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Effect of integrating entomopathogenic fungi and botanical extracts against Russian wheat aphid, *Diuraphis noxia* Mordvilko (Hemiptera: Aphididae) in West Showa, Ethiopia

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Ethiopia is the only country in Sub-Saharan Africa where smallholder wheat production meets more than 70% of the national consumption demand. Russian wheat aphid (RWA; *Diuraphis noxia* (Mordvilko) (Hemiptera: Aphididae) is one of the major insect pests of wheat throughout the world including in Ethiopia. Wheat yield loss due to RWA damage in Ethiopia was estimated to be 70%. Therefore, the present study was carried out to determine the effect of integrating the potentials of the plant extracts of Neem, *Azadirachta indica* L. (leaf and seed) and the Entomopathogenic fungi (EPF); *Beauveria bassiana* and *Metarhizium anisopliae* against the RWA. After 7 days of applications; the combined mixture of *B. bassiana* with *M. anisopliae* caused the maximum loss (73.4%) of aphids, while the combination of Neem leaf with seed extracts recorded the least (57.82%) abundance of aphids, which were negatively affected by single and combined treatments. The lowest abundance was recorded when the aphid was treated by the combination of both *B. bassiana* and *M. anisopliae* as well as the combined mixture of Neem leaf and seed extracts. The results indicated that bio-pesticides, based on entomopathogens and botanical extracts (Neem) can be the best replacements for synthetic chemicals and caused significant reduction in survival and abundance of RWA. Therefore, they could be used as promising natural alternatives to synthetic insecticides against RWA in Ethiopia.

Key words: Russian wheat aphid, Diuraphis noxia, Beauveria bassiana, Metarhizium anisopliae, Neem, efficacy.

INTRODUCTION

Wheat *(Triticum aestivum* L.*)* is one of the world's leading cereal grains serving as a stable food for more than one third of the global population and also one of the major cereal crops grown in the highlands of Ethiopia (Figueroa et al., 2017). Ethiopia is the only country in Sub-Saharan Africa where smallholder wheat production meets more than 70% of the national consumption demand (Shiferaw et al., 2011). In Ethiopia, wheat is widely cultivated both in uni-modal and bi-modal rain fall areas and the major wheat production areas are West Shewa, Western

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Hareghe, Illubabur, Bale, Arsi, Shewa, Sidamo, Tigray, Northern Gondar and Gojam zones in Ethiopia (Hundie et al., 2000). The area under wheat production in Ethiopia is estimated to be about 1.5 million hectares and it is cultivated in a wide altitude range from 1500 to 3000 m. a. s. l. However, the most suitable agro-ecological zones for wheat production fall between 1900 and 2700 m. a. s. l (Beyene*,* 2017; Hill and Fuje, 2017).

Bread wheat (*Triticum aestivum* L.) and durum wheat (*T. turgidum* L. *var. durum*) are the two species which are mainly cultivated by small scale farmers in Ethiopia which are grown in the country both for food and income generation purposes. The crop has high economic value in Ethiopia and the area cultivated under wheat had increased production and productivity of the crop in the last few years. The national average yield is estimated to 2.4 tons/ha (CSA, 2015). According to CSA (2016/17), in Ethiopia wheat is produced on 1.7 million hectares of land with annual average production of about 2.6 metric tons, which is by far below the world's average yield/ha which is about 3.3 tones/ha (Curtis et al., 2002). The low productivity of the crop is attributed to number of factors including biotic (diseases, insects and weeds), abiotic (drought, acidity, alkaline, extreme temperatures, depleted soil fertility and snow) and low adoption of new agricultural technologies (Ayele et al., 2008). The wheat production is decreased due to biotic factors, such as incidence of insect pests and diseases. Aphids cause yield losses either directly (35-40%) by sucking the sap of the plants or indirectly (20-80%) by transmitting viral and fungal diseases (Aslam et al., 2005). Among the different insect pests, the Russian wheat aphid (RWA), *Diuraphis noxia* (Hemiptera: Aphididae) is one of the major insect pest problem of wheat worldwide (Botha et al.*,* 2006) including Ethiopia. This species causes serious yield losses by direct feeding and indirectly by spreading wheat pathogens during early stages of wheat crop (Aslam et al., 2005). Wheat yield loss due to RWA damage in Ethiopia was estimated to be 70% (Miller and Haile, 1988); this occurred during years of rainfall shortage, which favors the development of the pest population (Kinelskaya, 2010).

Bio-pesticides, based on entomopathogenic fungi (EPF) and botanicals are the best replacements for the synthetic chemicals (Ali et al.*,* 2018). Among the EPF, *Beauveria bassiana and Metarhizium anisopliae* are broadly used for the bio-control of many insect pests because of its virulence (Barbarin et al.*,* 2012; Reddy et al., 2014). Some botanicals possess an insecticidal activity. Among different botanicals, Neem products can result in different concentrations of the active compounds, as well as different biological effectiveness (Roychoudhury, 2016). The compatibility between EPFs and botanicals are the selection of appropriate products under IPM programs (Neves et al., 2001). Such combined applications can improve the efficacy of control by reducing the applied amounts, minimizing environmental pollution threats and build-up of pest resistance (Usha et al.*,* 2014). Therefore, the present study was conducted to determine the effect of integrating the potentials of the plant extracts of *Azadirachta indica* (Neem leaf and seed) and the entomopathogenic fungi; *Beauveria bassiana* and *Metarhizium anisopliae* against RWA under field conditions.

MATERIALS AND METHODS

The experimental study was conducted in selected districts of West Showa Zone, Ethiopia during the off season of 2018/19 separately. The study localities have a bimodal rainfall distribution and also a typical sub-humid, high altitude agro-climatic zone. The West Shewa zone is located at latitude (N) 8°57' and longitude (E) 38°07', the altitude ranging from 1380 to 3300 m. a. s. l. and the average rainfall of 600 to 1900 mm. The mean minimum and maximum air temperature of the area is 17.2 and 24.4°C, respectively. The soil of the experimental study site is vertisol with light black in color and sandy loam soil type with pH value of 6.8.

Experimental materials used

The entomopathogenic fungi, *Beauveria bassiana (*PPRC-56) and *Metarhizium anisopliae* (PPRC-2) were obtained from Ambo Plant Protection Agricultural Research Center, Ethiopia. The fresh leaves and seeds of Neem (*Azadirachta indica L*.) were collected from Melkassa Research Center, Melkassa, Ethiopia.

Preparation of Neem leaves and seed extracts and EPF

The fresh leaves and seeds of Neem were shade dried and then separately made powdered form using an electric grinder. The dried plant extracts were prepared by the method of Ali et al. (2018). After evaporation by using rotary evaporator at 60°C under vacuum, the dried plant extracts were brought to constant volume, using a hot air oven (60°C). These extracts were stored under 4°C till used as a stock solution. Seven percent concentration of Neem leaf and seed extracts were prepared by dilution with distilled water from the stock solution for experimental evaluation. The entomopathogenic fungi, *B. bassiana* (PPRC-56) and *M. anisopliae* (PPRC-2) were prepared at CFU count of 10^{-8} fungal spores/g. The concentrations of 10^{-8} spores/ml of EPF were obtained by dissolving 1 g EPF formulations in 100 ml of water to apply on the aphids.

Experimental design, treatments and applications

The field trial consisted of two entomopathogenic fungi (alone and in combination) and one botanical, Neem (leaf and seed alone and in combination), two standard checks (Dimeto 40% WP and Malamar 50% WP insecticides) and one untreated control. The experimental design was randomized complete blocks with nine treatments replicated three times that were evaluated against RWA during the off - cropping season of 2018-2019.

The following treatments were:

T1 - *Beauveria bassiana alone*
T2 - *Metarhizium anisopliae alone*
T3 – Combination of both EPF
T4 - Neem leaf extract alone
T5 - Neem seed extract alone

T6 – Combination of both plant extracts.
T7 - Dimeto 40% EC (Standard check)
T8 - Malamar 50% EC (Standard check)
T9 - Control

Plot size was consisted of 1.5 m × 3 m and row spacing of 30 cm with 5 seedlings in each row with three rows per plot harvested and each row accommodating 20 plants. The two bio-control agents of EPF were applied as suspension of the concentrations of 10^8 spores/ml were obtained by dissolving 1 g EPF formulations in 100 ml of water to apply on the aphids at the time of sowing on the field. The EPF, *B. bassiana* causes white muscadine disease in insects (Groden, 1999). When spores of this fungus come in contact with the insect cuticle, they germinate and grow directly through the cuticle to the inner body of their host even without being consumed and proliferate throughout the host's body producing toxins, and eventually killing it. Once the fungus has killed its host, it grows back out through the softer portions of the cuticle covering the insect with a layer of white mold that gives rise to its common name "white muscadine disease".

Seven percent concentration of Neem leaf and seed extracts were prepared by dilution with distilled water from the stock solution for experimental evaluation sprayed on the aphids at the time of sowing on the field. Neem products work as insect growth regulators, anti-feedants, ovi-position detterants, sterilants, repellents and comprise residual insecticidal properties. All the recommended agronomic practices for wheat production were used as described by Anon (1989). Weeding was performed twice, after two weeks of the plant emergence and 25 days after emergence.

Data collection and statistical analysis

The daily percent efficacy (N±SE) of aphids present in the field was recorded after the treatment with different botanicals and entomopathogenic fungi alone and in combinations. The data collected from different sources was analyzed using descriptive statistics (mean, frequency etc). The mean comparisons of the parameters were tested by significance difference level and probability at 5% and simple correlation analysis was made. The evaluation of the tested materials and techniques was based on the incidence percentage and corrected by Abbot's formula (Abbott, 1925).

$$\text{Efficacy\%} = \left(\frac{\text{pre spray count} - \text{Post spray count}}{prespraycount}\right) * 100$$

The efficacy percentage data were subjected to factorial analysis of variance (ANOVA), using Statistically Analysis Software (SAS, 2000). The means comparisons were compared by applying Duncan's Multiple Range Test (DMRT) at 5% level of significance to evaluate the impact of treatments on abundance of treated Russian wheat aphids. The results was summarized and presented in tabular and graphical forms.

RESULTS AND DISCUSSION

Effect of Neem leaves and seeds and entomopathogenic fungi against Russian wheat aphids

The combinations of EPF, *B. bassiana* with *M. anisopliae* recorded great latent by causing significantly higher efficacy within 168 h as compared to other treatments against RWA (LSD= 1.013, CV= 23 and SE±=5.48) which was followed by the combinations of neem leaves and seed extracts that recorded significantly after 168 h, while the lowest efficacy (38.33%) was recorded in the control treatment after 168 h. The remaining treatments caused half way efficacy among the treated aphids. More than half of the treated aphids recorded high after 96 h in T4, T5 and T6, while in other treatments, the efficacy was comparatively intermediate, but higher than the control treatment (Table 1). T1, T2 and T3, also showed little efficacy among treated aphids after 96 h, but, Vu et al. (2007) and Kim et al. (2013) were reported, the EPF was recorded the substantial efficacy in aphids after 24 h. In this study, after 7 days, total aphids efficacy percentage (corrected by using Abbot's formula) indicated that the binary mixture of *B. bassiana* and *M. anisopliae* caused the maximum efficacy (73.4%). Neem leaf and seed extract also gave significantly higher (57.82%) efficacy percentage among the treated aphids, after 7 day with respect to other treatments. The binary mixture of *B. bassiana* with *M. anisopliae* showed the maximum efficacy percentage (53.4%) after 144 h, while the other treatments imposed the intermediate rates by 38.33% after 7 days of botanical applications (Figure 1). The results is similar with Kim et al. (2013) and Saleh et al. (2016) which reported that *B. bassiana* and *M. anisopliae* showed deleterious effects to different aphid species quoted by Sajjad et al. (2018). The variations among efficacy percentages were not significantly different at all the treatments (LSD = 0.865, CV = 20, and SE± = 4.00). The present study showed the virulence of EPF (*B. bassiana* and *M. anisopliae*) and botanical extracts (neem) against the RWA confirmed with Sajjad et al. (2018) that compatibility of entomopathogenic fungi and botanical extracts against the wheat aphid, *Sitobion avenae* (Hemiptera: Aphididae). *B. bassiana* was significantly effective when applied singly or in combination with *M. anisopliae*. This result is similar with Kim et al. (2013), that biological control of aphid using fungal culture and culture filtrates of *Beauveria bassiana*. According to Sajjad et al. (2018) reported that EPF, *B. bassiana* caused high losses among the treated wheat aphid due to mycosis, secreting specific hydrolytic enzymes such as proteinase, chitinase and lipase.

Metarhizium anisopliae had less efficacy compared to *B. bassiana.* However, *B. bassiana was* caused more than (73.82%) efficacy percentage at a single or a binary mixture treatment. The results confirmed that with Castiglioni et al. (2003) reported that compatibility between *B. bassiana* and *M. anisopliae* in the control of *H. tenuis*. The previous study of Kim et al. (2013) and Saleh et al. (2016) also acknowledged that *B. bassiana* and *M. anisopliae* showed deleterious effects to different aphid species which is cited by Sajjad et al. (2018). Neem extracts also caused higher efficacy rates in the RWA followed by *B. bassiana* and *M. anisopliae*. Also according to several authors reported that, fatalities

Table 1. Mean percent efficacy % (N±SE) of botanicals and EPF under field conditions.

Treatments*	Mean percentage						
	24 h	**48 h**	**72 h**	**96 h**	**120 h**	**144 h**	**168 h**
T1	14.89±0.26^{b}	21.47±0.37^{bc}	35.3±0.62^{bc}	35.3±0.62^{bc}	43.05±0.75^{c}	46.95±0.82^{c}	50.7±0.89^{bc}
T2	14.89±0.26^{b}	31.05±0.54)^{bc}	31.05±0.54^{bc}	43.05±0.75^{bc}	43.05±0.75^{c}	43.05±0.75^{bc}	46.95±0.82^{bc}
T3	21.47±0.37^{bc}	26.57±0.46^{bc}	43.09±0.75^{cd}	39.23±0.68^{bc}	46.95±0.82^{c}	46.95±0.82^{c}	58.95±1.03^{cd}
T4	16.02±0.28^{cd}	28.72±0.50^{bc}	28.72±0.50^{bc}	33.66±0.59^{bc}	38.25±0.67^{c}	38.25±0.67^{bc}	38.25±0.67^{b}
T5	14.87±0.26^{b}	14.89±0.26^{b}	21.47±0.37^{b}	26.57±0.46^{b}	31.05±0.54^{b}	35.3±0.62^{b}	43.05±0.75^{b}
T6	16.02±0.28^{bcd}	16.02±0.28^{bc}	28.72±0.50^{bc}	33.66±0.59^{bc}	38.26±0.67^{c}	41.39±0.72^{bc}	49.5±0.86^{b}
T7	37.78±0.66^{d}	41.39±0.72^{c}	55.96±0.98^{d}	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00)^{e}	0.00±0.00^{e}
T8	38.55±0.67^{d}	45±0.79^{c}	61.91±1.08^{d}	61.91±1.08^{d}	61.91±1.08^{d}	65.95±1.15^{d}	70.05±1.22^{d}
T9	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{e}
LSd at 0.01	**0.970**	**1.615**	**1.49**	**1.033**	**0.97**	**0.865**	**1.013**
CV%	**12.52**	**22.49**	**24.54**	**19.42**	**20.37**	**20.00**	**23.59**
SE±	**5.04**	**13.93**	**11.99**	**5.70**	**4.59**	**4.00**	**5.48**

Means showing the same letter within a column are not significantly different (P ≤ 0.05) *T1 = *Beauveria bassiana alone*; T2 = *Metarhizium anisopliae alone*; T3 = Combination of *B. bassiana* + *M. anisopliae*; T4 = Neem Leaf extracts alone; T5 = Neem seed extracts alone; T6 = Combination of neem leaf + seed extracts; T7 **=** Dimeto 40% EC Standard Check, T8 = Malamar 50% Standard check EC W/V, T9 = Control/untreated.

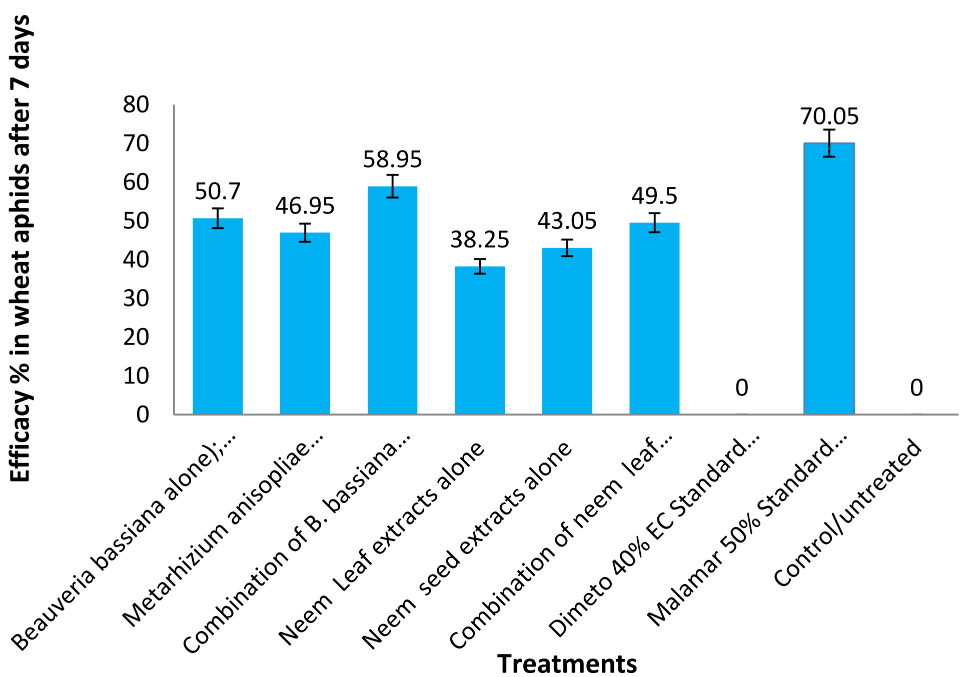

Figure 1. Effectiveness of botanicals and entomopathogenic fungi in controlling Russian wheat aphids after 7 days of application both in combination and alone.

caused by neem extracts were well documented showing that neem-based products and certain botanicals had a good potential to minimize the populations of different aphid species by reducing their survival and productiveness with their physical and biological actions (Pavela et al., 2004; Patil and Chavan, 2009; Nia et al., 2015; Sharma et al., 2016). The highest efficacy percentage was recorded when *B. bassiana* was applied in combination with *M. anisopliae* (Figure 1). The study result is similar to previous study of Wang and Knudsen (1993) which shows the effect of *B. bassiana* on fecundity of the RWA. The study also agree with, Castiglioni et al. (2003), which gave an accounted on the compatibility between *B. bassiana* and *M. anisopliae* with Nimkol-L in the control of *H. tenuis*. Neem extract possesses chemical compounds, which can cause disturbance in digestion,

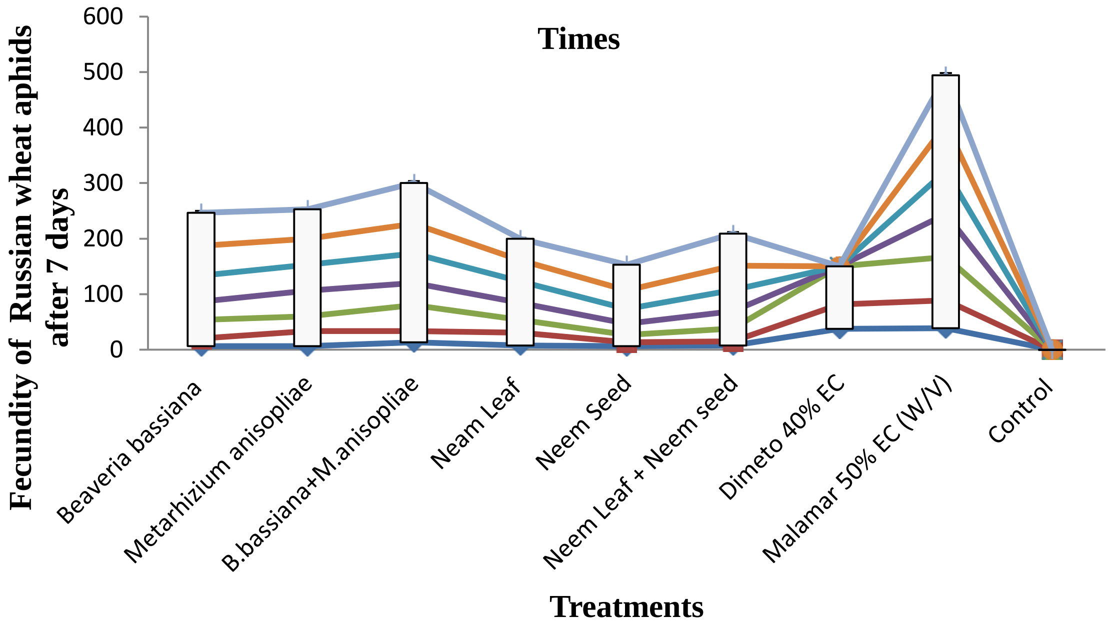

Figure 2. Fecundity of Russian wheat aphids 7 days after application of botanicals and entomopathogenic fungi alone and in combination.

Table 2. Correlation matrix for total fecundity of Russian wheat aphids after 7 days of application of botanicals and entomopathogenic fungi alone and in combination.

	24 ha	48 ha	72 ha	96 ha	120 ha	144 ha	168 ha
24 ha	1.00						
48 ha	0.898**	1.00					
72 ha	0.948**	0.892**	1.00				
96 ha	0.150	0.316	0.288	1.00			
120 ha	0.000	0.142	0.182	0.968**	1.00		
144 ha	-0.015*	0.085	0.152	0.953**	0.991**	1.00	
168 ha	-0.122*	-0.081*	0.055	0.881**	0.952**	0.966**	1.00

*=Correlation is not significant at the 0.01 level; **=Correlation is significant at the 0.01 level and not significant at the 0.05 level.

growth inhabitation, affect maturation and reproductive ability by decreasing appetite in exposed insects leading to death (Russo et al*.,* 2015). This study also have the same opinion with that, Depieri et al., (2005), they concluded the compatibility of plant extracts neem leaf and seed as well as EPF, *B. bassiana* and *M. anisopliae* that might be due to inconsistent concentrations of phytoalexins, sulfurade, terpenoids, and triterpenoids compounds. Also Ribeiro et al. (2012) reported the compatibility of EPF, *B. bassiana* that depends on the qualitative and quantitative variations in secondary metabolites composition, which may have a negative impact on the entomopathogenic microorganisms. According to the information of Mohan et al. (2007), it is also important that the genetic variability of fungal isolates may also produce varied grades of susceptibility or compatibility to different phytosanitary products.

Effect of botanicals and entomopathogenic fungi on fecundity of RWA

The control/untreated and neem leaf and seed extracts showed the maximum and minimum richness in 7 days respectively. Russian wheat aphid treated with *M. anisopliae* is low during the same time. The lowest fecundity was recorded at *B. bassiana* and *M. anisopliae* (binary mixture) treatment in 7 days (Figure 2). This agreed with Usha et al. (2014) that reported on detection of compatibility of entomopathogenic fungus *B. bassiana* (bals.) Vuill. with pesticides, fungicides and botanicals. The binary mixtures of *B. bassiana* and *M. anisopliae* also showed substantial decrease in the success of Russian wheat aphid after exposure to the pests. So, the females in control treatment possessed lower reproductive fitness than at all other treatments (LSD = 23, CV = 23, and P < 0.001). Fecundity is a critical aspect of insect population, which can be affected by entomopathogens and plant extracts as reflected in terms of lowered abundance, when *M. anisopliae* exposed to them in the study although variations were observed under different treatments. Treated aphids reproduced at slower rate than untreated ones, except when they were exposed to *M. anisopliae* and *B. bassiana* binary mixture. In the same way, Bayhan et al. (2006), neem extracts negatively affected the longevity as well as fecundity of cotton aphids. Similarly, Pavela et al. (2004) reported the lowest fecundity in cabbage aphids after exposure to systemic neem extract. Citrus aphids also produced lowered number of nymphs after they were exposed to neem seed extracts (Tang et al., 2002). The negative effects of fungal infections on abundance and egg fertility were also reported, using fungal species *B. bassiana and M. anisopliae* on Russian wheat aphid (Wang and Knudsen, 1993)

Pearson's correlation coefficients for fecundity of Russian wheat aphids after 7 days of application of botanicals and entomopathogenic fungi alone and in combination are presented in Table 2. A significant negative relationship was observed after 24 h and 144 h (r = 0.015, p < 0.05), indicating that not effective with a shorter time had lower efficacy during the initial application period. 24 h was also negatively correlated with 144 and 168 h. This means that the efficacy with the shortest after application time has a less effective rate of botanicals and entomopathogenic fungi alone and in combination. After 48 h application of botanicals and entomopathogenic fungi alone and in combination was negatively correlated with 168 h, meaning that these parameters cannot be used to predict the effectiveness of botanicals and entomo pathogenic fungi alone and in combination against RWA.

After 96 h applications of botanicals and entomopathogenic fungi alone and in combination had a significant positive correlation with 120, 144 and 168 h. After 120 h applications of botanicals and entomopathogenic fungi alone and in combination was positively correlated with 144 and 168 h, whereas 120 h was strongly correlated with 144 and 168 h. There was no correlation between 24, 48 and 72 h.

CONCLUSION AND FUTURE PROSPECTS

In the end, the study showed that the binary mixtures or combinations effect of EPF and botanicals are particular in action. The control of Russian wheat aphid (*D. noxia*) was benefitted by the applications of the binary mixtures of EPF and botanicals, but their efficacy depended upon the mixing components. A significant negative relationship was observed after 24h, and 144h (r = 0.015, p < 0.05), indicating that not effective with a shorter time had lower efficacy during the initial application period.24h was also negatively correlated with 168h. This means that the efficacy with the shortest after application time has a less effective rate of botanicals and entomopathogenic fungi alone and in combination. After 48h application of botanicals and entomopathogenic fungi alone and in combination was negatively correlated with 168h, meaning that these parameters cannot be used to predict the effectiveness of botanicals and entomopathogenic fungi alone and in combination against RWA with in shortest time. After 96h applications of botanicals and entomopathogenic fungi alone and in combination had a significant positive correlation with 120h, 144h and 168h. After 120h applications of botanicals and entomopathogenic fungi alone and in combination was positively correlated with 144 and 168 h, whereas 120 h was strongly correlated with 144 and 168 h. There was no correlation between 24, 48 and 72 h. However, more research is needed to fractionate and isolate the active ingredients responsible for the loss of aphids and to test their hazards and well-suited for a better biological control of Russian wheat aphids.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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