EFFICIENCY OF TWO DIFFERENT ENTOMOPATHOGEN FUNGI *BEAUVERIA BASSIANA* AND *PURPUREOCILLIUM LILACINUM* TR1 AGAINST *TETRANYCHUS URTICAE*

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Abstract. Two-spotted red spider/red spider mite is an important pest of cultivated crops. Resistance is one of the most important problems in the intensive chemical control used in traditional management methods. Therefore, researchers have been using alternative control methods in pest management to overcome resistance problem. This study was conducted between 2015 and 2017 to investigate the effects of entomopathogenic fungi (EPF) *Beauveria bassiana* and *Purpureocillium lilacinum* TR1 on *Tetranychus urticae* (Koch) (Acarina: *Tetranychidae*). The doses used for *P. lilacinum* were 10^4, 10^5, 10^6, 10^7, 10^8 and 1.6 10^8 conidia ml^{-1} and for *B. bassiana* 10^4, 10^5, 10^6, 10^7, 10^8, 10^9 and 3.7×10^9 conidia ml^{-1}. After five days the results showed that mortality of *T. urticae* adults were started with 10^5 conidia ml^{-1} concentration of *B. bassiana* and after seven days 10^4 conidia ml^{-1} concentration of *P. lilacinus.* At the end of the trial, the mortality rate recorded by the highest doses, 10^8 conidia/ml, of both EPFs were 28.3 and 66.6% with *P. Lilacinus and B. bassiana*. Since many EPF fungi are thought to be epizootic, *B. bassiana* and *P. lilacinum* can be effectively used against *T. urticae* control as biological agents.
Keywords: *two spotted spider mites, entomopathogenic fungi, control, Turkey*

Introduction

The polyphagous pests are among the most harmful pest groups of agricultural products (Vacante, 2016). *Tetranychus urticae* is also an important pest of cultivated crops. Hatched two-spotted spider mites ((TSSM) mostly fed on the lower surface of leaves. The TSSM causes destruction in leaves by feeding in approximately 18-22 plant cells in a minute. After inserting the stylet like mouthparts into leaf cells, secretes the enzyme and absorbs the cell contents. Consequently, leaves are turned on pale, yellowish, gray or bronze-colored spots and causing drying and defoliation. In addition, adults cause damage by spinning tight and silk webs on the plants (Anonymous, 2008). Approximately 4000 different host plant species of *T. urticae* have been identified in worldwide (Migeon and Dorkeld, 2010, 2017) as well as in different studies conducted in Turkey (Yabaş and Ulubilir, 1995; Bulut, E., 1999; Yeşilayer, 2009).

The most preferred method in TSSM management is chemical control which is inexpensive and easy to adapt. However, one of the most important disadvantages of continuous use of chemical control is the resistance of *T. urticae* to the pesticides over time (Keena and Granett, 1987; Herron and Rophail, 1998; Van Leeuwen et al., 2004). New methods which can be alternative or complementary to chemical control of pests have recently been studied and especially importance of biological control is increasing as a suitable alternate method. Biological control is an appropriate method to sustainable agricultural techniques and sensible to human and animal health. The main components of this method used in pest control are parasitoids, predators and entomopathogens (Kılınçer et al., 2010; Dermauw et al., 2013). Studies conducted to date have reported approximately 500 fungi species as pathogenic in insects The

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entomopathogenic fungi species of *Lagenidium, Entomophaga, Neozygites, Entomophytora, Erynia, Aschersonia, Lecanicillium, Nomuraea, Hirsutella, Metarhizium, Beauveria* and *Isaria* have gained importance in the field of plant protection (Erkılıç and Uygun, 1993; Kılıç and Yıldırım, 2008). *P. lilacinus* is known as a nematophagus fungi and used to control mites and insects. In addition, EPN activity of entomopatogen fungus *B. bassiana* against *T. urticae* and insects has also been demostrated (Örtücü and Albayrak İskender, 2017).

Thee fungi do not develop resistance in mites, insects and like other pesticides, have absence of any toxic effects on ecology and have potential for future biotechnological developments. The longtime control, infecting the development period of their hosts, applicability with many insecticides and easiness in for mass production are also other advantages of these fungi (Demirbag, 2008). Fungi directly enter from the cell wall. The spores on cuticle settle here and germinate. The germinating spores enter due to the appressorium (penetration peg). The hyphae in epidermis and hypodermic grow, continue to proliferation in the insect body and blood cells and cause the death of insect (Ortiz-Urquiza, 2013). Commercial preparation of some entomopathogenic fungi as mycoinsecticides (*B. bassiana, Metarhiz anisopliae, Hirsutell thompsoni*) are available in the world and Turkey (Kılınçer et al., 2010). Recent studies have recorded the efficiency of some EPFs such as B. *bassiana*, *Verticillium lecanii* and *M. anisoplia* against two spotted spider mites (Chandler et al., 2005). The aim of this study was to evaluate the efficiency of two different entomopathogenic fungi, *Purpureocillium lilacinum* and *Beauveria bassiana* against *Tetranychus urticae*.

Material and methods

Plant and mites culture

Bean used as host plant *Phaseolus vulgaris* L. (Fabaceae) was grown in production cabinets at 25 ± 2 °C, 65 ± 5% relative humidity and 16 h lights: 8 h darkness photoperiod in the Plant Protection Department of Faculty of Agriculture, Gaziosmanpaşa University in Tokat-Turkey. The plants, at 5-6 leaves stage were transferred to the production cabin to be used in the production of two-spotted red spiders. Cultures of *Tetranychus urticae* were reared in climate chambers of Plant Protection Department, Faculty of Agriculture, Gaziosmanpasa University. The infected bean plant leaves with mites were cut and placed on non-infected plants to infect.

Cultures of entomopathogenic fungus

The EPFs used in the study, *Purpureocillium lilacinum* (syn: *Paecilomyces lilacinus* (Thom) Samson) TR1 and *Beauveria bassiana* (Balsamo) Vuillemin were obtained from the stock cultures of Prof. Dr İlker KEPENEKÇİ (Department of Plant Protection, Faculty of Agriculture, Gaziosmanpasa University) and from Prof. Dr. Fikret DEMİRCİ (Department of Plant Protection, Faculty of Agriculture, Ankara University), respectively. The fungus was produced at the PDA medium to obtain sufficient spore suspension. Pure entomopathogenic isolates were planted using with glass hokey stick. After 4 weeks, 5 ml of 0.02% Tween 80 solution was added to the petri dishes containing the cultures and homogenous mixing of fungi spores was achieved by spreading with glass hokey stick. The resulting suspension was then filtered through a sterile material to remove particulates, and transferred to 15 ml and 50 ml centrifuge

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tubes. The centrifuge tubes were shaken for 5 min on a Vortex shaker to separate the clustered fungi spores in the fungi suspensions. The spore intensity was determined using Thoma slide and light microscopy (Gabarty et al., 2014). Afterwards, each fungi isolate was diluted and the number of spores was adjusted.

Bioassay

Total of 13 different conidial concentrations were prepared to study the efficiency of entomopathogenic fungi on *T. urticae*. The conidial concentrations included 7 different doses of *B. bassiana* (1×10^4**,** 1×10^5, 1×10^6, 1×10^7, 1×10^8, 1×10^9 and 3.7×10^9 conidia ml^{-1}) and 6 different intensities of *P. lilacinum* (1×10^4**,** 1×10^5, 1×10^6, 1×10^7, 1×10^8 and $1 \times 1.6\ 10^8$ conidia ml^{-1}). For each application, 10 ml suspensions of different spore-density solutions were shaken for 2-3 min on the Vortex shaker. The EFPs, then, were sprayed to the leaves using a hand sprayer (2.5 ml). For each application, 10 *T. urticae* adults were used. Fungi solutions were sprayed to the down side of bean leaf disc and placed on a moist cotton in Petri dishes. Adult mites were transferred with a fine brush to the surface of leaf disc. Sterile distilled water (dH_2O) containing 0.02% Tween 80 was sprayed onto the leaves in the control. Mortality values were counted on days 1, 3, 5, 7 and 9 after the application, and mycosis rates observed on days 7, 11 and 19 were recorded. Mites, mortality in mycosis experiment were placed on a moist filter paper in 9 cm sterile glass petri dishes and fungal development was observed.

Daily maintenance and humidity control were carried out during the experiments. The experiment was set up with three replicates and repeated twice.

Statistical analyses

Data was subjected to the one-way analysis of variance (One-Way ANOVA), and mean values were compared by Tukey's test at $P = 0.05$ significance level (SPSS, 2011).

Results

Total of 13 concentrations of two different entomopathogenic fungi were applied to the adults of *T. urticae* under the laboratory conditions. Mortality using single dose of 10^5 started to occur on days 3 and 5 after applications of *P. lilacinum* and *B. bassiana*, respectively. Mortality of *T. urticae* adults among the application of other concentrations after the single dose experiment, were observed from day 5 at 10^6 concentration of *P. lilacinum*. The mortality rate at 10^6, 10^7 and 10^8 conidia ml^{-1} doses on day 9 was 28.3% and the mortality rate at the highest concentration ($1 \times 1.6\ 10^8$ conidia ml^{-1}) on day 11 was 76.6%. The mortality rates were linearly increased with increasing the doses and days (*Table 1*). The counting results in day 3 of *P. lilacinum* application in controlling the adult mites was not significantly different ($P > 0.05$). The mortality rates counted on days 5, 7 and 9 at 10^7 conidia/ml dose were statistically significant ($P < 0.05$) and the mortality rate ranged from 20 to 61.6% (*Table 1*).

The deaths of two-spotted adult mites at the highest concentration (3.7×10^9 conidia ml^{-1}) of *B. bassiana*, the second EPF used in the study, started on day 3 and mortality rate reached to 20%. The mortality rate was recorded as 91% on the last day (*Table 2*).

The mortality rates on days 5 (13.3%) and 7 (16.6%) at 10^7 and 10^8 conidia ml^{-1} concentrations of *B. bassiana* were statistically significant ($P < 0.05$). In contrast to *B.*

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bassiana, the difference between mortality rates obtained with *P. lilacinum* starting from 10^8 conidia ml^{-1} concentration on day 3 was significant ($P < 0.05$). The differences between mortality rates on days 5 and 7 were similar, though statistically significant ($P < 0.05$). Mortality has not been observed after 3 days treatment with 10^4 but mortality was 48.3% at end of the day 9.

Table 1. *Effect of P. lilacinum on T. urticae (% mortality rate±SE)*

Concentration (conidia ml^{-1})	3th	5th	7th	9th
Control	0.00±0.00a	0.00±0.00a	1.66±1.66a	1.66±1.66a
10^4	0.00±0.00a	0.00±0.00a	10.00±0.00a	20.00±0.00a
10^5	0.00±0.00a	0.00±0.00a	11.66±1.66a	21.66±0.18 a
10^6	0.00±0.00a	1.66±2.43a	11.66±1.66a	28.33±1.23a
10^7	0.00±0.00a	3.33±2.10b	15.00±2.23ab	28.33±1.23a
10^8	0.00±0.00a	10.00±1.56b	20.00±1.45b	28.33±1.23a
$1 \times 1.6\ 10^8$	0.00±0.00a	28.33±1.33c	38.33±1.66c	61.66±2.32b

Means followed in the same column by different letters are significantly different $P < 0.05$, Tukey test

Table 2. *Effects of B. bassiana on T. urticae adults (% mortality rate ± SE)*

Concentration (conidia ml^{-1})	3th	5th	7th	9th
Control	1.66±1.66a	1.66±1.66a	1.66±1.66a	13.33±2.10a
10^4	0.00±0.00a	1.66±2.43a	1.66±2.43a	48.33±12.75abc
10^5	0.00±0.00a	8.33±3.01ab	8.33±3.01ab	31.66±2.56a
10^6	0.00±0.00a	11.66±1.66ab	11.66±1.66ab	41.66±3.06ab
10^7	0.00±0.00a	13.33±2.10ab	13.33±2.10ab	58.33±2.86bc
10^8	10.00±0.00b	16.66±2.10b	16.66±2.10b	66.66±2.10cd
10^9	16.66±2.10c	45.00±6.70c	45.00±6.70c	88.33±3.42de
3.7×10^9	20.00±2.58c	45.00±2.23c	45.00±2.23c	91.38±0.45e

Means followed in the same column by different letters are significantly different $P < 0.05$, Tukey test

Mycosis study with EPF fungus

Mycosis observations were conducted on days 7, 11 and 19 within the efficiency study against *T. urticae* adults included the different concentrations of the two EPF fungi. The mycosis rate on the end of day 7 at the lowest concentration (10^4 conidia ml^{-1}) was 10% and it was 51.6% at the end of the 19th day. The mycosis development rates of six different *P. lilacinum* concentrations on various days showed mycosis development on day 7 at 1×10^5, 1×10^6 and 1×10^7 conidia/ml concentrations, but the difference was not statistically significant ($P > 0.05$). The rate of mycosis development at 1×10^8 and $1 \times 1.6\ 10^8$ conidia ml^{-1} concentrations on day 7 was statistically significant ($P < 0.05$) (*Table 3*).

Similar to *P. lilacinum* (*P. lilacinus*), the mycosis development rates conducted at different concentrations of *B. bassiana* started to be observed from the day 7. The mycosis development rate at the highest concentrations of 1×10^7, 1×10^8 and $1 \times 1.6\ 10^8$ conidia ml^{-1} ranged from 20 to 45% and it was statistically significant ($P < 0.05$). The rate of mycosis development, which increased to over 50% at the highest

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concentrations from the day 11, linearly increased. The minimum mycosis development rate at 10^4 conidia ml^{-1} concentration on the end of day 19 which was the last day was 58.3%. The mycosis development rate at 1×10^8, 1×10^9 and 3.7×10^9 conidia ml^{-1} concentrations was 100% and the difference was statistically significant ($P < 0.05$) (*Table 4*).

Table 3. *P. lilacinus of mycosis rate (%)*

Concentration (conidia ml^{-1})	7th	11th	19th
Control	0.00±0.00a	0.00±0.00a	0.00±0.00a
1×10^4	10.00±0.00a	21.66±1.66b	51.66±3.45b
1×10^5	13.33±2.10b	28.33±1.66b	65.00±2.23c
1×10^6	13.33±2.10b	38.33±1.66c	81.66±3.07d
1×10^7	20.00±2.52b	45.00±2.23c	100.00±0.00e
1×10^8	31.66±1.66c	55.00±3.42d	100.00±0.00e
$1 \times 1.6\ 10^8$	45.00±5.00d	60.00±2.58d	100.00±0.00e

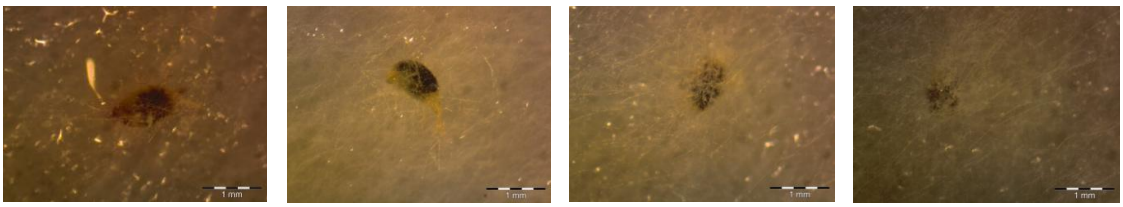
Means followed in the same column by different letters are significantly different $P < 0.05$, Tukey test)

Table 4. *B. bassiana of mycosis rate (%)*

Concentration (conidia ml^{-1})	7th	11th	19th
Control	0.00±0.00a	0.00±0.00a	0.00±0.00a
1×10^4	11.66±1.66b	28.33±1.66b	58.33±3.66b
1×10^5	15.00±2.23b	40.00±2.06c	71.66±1.66c
1×10^6	16.66±2.10b	43.33±2.10c	81.66±1.66d
1×10^7	21.66±1.66b	53.33±3.21d	86.66±2.10d
1×10^8	36.66±2.10c	66.66±2.10e	100.00±0.00e
1×10^9	50.00±4.47d	78.33±3.44f	100.00±0.00e
3.7×10^9	70.00±3.16e	78.00±3.00f	100.00±0.00e

Means followed in the same column by different letters are significantly different $P < 0.05$, Tukey test

The difference between observed mycosis rates of both EPF applications was statistically significant ($P < 0.05$). The mycosis development rate at the lowest concentration on the day 11 varied from 21 to 60% for *P. lilacinum* and from 28 to 78% for *B. bassiana*. The mycosis development rates of two different EPFs at different concentrations on days 7, 11 and 19 are presented in *Figure 1a-d*.



a **b** **c** **d**

Figure 1. *The mycosis development rates a- P. lilacinus 10^5, b- P. lilacnius 10^7, c- B. bassiana 10^5, d- B. bassiana 10^7 micosis*

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The mycosis rates at 10^4 conidia ml^{-1} concentration of *B. bassiana* and *P. lilacinum* were recorded on days 7, 11 and 19. The mycosis development at 10^4 conidia ml^{-1} concentration in both fungi application was similar at the end of day 19 day. The lowest mycosis rates at 10^4 conidia ml^{-1} concentration on day 7 were 13.3 and 15% for *P. lilacinum* and *B. bassiana*, respectively. The mycosis rate on day 19 was lower in *P. lilacinum* than in *B. bassiana*. The lowest mycosis rates at 10^6 conidia ml^{-1} concentration on day 7 were 13.3 and 16% for *P. lilacinum* and *B. bassiana*, respectively. The mycosis rate of *P. lilacinum* from day 11 at 10^7 conidia ml^{-1} concentration was 45% while that of *B. bassiana* was over 50%. The mycosis rate at the highest common concentration (10^8 conidia ml^{-1}) on day 11 was recorded over 50% for both fungi while a mycosis rate of 100% was recorded on day 19.

Discussion

The lowest mortality rate for both fungi, *P. lilacinum* and *B. bassiana*, used in the study at the lowest concentration of 10^4 conidia ml^{-1} on day 9 was 20% and 48.3%, respectively. The mortality rate at the highest concentration on day 5 was found to be 28.3% for *P. lilacinum* and 45% for B. *bassiana*. The mortality and mycosis rates in the *B. bassiana* treatment were higher than that of *P. lilacinum* and the mortality rate of *T. urticae* at 7 different concentrations ranged from 10 to 100% between days 3 and 9. Similarly, Shi et al. (2008b) reported that *B. bassiana* application caused 31.9 to 87.7% mortalities of *Tetranychus cinnabarinus*, a red spider, in a study with three different EPFs. In laboratory conditions, application of two different isolates of *B. bassiana* caused mortality between 22.1 and 82.6 of adult females of *T. evansi* mites (Wekesa et al., 2005). In another study conducted with four different EPFs, *M. anisopliae* V275 and *M. anisopliae* led to quite high mortality on adult stages of *T. urticae*. In the same study, *M. flavoviride*, *L. lecanii* and *B. bassiana* were found effective in the adult stage by 57.8, 50 and 45.8%, respectively, and the differences in adult mortality rates caused by entomopathogenic fungi were statistically significant (Doğan, 2016). Shi and Feng (2009) and Wekesa et al. (2006) reported that entomopathogenic fungus applications resulted in deaths in *T. urticae* mites and also reduced their reproductive potentials. Tamai et al. (2002) investigated the effects of 45 isolates belonging to *Aschersonia aleyrodis* (1), *Beauveria bassiana* (32), *Metarhizium anisopliae* (10), *Hirsutella* sp. (1) and *Paecilomyces farinosus* on *T. urticae*. The concentration of entomopathogenic fungi were 5×10^7 conidia ml^{-1} except for *Hirsutella* sp. which was 1.7×10^7 conidia ml^{-1}. *B. bassiana, Hirsutella sp*. and *M. anisopliae* were found more pathogenic than other fungi used in the study, and these fungi have been reported causing mortalities of mites from the third day after inoculation. Similar to the findings of others, the mortality of mites in this study started after the third day of 10^8 and 10^9 conidia ml^{-1}. *B. bassiana* application.

The highest mortality rate of *B. bassiana* at 1×10^7 conidia ml^{-1} concentration in this study was 58.3%, while Doğan et al. (2017) reported 80% mortality on *T. urticae* at 1×10^7 concentration of the same fungus. Wu et al. (2016) reported 37 to 49% adult mortality at 1×10^7 concentration of different *B. bassiana* strains, and fungal virulence was attributed not only to strains but also to the concentration, frequency of application and formulation. Therefore, 80% mortality was reported from *T. evansi* and *T. cinnabarinus* strains of *B. bassiana* (Wekasa et al., 2005; Shi et al., 2008a). Bugeme et al. (2014) found that 1×10^7 conidia ml^{-1} concentration of *B. bassiana* and *M.*

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anisopliae increased the mortality rates of *T. urticae* nymphs and adults and both EPFs were found effective.

The *P. lilacinum*, in the experiment, was very effective in two-spotted spider mites. The mortality rate linearly increased with the increase in concentrations. The mortality rate at 1×10^6, 1×10^7 and 1×10^8 conidia ml^{-1} concentrations ranged from 80 to 100%. Amjad et al. (2012) applied three different doses (1×10^6, 1×10^7 and 1×10^8 conidia ml^{-1}) of *Metarhizium anisopliae, Paecilomycis fumosoroseus* (Wize) and *Verticillium lecanii* against *T. urticae* and reported 90% mortality with *P. fumoroseus* application. In this experiment, the mortality rate at $1 \times 1.6\ 10^8$ conidia ml^{-1} *P. lilacinum* application was %66.3. In addition, development of mycosis was observed on day 7 of *P. lilacinum* applications against mites tested. Amjad et al. (2012) have also observed mycosis development on adult female mites from day 7 of *P. lilacinus* application against *Tetranychus kanzawai*.

The results of efficacy study conducted in Tokat province of Turkey revealed that *B. bassiana* and *P. lilacinum,* entomopathogenic fungi, caused effective mortality of mites at the end of 72 h (3 days) and 120 h (5 days), respectively. The development of mycosis has been intense from the day 7 at both fungi application. The *B. bassiana*, comparing the mortality and mycosis rates, was more effective against red spider mites than *P. lilacinum*.

In conclusion, we may conclude from the laboratory results that *B. bassiana* and *P. lilacinus* can be successfully used in controlling *T. urticae*. However, greenhouse and field trials are needed to support the findings of this study and to obtain more effective control against *T. urticae*. The use of entomopathogens in management of two-spotted red spiders has more advantageous to practice in terms of human, environment, benefices and predators. In addition, mass production, formulation development, licensing and further research efforts are required on entomopathogens fungi.

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APPENDIX

ANOVA (BB)

		Sum of Squares	df	Mean Square	F	Sig.
day1	Between Groups	.000	7	.000	.	.
	Within Groups	.000	40	.000		
	Total	.000	47			
day3	Between Groups	2014.583	7	287.798	18.668	.000
	Within Groups	616.667	40	15.417		
	Total	2631.250	47			
day5	Between Groups	10997.917	7	1571.131	96.685	.000
	Within Groups	650.000	40	16.250		
	Total	11647.917	47			
day7	Between Groups	7247.917	7	1035.417	55.222	.000
	Within Groups	750.000	40	18.750		
	Total	7997.917	47			
day9	Between Groups	26349.479	7	3764.211	179.783	.000
	Within Groups	837.500	40	20.938		
	Total	27186.979	47			

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ANOVA (PTR)

		Sum of Squares	df	Mean Square	F	Sig.
day1	Between Groups	.000	6	.000	.	.
	Within Groups	.000	35	.000		
	Total	.000	41			
day3	Between Groups	61.905	6	10.317	.867	.529
	Within Groups	416.667	35	11.905		
	Total	478.571	41			
day5	Between Groups	3661.905	6	610.317	42.722	.000
	Within Groups	500.000	35	14.286		
	Total	4161.905	41			
day7	Between Groups	3447.619	6	574,603	33,519	.000
	Within Groups	600.000	35	17,143		
	Total	4047.619	41			
day9	Between Groups	7223.810	6	1203.968	54.964	.000
	Within Groups	766.667	35	21.905		
	Total	7990.476	41			

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