THE EFFECTS OF WOOD VINEGAR ON SOME SOIL MICROORGANISMS

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Abstract. This study was carried out in order to determine the enzyme activity of the wood vinegar obtained from the hazelnut shells with the potential of bio-pesticide in agro-ecosystem soil, and its effect on the microfungi and heterotrophic bacteria in total. The study was realized in the production seasons of 2014-2015 and 2015-2016, in the ecological conditions of the province Muş (in Turkey) on winter wheat field and with four repetitions according to Randomized Blocks Experimental Design. The treatments within the scope of the experiment were conducted as the six different doses of wood vinegar at 0.5%, 1.0%, 2.0%, 3.0%, 4.0%, and 5.0% mL, and the control treatment which was only provided with tap water. Repeated Measurement ANOVA was used to determine the effect of wood vinegar at different doses, the production seasons and periods (pre- and post-treatment) on relevant features. As based on the statistical inferences, only the Treatment × Year and Period × Year interaction effects in terms of the number of heterotrophic bacteria (P = 0.000 and P = 0.011); and only the Period × Year interaction effects for the number of microfungi (P = 0.000) were found statistically significant. Therefore, the effect of the treatments made on the number of heterotrophic bacteria varied by the production seasons. In a similar way, the effect of the production season and periods on the number of microfungi also changed as based on the term. While the effect of period (P = 0.000) and the Treatment × Period interaction (P = 0.014) effect were significant for Alkaline Phosphatase activity, Year × Period (P = 0.001) and Treatment × Period interactions (P = 0.000) were found significant as related to Beta Glucosidase activity. In conclusion, it may be stated that the wood vinegar used at different doses with the purpose of protecting plant and/or crop in wheat agro-ecosystem does not have a negative effect on microbial factors determined in the soil, and especially, the treatment of 3% mL has a positive effect on bacteria number and Beta Glucosidase enzyme activity.
Keywords: *agroecosystem, heterotrophic bacteria, microfungi, soil enzyme activities, wood vinegar*

Introduction

The primary objective of agricultural practices is to obtain abundant and high-quality crops without disturbing ecological balances (Topal, 2011). Within the scope of sustainable plant protection studies, herbal products may have a considerable part as stabilizer for synthetic pesticides or effecting the actions of synthetic pesticides in soil (Hagner, 2013). In the production of herbal pesticides, a variety of plants and various technologies have been used (Tiilikkala et al., 2011). Wood vinegar (WV) and other weak pyrolysis liquids are produced as a by-product of carbonization processes, and in the archeological studies carried out so far, it has already been detected that they were used in the Neanderthal's era (Tiilikkala et al., 2010). WV consists of water by 80-90% and the rest amount is of more than 200 organic compounds (Kim et al., 2008). The main components of WV are acetic acid and also organic acids, phenolic, alkane, alcohol and ester compounds (Jothityangkoon et al., 2008). WV is a substance that is toxic or slightly toxic to the non-target organisms in water and soil (Hagner, 2013). It has been detected that WV obtained from *Vitex pubescens* displayed antifungal effect (Orahami and Yoshimura, 2013). Baimark and Niamsa (2009) stated that WV could restrain the growth of fungus, as its involving strong phenolic compounds. Velmurugan et al. (2009) found out that the neutralized WV showed a strong antifungal effect. Jothityangkoon et al. (2008) detected that the contamination decreased, in their studies for the effect of WV on fungi producing aflatoxine. Namlı et al. (2014) stated that the case in which WV is treated as biocide in in-vivo conditions would provide beneficial outcomes, in accordance with the data they have already obtained in in-vitro conditions. Orahami et al. (2018) indicated in their studies that WV has had antifungal effect. Eric et al. (2012) stated that distilled and non-distilled WV had inhibited the bacteria. WV can activate the edaphon number in a short time and at maximum, and also has a promoting role in increasing the number of bacteria especially in the root parts of vegetables (Shi, 2003). Duan et al. (2016) put forth that the four types of WV, which they had involved in their studies, had a strong blocking effect on three sorts of bacteria. In plant protection activities, WV stands for a promising solution in terms of preventing the growth of pathogenic bacteria and fungi (Chalermsan and Peerapan, 2009). Lee et al. (2010), in their studies, pointed out that WV had a strong antimicrobial effect. Additionally, it was stated that the applied WV treatments had increased the phosphatase, protease, urease and invertase enzyme activities (Aleandri et al., 2012). It was also observed that the WV application at different concentration in sandy soil created an effect on microbial biomass and enzyme activity (β-Glucosidase, alkaline phosphatase and dehydrogenase) (Du et al., 2016). Koç et al. (2018) detected that WV was promising in increasing the enzyme activities in some of its applications. We consider that the determination of the most effective and appropriate WV dose is a highly important issue for plant protection activities. In this way, to maintain a pest control process without using any chemical substance will become possible. The objective in this research is to determine the effect of the wood vinegar obtained by means of the carbonization of hazelnut shells in the potential of bio-pesticide on alkaline phosphatase and beta glucosidase enzyme activities, bacteria and microfungi populations in agro-ecosystem soil.

Materials and methods

This study has been carried out in Krasunia odeska wheat field that belongs to BERCE Alparslan Agricultural Administration (height: 1276, lateral: 380 47' 33. 1815", long: 410 32' 45. 700") located at a distance of 12 km to the central part of the province Muş (in Turkey) (*Fig. 1*). The study was made in accordance with the

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Randomized Block Design and with four repetitions. Each parcel in experiment has the size of 25 m^2 and at least a 2-m-gap was spaced out between blocks and parcels (Anonymous, 2016a). The treatments made in the experiment was realized in the way of six different doses of WV at 0.5%, 1.0%, 2.0%, 3.0%, 4.0% and 5.0% mL, and the control provided with tap water only. WV treatments were carried out as to the schedule for fertilizing and agricultural spraying by BERCE Alparslan Agricultural Administration. Treatments were made by means of 16 L backpack sprayer (AnadoluPower APW-16, mode of operation: mechanical/operating by side pressing, filling chamber with filter, having an internal tank stirrer, can spray the liquid up to a maximum distance of 4 m, with an adjustable nozzle at 4 different types, 2.70 kg in weight). The classification by the texture of soil in the experimental area is argillaceous soil with the rate of clay by 63.29%, silt ratio by 25.8% and sand ratio by 10.9% (Koç, 2017). The climatic data of the research area, respectively, the 1st year (2014-2015), the 2nd year (2015-2016) and areal precipitation, average temperature and average relative humidity values of the last decade (long period average) were presented in *Table 1* (Anonymous, 2016b).

***Figure 1.** Experimental area*

***Table 1.** Some climate data for 2014-15, 2015-16 years and last ten years (LTY) in Muş province*

Total rainfall (mm)			**Mean temperature (°C)**			**Mean relative humidity (%)**		
2014-15	2015-16	LTY	2014-15	2015-16	LTY	2014-15	2015-16	LTY
740.4	790.1	740.5	11.55	11.48	10.62	55.02	54.00	60.79

Wood vinegar (WV) used in this study has been supplied from an establishment developing bio-coal and wood vinegar products by the gasifier of hazelnut shells (Namlı et al., 2014). Wheat seeds were sown by No-Till method. Inputs used in the treatment: bottom fertilizer (NP 20-20-0, 13.700 g/da) upon sowing on the 16th October of 2014 and height fertilizer in bolting period (46% urea, 10 kg/ha^{-1}) were applied. Furthermore, on the 26th May of 2015, to the parcels for which WV was applied, WV at different doses and to the control parcels only tap water was applied. Upon sowing, on the 12th September of 2015, bottom fertilizer (NP 20-20-0, 13.7 kg/ha^{-1}) and height fertilizer in bolting period (46% urea, 10 kg/ha^{-1}) were applied. Moreover, to the parcels which WV was applied on the dates of April 24th, April 30th, May 8th and June 6th, 2016, WV application at different doses was performed and the control parcels were provided with only tap water. The average humidity (%) content of soil samples in the experimental area was detected (*Table 2*).

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Table 2. *Average amount of moisture (%), according to production seasons in the experimental area*

Years	Sample time	Average moisture (%)
2014-2015	19.05.2015	24.90
	25.06.2015	19.60
2015-2016	21.04.2016	27.97
	25.06.2016	16.27

Soil sampling process involved the soil samples obtained from 8 different parts of each parcel (Yardım, 1996) by means of nematode sampling instrument (from a depth of 10 to 30 cm), and those samples taken were blended well to be aggregated. Samples were collected in sterile polyethylene nylon bags and preserved at +4 °C in the laboratory for further analysis. Treatments were realized once in 2014-2015 and four times in 2015-2016. The determination of humidity percentage in soil samples according to Craze (1990), the detection of total number of microfungi and bacteria according to Benson (2001), and the determination of Beta-glucosidase and Alkaline phosphatase enzyme activities were done according to Naseby et al. (1997). Repeated Measurement ANOVA has been used in analyzing data sets. Analysis results have presented as graphically (*Figs. 2–8*). All statistical analyses have been performed by using IBM SPSS (Ver. 24).

Statistical model used for analyzing data set was:

$$Yijkl = \mu + \alpha i + \beta j + \alpha\beta ij + \pi l(ij) + \gamma k + \alpha\gamma ik + \beta\gamma jk + \alpha\beta\gamma ijk + \gamma\pi kl(ij) + \varepsilon m(ijkl)$$

$Yijkl$: observed value for the number of heterotrophic bacteria, the number of microfungi and enzyme activity in k period of lth experimental unit of jth year in the ith treatment,
μ: overall population mean,
αi: effects of ith treatment (i = 1, 2, 3, …,7),
βj: effects of jth year (j = 1, 2),
$\alpha\beta ij$: treatment by year interaction,
π l(ij): random effect of the experimental unit l in ith treatment and jth year,
γk: effect of k th period (k = 1 = before, k = 2 after),
$\alpha\gamma ik$: treatment by period interaction,
$\beta\gamma jk$: year by period interaction,
$\alpha\beta\gamma ijk$: treatment × year × period interaction,
$\gamma\pi kl(ij)$: experimental unit l by period interaction in ith treatment and jth year,
ε m(ijkl): random error term (Mendeş, 2013).

Results and discussion

Repeated Measurement ANOVA has been used for investigating effect of WV applications on the number of heterotrophic bacteria, the number of microfungi and enzyme activity (Winer et al., 1971; Mendeş, 2013). And the results of the Repeated Measurement ANOVA have been presented below:

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Effect of wood vinegar treatments on the number of heterotrophic bacteria

As based on the repeated measurement ANOVA results, only Treatment × Year and Period × Year interaction effects have been found statistically significant (P = 0.000 and P = 0.011). Therefore, the effects of treatments on heterotrophic bacteria have varied by years (2014-2015 and 2015-2016). Similarly, on the basis of period (pre- and post-treatment), the effect of year on heterotrophic bacteria have also differed. Treatment × Year and Period × Year interaction have been presented in *Figures 2* and *3*, respectively. As is seen in *Figure 2*, it has been determined that WV is effective in the treatments for 2014-2015 in terms of the decrease in heterotrophic bacteria, and however, there have been great differences in the case for 2015-2016.

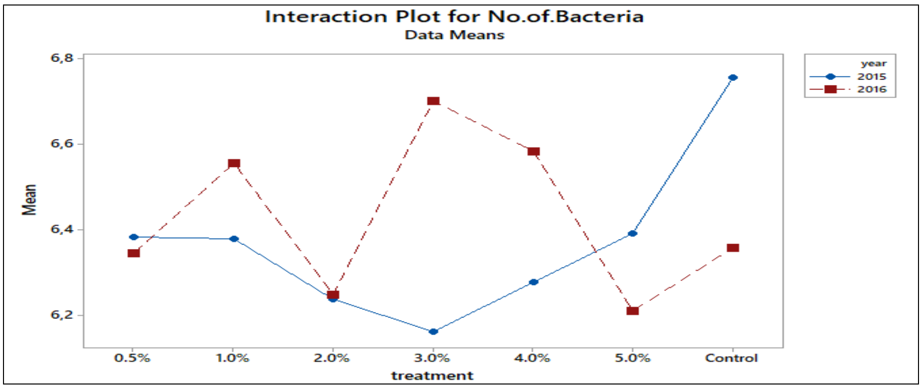

Figure 2. *Interaction plot for treatment by year in terms of number of heterotrophic bacteria*

When the Period × Year interaction plot is analyzed (*Fig. 3*), it is seen that the number of bacteria before the treatment in 2014-2015 is a bit more than the number of bacteria after the treatment. On the other hand, it can be observed that the post-treatment bacteria number in 2015-2016 is considerably more than the number for pre-treatment.

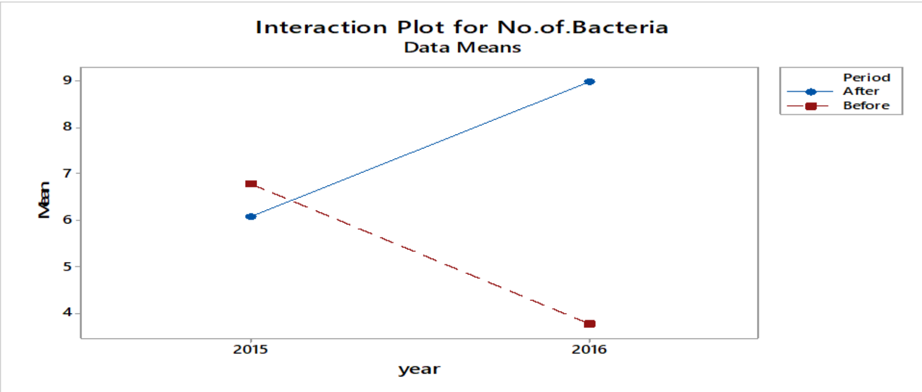

Figure 3. *Interaction plot for period by year in terms of number of heterotrophic bacteria*

Effect of wood vinegar treatments on the number of microfungi

Based on the Repeated Measurement ANOVA results, it has been seen that the only Period × Year interaction effect is statistically significant (P = 0.000). For this reason,

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the effect of year on the number of microfungi has varied by the period (before and after treatment). The Period × Year interaction plot is given in *Figure 4*. As it is seen in this plot, microfungi number in 2014-2015 is rather more, when compared to the period by 2015-2016, independently of treatment number. It is realized that the number of microfungi after treatment in 2015-2016 is greater than it is in 2014-2015.

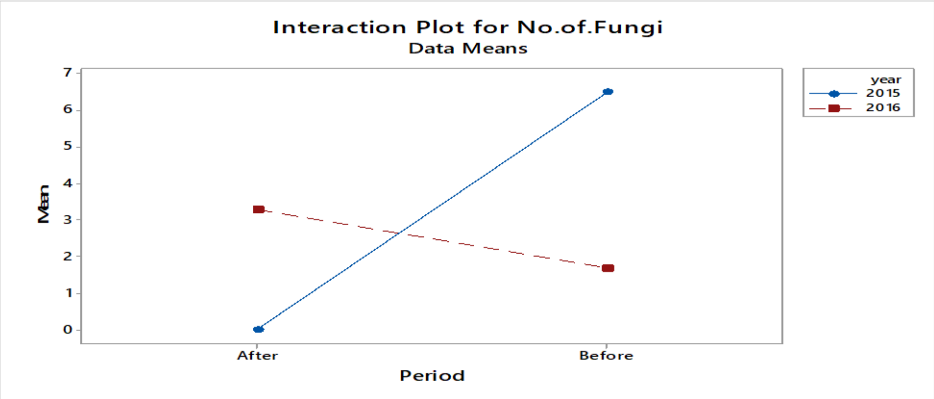

Figure 4. *Interaction plot for period by year in terms of number of microfungi*

Effect of wood vinegar treatments on enzyme activity

Considering the results of statistical analysis, it has been seen that the period effect (P = 0.000) and Treatment × Period interaction (P = 0.014) is significant. Therefore, the effects of treatments on Alkaline Phosphatase activity have varied by period (before and after treatment). Main effect plot for period and Treatment × Period Interaction plot are provided in *Figures 5* and *6*, respectively.

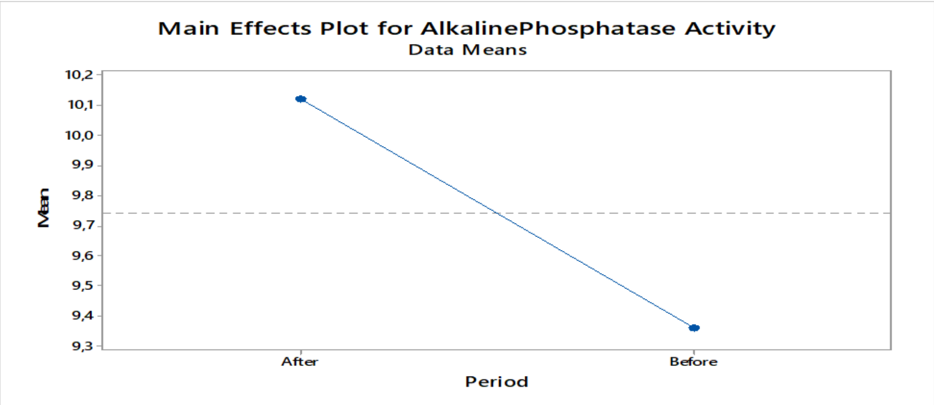

Figure 5. *Main effect plot for period in terms of alkaline phosphatase activity*

As it can be seen in *Figure 5*, the value of Alkaline Phosphatase activity after treatment is significantly higher than before treatment, independently of year and treatments. Treatment × Year interaction effect plot is given in *Figure 6*. When *Figure 6* is considered, the value of Alkaline Phosphatase activity has varied by treatments and years. However, the Alkaline Phosphatase activity at maximum is gained from the WV treatment of 1.0% mL, in 2014-2015. For 2015-2016, the highest value was obtained

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from the control treatment. As the reason for these results, it has been estimated that the case may be based on the extent, namely the highest, of WV doses used.

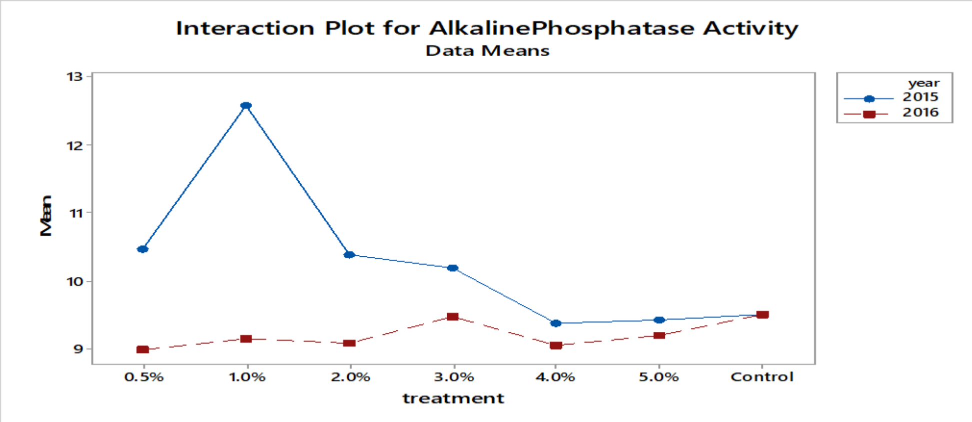

Figure 6. *Interaction plot for treatment by year in terms of alkaline phosphatase*

ANOVA results as related to Beta Glucosidase activity have revealed that Year × Period (P = 0.001) and Treatment × Period interactions (P = 0.000) are statistically significant. Therefore, the effect of year and treatments on Beta Glucosidase activity has varied by period (before and after treatment). Interaction plots for Year × Period and Treatment × Period are presented, respectively, in *Figures 7* and *8*. As it can be seen in *Figure 7*, the difference between pre-treatment and post-treatment is rather obvious, especially in 2015-2016.

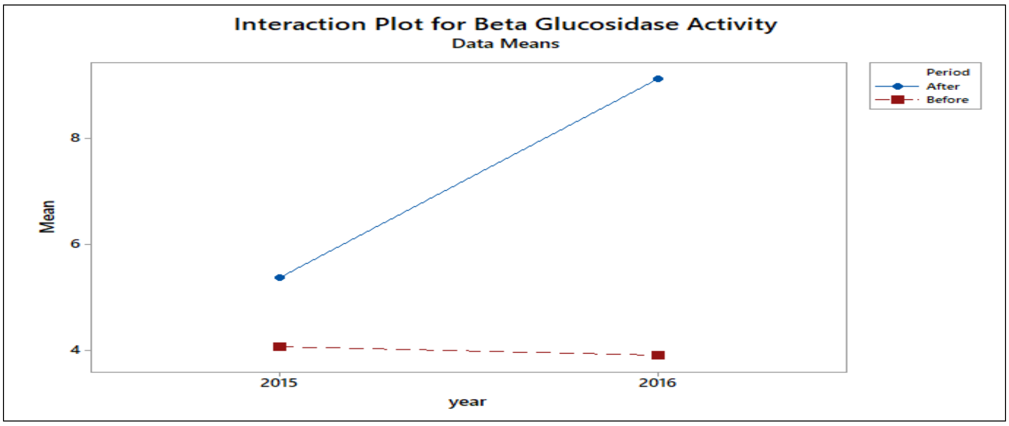

Figure 7. *Interaction plot for period by year in terms of beta glucosidase activity*

When the effect of treatments on Beta Glucosidase activity is considered (*Fig. 8*), it is seen that Beta Glucosidase activity has varied by treatment and years. In addition to this, while the highest value of Beta Glucosidase activity has been gained from the 3.0% mL treatment for the year 2014-2015, the maximum value for Beta Glucosidase activity has been received in the control treatment for 2015-2016. This result, as is in Alkaline Phosphatase activity, is thought to be arisen from that WV doses have been applied at higher levels, as well.

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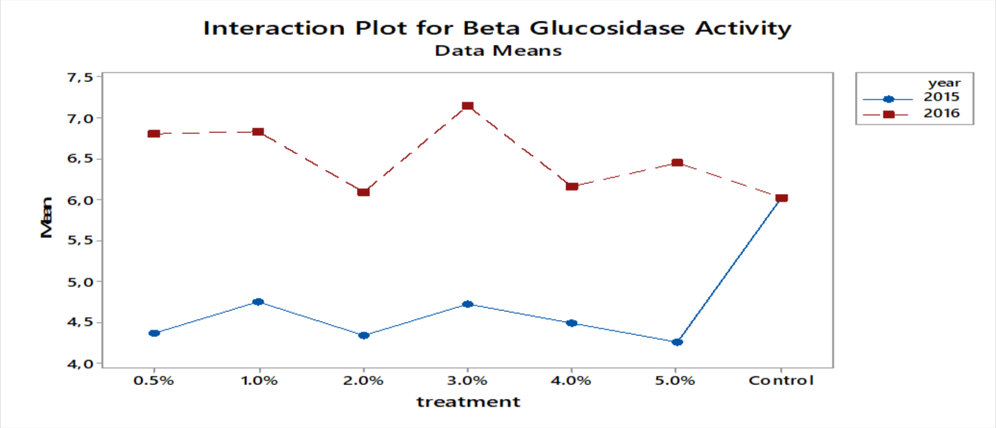

Figure 8. *Interaction plot for treatment by year in terms of beta glucosidase*

The utilization of bio-pesticides in agricultural practices as an alternative to the common pesticides is essential with regard to secure food, preservation of biological diversity and prevention of environmental pollution. In this study, the probable effect of wood vinegar obtained from the carbonization of hazelnut shells having the bio-pesticide potential on some microbial factors in agro-ecosystem soil has been investigated. It is realized that the findings reached at within the scope of this study are supported by the results of some similar studies that have been carried out before. For instance, the obtained findings as being relevant to the number of heterotrophic bacteria share similarity with the results in Shi (2003); however, it has been detected that they indicate some differences compared to the studies carried out by Nurhayati et al. (2005), Chalermsan and Peerapan (2009), Mao et al. (2010), Eric et al. (2012) and Duan et al. (2016). It has been thought that these differences may result from the changes related to the specific conditions, like climatic conditions, in the experimental area. When considered in terms of microfungi, especially in the production season of 2014-2015, it has been determined that WV treatments have effect on the decrease in the number of microfungi. Also, it has been noticed that the results similar to these findings have already been reached in the studies by Jothityangkoon et al. (2008), Baimark et al. (2008), Baimark and Niamsa (2009), Chalermsan and Peerapan (2009), Velmurugan et al. (2009), Lee et al. (2010), Ibrahim et al. (2013), Oramahi and Yoshimura (2013), Saberi et al. (2013), Namlı et al. (2014), Chuaboon et al. (2016), Ahadiyat et al. (2018) and Oramahi et al. (2018). It has been observed that the effect of WV treatments applied in this study on the enzyme activities (Alkaline phosphatase and Beta-glucosidase) varies according to before and after treatment. However, it is understood that the enzyme activities generally increase after WV treatments, and similar results have also revealed in the studies by Aleandri et al. (2012) and Koç et al. (2018). It can be said that WV treatments will be able to affect microbial biomass, as based on the findings obtained from this study, as Du et al. (2016) and Rui et al. (2014) stated before in their researches.

Conclusion

Pesticides which are widely used in agricultural practices harm the food safety and/or security, environment, biological diversity and biological chain. This issue affects soil

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productivity and human health in a serious and negative way, and these negative effects become more prominent in time. In accordance with the 2009/128/EC Framework Directives, the usage of pesticides must be minimized, and primarily the low-risk pesticides like bio-pesticides must be taken into consideration. Wood vinegar is a product which has a very low environmental risk and can quickly decay in the soil as a result of microbial activities. At this point, we consider that wood vinegar can be used as a bio-pesticide, i.e. as an alternative to the pesticides. In this study, it has been detected whether wood vinegar used in wheat agricultural eco-system has a negative effect on microbial factors determined in the soil, or not. As based on this, it can be put forth that wood vinegar will be able to be used securely as a pesticide. Additionally, the increase in enzyme activities and productivity may indicate that using WV practically is able to create a positive effect in crop production. Considering wood vinegar treatments, it can be stated that especially its doses and use frequencies have an effect on the microbial factors in soil. It is estimated that the dose of 3.0% mL will especially create a positive effect on the number and activity of biological factors in soil, in general.

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