only partial control.

Control of *X. dispar* must rely on a combination of different methods. All measures promoting plant health are essential. Attacked plant parts must be removed in time. In orchards with a risk of damage, alcohol traps should be placed for monitoring or for control. Only in exceptional cases do insecticide sprays become necessary. Eventually, it may be possible to develop the use of non-host volatiles as repellents to prevent, or at least reduce, attacks (Borden et al., 2003).

Chemical pesticides have been the main control methods for these pests in crop production both world and Turkey. Ambrosia beetles, *Xylosandrus germanus* and *Xyleborus dispar* have got natural enemies but, these do not adequately control high populations. Several mycopesticides have been developed and used in several countries. An entomopathogenic fungus can act as a parasite of insects and kills or seriously disables them. Naturally occurring entomopathogenic fungi (EPFs) are considered to be one of the best alternative to existing chemicals (Hajek & Leger, 1994).

More than 400 entomopathogenic fungi have been defined, but studies are noted to have concentrated only on 20 of these (Hall & Papierok, 1982; Zimmermann, 1986). Among these, the genera *Lagenidium, Entomophaga, Neozygites, Entomopytora, Erynia, Aschersonia, Lecanicillium, Nomuraea, Hirsutella, Matarhizium, Beauveria* and *Paecilomyces* are stated to be of importance (Roberts & Wraight, 1986). Entomopathogenic fungi such as *Lecanicillium* sp. (Jung et al. 2006), *Beauveria bassiana* (Quesada et al., 2006; Wakil et al., 2011), *Metarhizium anisopliae* (Wright et al., 2004), *Paecilomyces* spp. (Shia & Feng, 2004) and *Nomuraea rileyi* (Devi et al., 2003) are being used for the control of aphids, mites and other insect pests.

Purpureocillium lilacinum (Thom) Luangsaard, Hywel-Jones, Houbraken and Samson (syn: *Paecilomyces lilacinus*) (Sordariomycetes: Hypocreales) is a typical soil-borne fungus with a good potential for biological control (Luangsa-Ard et al., 2011). This species has been described as being as efficient as the commonly used nematicides (Dube & Smart, 1987; Schenck, 2004; Mendoza et al., 2007); it is also a controller of insects (Posada et al., 1998; Suh et al., 2002; Gökçe & Er, 2005; Wakil et al., 2012) and others arthropods (Fiedler & Sosnowska 2007; Shin et al., 2011; Angelo et al., 2012). According to Bellows (2001), Headrik & Goden (2001) and other authors, the use of entomopathogenic fungi is an excellent method for the biological control of insects.

Purpureocillium lilacinum was isolated firstly from insects in tropical regions. It has been recorded in many regions of the worlds, but is mainly seen in warm regions (Domsch et al., 1980). *P. lilacinum* was determined to cause infection in many insect, nematodes and acari species (Anonymous, 2011). There are few literature records about effects of entomopathogenic fungi originating in our country on the pest groups of economical significance in our country [whiteflies (*Trialeurodes vaporariorum*), potato beetle (*Leptinotarsa decemlineata*), green peach aphid (*Myzus persicae*), apple rust mite (*Aculus schlechtendali*) and citrus wooly aphid (*Planococcus citri*)] (Gökçe & Er, 2005; Kılıç & Yıldırım, 2008; Boztaş et al., 2009; Demirci & Denizhan, 2010; Demirci et al., 2011).

In this paper, we evaluated the control potential of *Purpureocillium lilacinum* TR1 against adults of ambrosia beetles, *Xylosandrus germanus* and *Xyleborus dispar* in the laboratory.

MATERIALS AND METHODS

Fungi Sources

The culture of *Purpureocillium lilacinum* TR1 was provided by the Gaziosmanpasa University, Faculty of Agriculture, Department of Plant Protection, Tokat (Turkey) and İnönü University, Faculty of Agriculture, Department of Plant Protection, Malatya (Turkey). It was firstly isolated from the eggs of root-knot nematode [*Meloidogyne incognita* (Kofoid & White)] collected from the greenhouse in Sarıcakaya (Eskişehir, Turkey) (Kepenekci et al., 2009). And it was identificated based on morphometric and molecular data (Kepenekci et al., 2015b).

Insect Sources

Ambrosia beetles, *Xylosandrus germanus* and *Xyleborus dispar* adults were obtained from the laboratory colony maintained at the entomology division, Plant Protection, Fac. of Agriculture, Ondokuz Mayıs Univ. in Samsun, Turkey. *X. germanus* and *X. dispar* adults were collected from the hazelnuts orchard from Taflan district, Kayagüneyi village, Samsun (Turkey).

Insects were extracted from the different nuts branches and selected healthy. The insects collected from the hazelnut gardens and brought to the laboratory were kept at 4 °C for 2 days and then used in experiments.

Mass-Culturing of *Purpureocillium lilacinum*

Purpureocillium lilacinum TR1 was subcultured on Potato Dextrose Agar with the help of sterilized bacteriological loop and the plates were closed by parafilm at 25±1°C for 14 days. The conidia were harvested using sterilized rubber loop attached to 1 ml borosilicate pipette at the angle of 45°. The scraped material was shifted into sterilized petri plates and stored at 4°C in refrigerator. The harvested fungal conidia were incorporated in to sterile 0.05% Tween-80 solution and the material were stirred for complete homogeneity.

The serial dilutions were prepared and the number of conidia was measured to achieve the 10^6, 10^7 and 10^8cfu ml^{-1} concentration under haemocytometer.

Bioassay

Trials were performed on June 2017 at Ondokuz Mayıs University, Faculty of Agriculture, Department of Plant Protection, Samsun (Turkey). Experiments were carried out in Petri-dishes with 5 individual adults of *X. germanus* and *X. dispar* per replicate and fungal isolate. Suspensions of *P. lilacinum* were applied at three different density (10^6, 10^7 and 10^8cfu ml^{-1}) at spraying applications (2 mL per dishes) the surface of the plates with beetles (after putting in 5 beetles and 4 replications). In the controls, sterile water with Tween 80 (0.01%) was applied to the surface of the plates with beetles. Each Petri plate contained moistened filter paper on the bottom and added to the bait, consisted of 2 pieces of branches of hazelnut tree approximately 4 cm length and 1 cm weight (favored bait) placed on the center of filter paper. The Petri plates were then placed in a growth chamber maintained at at 25±2°C and 60-65% R.H. in a dark laboratory. The petri plates tested were closed with a stretch for 48 hours and then opened. *X. germanus* and *X. dispar* mortalities were recorded on the 2nd, 4th, 6th and 8th days after application. Mortalities for beetles were evaluated in comparison to the control groups. The dead beetles adults were examined under a microscope to determine whether mortality was because of *P. lilacinum*, and mycelial development was checked. When necessary, these cadavers were placed in Petri dishes to follow up potential mycosis development.

Statistics

One-way ANOVA was used to compare the mortality of ambrosia beetles. Means were compared at the P=0.05 level, and Tukey's test was used to separate means (SPSS, 1999). Arcsine transformation was carried out on mortality (%) before analyses.

RESULTS

The data generally showed that all concentration were effective against the ambrosia beetles, *Xylosandrus germanus* and *Xyleborus dispar* adults (Fig. 1). When we look overall results of this study, 10^8cfu concentration had the highest effect on the 8th day both insect species.

The results of the study showed that although the effects at spraying applications the surface of the plates with *X. germanus* and *X. dispar* adults were very high. It was valid for both beetles species (*X. germanus* and *X. dispar*). The concentrations of 10^8cfu ml^{-1} at the *X. dispar* and *X. germanus* were found 100% and 94.72% effective 8th days after application. It is hoped that this first study in laboratory conditions will serve to the future studies on the control of the pest.

When the results of the study are evaluated in detail; for *X. dispar*, on day 2 there were 1.33% ±5.27 deaths in 10^6cfu ml^{-1} and 11.6% ±5.27 deaths in 10^8cfu ml^{-1}. No mortality was recorded in 10^7cfu ml^{-1} (F= *4,00*; df=3,15; P<0.035). The highest effect on day 4 was recorded as 75.39% ±1.21 in 10^8cfu ml^{-1} (F= *20,75*; df=3,15; P<0.000). On day 6, the mortality rate at the concentration of 10^8cfu ml^{-1} remained stable (F= *23,00*; df=3,15; P<0.000), but all insects that were exposed on day 8 were found to have died. (100%) (F= *26,99*; df=3,15; P<0.000). At the end of day 8, the same effect was obtained at 10^6 and 10^7cfu ml^{-1} concentrations, where the mortality rate was recorded as 94.72% ±6.99. In the experiments conducted, a mortality rate among the control groups on day 4 was 5.27% ±6.98 and no further deaths were recorded until the end of day 8.

For *Xylosandrus germanus*; on day 2 mortality was seen only in 10^7cfu ml^{-1} (1.33% ±5.27), there was not any mortality recorded in other concentrations. (*F= 1,00*; df=3,15; P<0.426).

At the end of day 4, very low mortality rates were recorded (11.27% ±14.83, 5.27% ±6.99 and 8.01% ±11.35 for 10^6, 10^7 and 10^8cfu ml^{-1} respectively). (*F= 1,05*; df=3,15; P<0.407).

The highest effect on day 6 was seen in concentration 10^8cfu ml^{-1} with 55.01% ±7.06. (*F= 9,11*; df=3,15; P<0.000). On day 8, 10^8cfu ml^{-1} had a high effect with 94.72% ±6.99 and the mortality rate was recorded as 75.40% ±1.21 for 10^6 and 10^7cfu ml^{-1}, which had the same effect (*F= 30,37*; df=3,15; P<0.000). In the control groups, 1.33% ±5.27 mortality was observed on day 6 and no further deaths were recorded until the end of day 8.

DISCUSSION

Mycoinsecticides can be effective not only when they make direct contact with the target host, when they are later acquired by a target host from a treated surface, when inocula are transferred to other target hosts (e.g., mates or offspring) elsewhere in the environment, and when infective spores on cadavers are acquired by additional target hosts. Ambrosia beetles, are difficult to control because only adults most often only adult females spend a relatively short time outside host trees during dispersal flights (Castrillo et al., 2011). Our experiments show that *Purpureocillium lilacinum* TR1 is virulent against *Xylosandrus germanus* and *Xyleborus dispar* adults and that inocula carried into the gallery can have a significant effect.

When the efficacy studies of *P. lilacinum* TR1 against important pests stored grain pests [*Tribolium castaneum* (rust red flour beetle), *T. confusum* (confused flour beetle) (Coleoptera: Tenebrionidae), *Sitophilus oryzae* (rice weevil), *S. granarius* (granary weevil) (Coleoptera: Curculionidae) and *Rhyzopertha dominica* (lesser grain borer) (Coleoptera: Bostrichidae)] are examined in Turkey; the maximum mortality 77.9% of *S. oryzae* and 97.6% *R. dominica* were recorded in 10th day and 10^8cfu ml^{-1} at concentration of 25°C. In all applications at other temperatures (15 and 30°C) and concentrations (10^6 and 10^7cfu ml^{-1}), mortality rates were found below 50% (Tülek et al., 2015).

In another study, *P. lilacinum* TR1 was used against *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) and *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomalidae), two of the most important pests of the potato plant. As a result of the research, *P. lilacinum* was found to be the most effective on the last larval instar of *L. decemlineata* (mortality was found 33.2% on 10th day of treatment) and *P. operculella* (mortality was found 43.3% on 10th day of treatment) after the application with the fungal concentration of 10^8cfu ml^{-1} at 25°C (Kepenekci et al., 2013).

The bean weevil *Acanthoscelides obtectus* Say. (Coleoptera: Bruchidae), an important warehouse pest in Turkey, has been tested at two different temperatures (15 and 20°C) against three different concentrations of *P. lilacinum* TR1 (10^6, 10^7 and 10^8cfu ml^{-1}). The data for mortality was recorded after 2, 4, 6, 8 and 10 days intervals. In this preliminary study, at 15°C, all treatments, effect was less than 50%. Highest effect at 10^8cfu ml^{-1} have been recorded as 47.87%. At 15°C, fungus did not exhibit more than 35% mortality at any concentration (Kepenekci et al., 2015a).

Kepenekci et al. (2014) studied the effect of *P. lilacinum* TR1 against Black Cherry Aphid *Myzus cerasi* Fabricus (Hemiptera: Aphididae) adults and the data

generally showed that all concentration were effective against the. When we look overall results of this study, at 25°C, 10^8cfu ml^{-1} concentration had the highest effect on the 8th day. This effect can not show any changes on the 10th day and remained constant. This effect is 2.5 times of a lower concentration (10^7cfu ml^{-1}) (to 83.64% from 33.25%).

No studies using *P. lilacinum* against *Xylosandrus germanus* and *Xyleborus dispar* have been found. However, there are studies on the effect of other entomopathogenic fungi (*Paecilomyces fumosoroseus*, *Beauveria bassiana* and *Metarhizium anisopliae*) against the *Ips* species (*Ips sexdentatus* and *I. typographus*) of the bark beetles. One of these studies was against the species we used (*Xylosandrus germanus*). *B. bassiana* isolate 426, 412 and 422 and *P. farinosus* isolate 290 (10^6cfu ml^{-1}) were tested against *Ips sexdentatus* adults at a single concentration (10^6cfu ml^{-1}), and a very high effect was obtained at 96.67% in *B. bassiana* isolate 426. Other isolates (*B. bassiana* isolate 412 and 422) of the same fungus were found to be effective (90.67% and 89.33% respectively). However, the *P. farinosus* isolate 290 and 290 had low effect (45.00% and 66.67% respectively). (Draganova et al., 2006).

In our study, it was 94.72% against *Xyleborus dispar* and 75.4% against *Xylosandrus germanus*, even in the *Purpureocillium lilacinum* TR1 10^6cfu ml^{-1}concentration.

In a study against another *Ips* species, *Beauveria bassiana* isolate BALS. and *Metarhizium anisopliae* izolate METSCH. against *Ips typographus* were tested and very high effect rates were obtained. In the *B. bassiana* applications almost all of the populations were killed (99% mortality rate). *M. anisopliae* has been reported to have a 97% effect (Mudrončeková et al., 2013). In our study, the effects were also found to be very high; in fact, the 10^8cfu ml^{-1} concentration was found to be 100% effective against *Xyleborus dispar*.

In another study, the effect of three different isolates (isolate *B. bassiana* GHA, *B. bassiana* Naturalis, and *M. brunneum* F52) of the entomopathogenic fungus used in our experiments with *Xylosandrus germanus*, were 6.7% ±6.7, 60% ±5.8 and 61.7% ±7.9 respectively after day 6.

However, it can be said that the concentration used is very low (approximately 600 conidia/mm^2) (Castrillo et al., 2011). In our study, we observed that as the concentration increased, so did the effectiveness, and the highest effect was recorded in the application of concentration 10^8cfu ml^{-1}. In fact, the 10^8cfu ml^{-1} concentration was found to be 100% effective against *Xyleborus dispar*. In similar studies related to this topic, it is reported that as the concentration increases (the intensity of the spor) so does the effectiveness.

Kreutz et al. (2004) used 4 strain types of *Beauveria bassiana* and a commercial composition Boverol® (Fytovita, Ostrožská Lhota, Czech Republic) to test the effectiveness of entomopathogenic fungi against *Ips typographus* of three concentrations (1×10^6, 1×10^7 and 1×10^8 conidia/ml). The results confirmed us about the correctness of concentration used in our experiments. The concentration 1×10^6 spores per ml reached the mortality of 93 percent, 1×10^7 and 1×10^8 spores per ml reached 100% mortality of the *I. typographus*. Similar results with a high mortality percentage with concentration of 1×10^8 conidia/ml also achieved authors like Ahmed et al. (2007), Kaeng et al. (2009), Bustillo et al. (2002), Prasad & Seyd (2010) and Steinwender et al. (2010). From this knowledge, we can sumarize: The higher the content of spores in the suspension , the more faster and likely is the onset of infection and death of the host . Draganová (2006) tested the infectivity ofisolates *Beauveria bassiana* and *Paecilomycetes farinosus* on *Ips sexdentatus* and *I. acuminatus*. Spruce bark

beetle mortality reached 96.67% by *B. bassiana* and by *P. farinosus* 66.67%. Another research in Slovakia was conducted by Jakuš & Blažec (2001) devoted to testing of *I. typographus* mortality in forests Spišská Magura, where the chosen cut trees invaded by bark beetles were treated by aqueous solution containing *B. bassiana*. The concentration of the spore suspension was 1×10^7 conidia/ml and they applied spray directly on the tree bark. This type of experiment could infect 28.75% of bark beetles. The authors believe that the anticipated success by entomopathogenic fungi did not reach the expected results due to expected conditions (temperature, relative humidity). And under laboratory conditions the efficiency of entomopathogenic fungi reaches 88 to 100% mortality of bark beetles (Vaupel et al., 1996; Kreutz et al., 2004; Kunca et al., 2009). These findings suggest us a strong influence of various factors on the viability of entomopathogenic fungi. Similar experiments were conducted on a wide range of insects with similar results: On termites (Krutmuanga & Mekchayb, 2005), *Spodoptera littoralis* (Ahmed et al., 2007), *Helicoverpa armigera* (Prasad & Seyd, 2010), *Boophilus microplus* (Kaeng et al., 2009), *Tribolium castaneum* (Lord, 2009).

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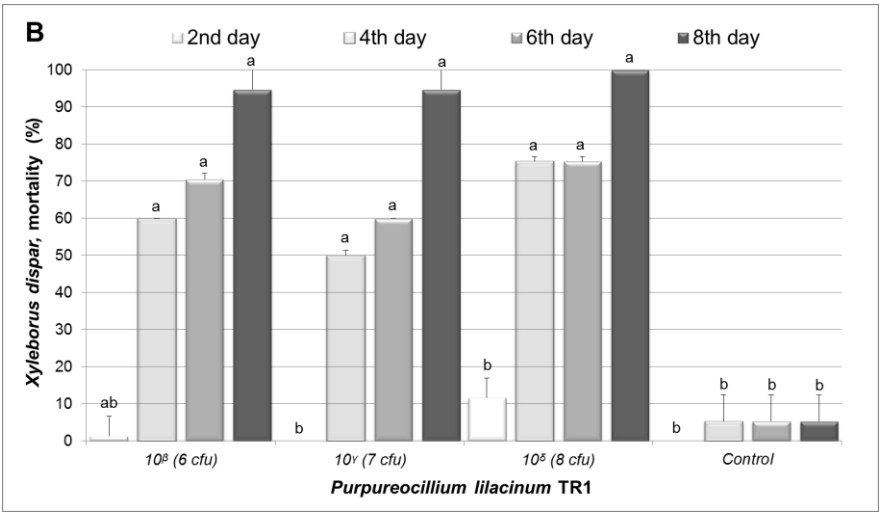

Figure 1. Mean adult mortality (%±SEM) of *Xylosandrus germanus* (A) and *Xyleborus dispar* (B) exposed for 2nd, 4th, 6th and 8th days on favored bait treated with *Purpureocillium lilacinus* TR1 (isolated from Turkey) (10^6, 10^7 and 10^8cfu ml^{-1} concentration) (means followed by the same letters are not significantly different at P=0.05).