The effectiveness of botanicals and fungus isolates from fish pound on the population reduction of Bemisia tabaci

Aslam M.
Department of Zoology, University of Agriculture, Faisalabad, Punjab, Pakistan
University of Agriculture, Faisalabad, Sub-Campus Burewala-Vehari, Punjab, Pakistan

Sarwar A.
Department of Zoology, University of Agriculture, Faisalabad, Punjab, Pakistan
University of Agriculture, Faisalabad, Sub-Campus Burewala-Vehari, Punjab, Pakistan

Khan S.Z.
University of Agriculture, Faisalabad, Sub-Campus Burewala-Vehari, Punjab, Pakistan

Awan M.I.
University of Agriculture Faisalabad Sub-Campus at Depalpur, Okara, Punjab, Pakistan

Munir M.
University of Agriculture, Faisalabad, Sub-Campus Burewala-Vehari, Punjab, Pakistan

Mushtaq M.N.
University of Agriculture, Faisalabad, Sub-Campus Burewala-Vehari, Punjab, Pakistan

Ahmad S.
University of Agriculture, Faisalabad, Sub-Campus Burewala-Vehari, Punjab, Pakistan

Hanif A.
University of Agriculture, Faisalabad, Sub-Campus Burewala-Vehari, Punjab, Pakistan

Masroor A.
University of Agriculture, Faisalabad, Sub-Campus Burewala-Vehari, Punjab, Pakistan, masroorakb@gmail.com

Ashraf M.R.
University of Agriculture, Faisalabad, Sub-Campus Burewala-Vehari, Punjab, Pakistan, rizwan.ashraf@uaf.edu.pk

Abstract

Whitefly (Bemisia tabaci) is a key pest in majority of the crops due to higher species evenness, over lapping generations, activity throughout the year, and transmission of different plant diseases. It is the time to work for effective alternative for conventional insecticides to achieve sustainability. In this study, the pathogenicity of two entomopathogenic fungi (Beauveria bassiana and Metarhizium anisopliae) isolates from fish pound and toxic potential of two botanicals (extract of Allium sativum, and Azadirachta indica) was evaluated against Bemisia tabaci. The individuals of Bemisia tabaci were exposed to three concentrations (0.4 ppm, 0.5 ppm and 0.6ppm) with one control treatment of fungi, and three concentrations (5%, 10% and 15%) with one control treatment of plant extracts. The Mean mortality was recorded after different time intervals (24, 48, 72 and 120 hours). The highest mortality (71%) was observed at highest concentration of Metarhizium anisopliae after 120 hours against nymph of Bemisia tabaci, whereas lowest (47%) towards A. indica. Metarhizium anisopliae was also significantly affective against adults of Bemisia tabaci with highest mortality percent (57%) and A. indica was observed least affective with lowest mortality percentage (37%). The reduced mortality was observed towards botanicals because the prolonged time to express their toxic potential and fast degradation; on the other hand enhance activity of the fungi was observed due to their fast growth on soft bodied insects. In conclusion, the fungus (Metarhizium anisopliae) could precisely be used in integrated pest control strategy for the control of Bemisia tabaci.

Keywords: *Beauveria bassiana, Metarhizium anisopliae, Allium sativum, and Azadirachta indica, Bemisia tabaci.*

INTRODUCTION

Whitefly (Bemisia tabaci) is a leading pest in agro-ecosystem and cause huge crop losses during the active period from April to October. In Pakistan, B. tabaci is obtained the status of a serious of 104 plants belonging to 24 different families. The pest is remained active throughout the year poses serious threat to different crops on only due to sucking behavior but also spreading different diseases. It has 12 generations and remainder energetic all the way through the time. Whitefly is a polyphagous insect with 12 generation and is well known for abrupt increase in their population. A solitary female can transmit virus in different species that further can swarm numerous other plants (Hussain et al., 1991). B. tabaci are nuisance creatures that distress an extensive assortment of produces all over the world. Even though more than, 1200 species have been designated (Bink-Moenen et al., 1990). The whitefly has a worldwide dissemination and wide-ranging inherited multiplicity (Dinsdale et al., 2010). Billions of tones of insecticides are imported every year to minimize the damage of these pests.

Biological pesticides are derivative from accepted resources comprising faunas, florae, microorganisms, in addition particular raw materials correspondingly recognized such as bio pesticides (Glare et al., 2012). The biological pesticides in profitable farming is presence experienced meanwhile extensive to sidestep difficulties connected by means of undifferentiating usage of insect killer (Kumar et al., 2003; Henry et al., 1999). These are decomposable, cost-effective and non-hazardous to anthropological life and advantageous creatures (Copping et al., 2000: Patel et al., 2009). The neem tree is maximum accustomed herbal by means of commanding insecticidal belongings consequently its improvement in 1966 (Mordue et al., 2005; Morgan, 2009). Neem has remained assisted as pest counteractive used for ages in subcontinent and is immobile common training now inaccessible ranges for warehoused particles in numerous amounts of world azadirachtin is a most important multifarious of neem with insecticidal belongings and has become maximum consideration in current centuries (Parakash and Srivastava, 2008). Nonetheless, numerous supplementary composites do have fluctuating gradation of insect preventive, nauseating, anti-feed ant, anti ovipositional and development changeable possessions like, deacetyl azadirachtinol, meliantriol, vepol, salannin, sulfur compounds, etc. (Atawodi and Atawodi, 2009; Diaz et al., 2010). Allium sativum, commonly known as lahsun in Hindi (Garlic in English) belongs to family Alliaceae and plant order liliales garlic is used to improve a variability of well-being difficulties due to its in height gratified of organosulfur complexes and antioxidant movement. The plant comprises a collection of constituents which hold safe controlling possessions (Sultan et al., 2014). Garlic (Allium sativum) is the second most widely cultivated spice used next to onion. It has long been recognized all over the world as a valuable spice for food Garlic comprises exceptional organosulfur compounds (Block, 1985), which deliver its representative essence and redolence and furthermost of its strong natural activity. The robust fragrance of fresh garlic and its capability to produce unfriendly gastric side properties (Heber, 1997, Moriguchi et al., 1997) have triggered numerous to errand nutritional garlic complements as an optimal choice for accumulative daily garlic consumption.

The entomopathogenic fungi are widely used against different crop pests. Their efficacy against different pest including B. tabaci is well documented in the laboratory and field as well. The Metarizium anisopliae and Beauveria bassiana are the recognized against many pests in different field conditions. The elevated efficacy of Metarizium anisopliae has been determined over Beauveria bassiana in microbial control of termites due to social behavior of termites and considerable higher fungal biomass production as compared to Beauveria bassiana (Sun et al., 2002). M. ansisopliae (strains) have been isolated from termites and are reported all over the world by using different techniques (Lai et al., 1982; Wells et al., 1995; Zoberi, 1995; Liu et al., 2002; Sun et al., 2003; Wright et al., 2005). The efficacy of fungus had been reported due to conidia develop and make a way into the insect body wall by a mixture of mechanical force. Further, the fungus affects the biochemical processes of insect by the manufacture of enzymes that assimilate into cuticle. Entomopathogenic fungi such as Beauveria bassianaare used to assist this attempt for the most part in circumstances, where B.tabaci population had increased and out of control (Mead et al., 1991). M. anisopliae was also able to inhibit the development of leaf-sucking and feeding pests (Ekesi et al., 1998). The entomopathogenic fungus Beauveria bassiana (Asco-mycota: Hypocreales) has been known for 170 years. It is an obviously happening, soil-dwelling fungus that grows as a saprophyte but it is also an extremely operative entomopathogenic fungus that sources illnesses in a diversity of insect classes, mostly in the orders Lepidoptera, Coleoptera, and Homoptera. In fact, B. bassiana has been one of the most considered entomopathogenic fungi, and a substantial capacity of collected works has accumulated on its biology, hosts, effects on non-target organisms, and protection towards vertebrates (Zimmermann, 2007).

The billions of tons of insecticides have been indulged in our ecosystem every year for the control of insect pests in different crops, orchards, forest trees and in house hold. The whitefly is a serious pest and possess sever damage to different plant species due to its elevated and uncontrolled population throughout the year. The critical investigation is required for the alternative control measures to different pest, in results the burden of insecticides will be shared by bio-pesticides for sustainable ecosystem. In current study two entomopathogenic fungi and two botanicals were investigated for the control of a polyphagous insect (whitefly). This will help us to control the existing pest for sustainable basis.

MATERIALS AND METHODS

Recent research work was accompanied in Cotton Insect Pest Management Laboratory, Department of Entomology, University of Agriculture, and Faisalabad during 2019-2020.

Insect Collection

The Bemisia tabaci (whiteflies) for research work collected from cotton field of University of Agriculture Faisalabad (UAF) Punjab, Pakistan and Ayub Research Center Faisalabad, Punjab, Pakistan. The nymph was collected manually with the help of aspirator from the cotton leaves.

Collection of Plant material

The plant extract such as leaves of Neem and Garlic was collected from Burewala District Vehari, Punjab, Pakistan.

Collection of Entomopathogenic Fungi

The cascade of animals including small fishes, insects and other arthropod were collected from a fish pound present at Department of fisheries, University of Agriculture. Only cascades with well fungus growth were collected. The fungus hyphae with sterilized needle were shifted to petri dishes poured with potato dextrose agar media. The petri dishes were incubated for 48 hours at 28°C. Different isolates of fungi were identified but only Beauveria bassiana and Metarhizium anisopliae were used for further experiments due to well documented pathogenicity.

Rearing of Insects

The collected whiteflies released in the rearing cages of different size in laboratory, which had cotton as host plant under properly maintained temperature, humidity, photoperiod and with appropriate supply of water. After 72 hrs leaves containing eggs was shifted in separate cages for hatching and to obtain F1 generation of Bemisia tabaci after few days.

Preparation of Plant Extracts

50 gram of powder in 150 ml Acetone for extracts by using rotatory shaker (120rpm) and shaking confirmed for 24hrs, and then filtered the extract through filter paper. Solvent from the crude extracts evaporated by rotator evaporator and stored in refrigerator at 4°C before use. From the stock solution, serial dilution (5%, 10% and 15%) prepared using distilled water as solvent. Three concentrations prepared along with one controlled treatment.

Preparation of Entomopathogenic Fungi

Each concentration of Fungi prepared such as (0.4ppm, 0.5ppm and 0.6ppm). Recommended dose of entomopathogrenic fungi 5g/litter distilled water for application was used.

Effect of plants extract and Entomophathogenic fungi on efficacy of whiteflyAdults

Current experimentation carried out to examine the toxic effects of plants extracts (neem and garlic) and Entomopathogenic fungi (Beauveria bassiana and Metarhizium anisopliae) on whitefly Adults. Three concentrations of plants Extracts (5, 10 and 15%) were applied with five replications and the entomopathogenic fungi were applied in three concentrations (0.2, 0.4 and 0.5ppm) by using dip method on cotton leaves. Agarose gel prepared and put into the petri dish. Treated Cotton leaf by leaf dip method Fatemeh Jafarbeigi et al., (2014) fixed in a petri dish. Twenty whiteflies were transferred per petri dish with the help of camel brush. Twenty Adult was placed in untreated petri dish (control treatment).

Effect of plants extract and Entomophathogenic fungi on whitefly Nymph

Three concentrations of plants Extracts (5, 10 and 15%) were applied with five replications and the entomopathogenic fungi were applied in three concentrations (0.2, 0.4 and 0.5ppm) with the help of hand spray method. Cotton leaves were taken and identified the premature stages of whitefly nymph by marking it with marker.

Statistical Analysis

Calculations

A completely random design (CRD) which used for ANOVA and mean value and mortality of both Adult and Nymph checked after 24, 48, 72h and five days. Calculate the corrected mortality percentage using the following Abbot (1925) formula:

$$\%\, Corrected\ Mortality = \frac{MO - MC}{(100 - MC)}$$

Where, MO = Observed Mortality and MC = Control Unit Mortality

Statistics was analyzed by analysis of variance (ANOVA) by Factorial using software Statisticx 8.1 and treatments were compared with the help of Turkey's Honest Significant Difference (THSD) test.

RESULTS AND DISSCUSION

In current study, we had investigated the toxic effects of various concentrations of plant extracts (garlic and neem) and pathogenic ability of Entomopathogrenic Fungi (Metarhizium anisopliae and Beauveria bassiana) for the control of whitefly (Bemisia Tabaci) by analysis of variance (ANOVA) of factorial using software Statisticx 8.1 and treatment was compared with the help of (THSD) test.

Effects of biopesticides on Bemicia tabaci nymph stage:

The table 1, showed mean mortality of nymph of Bemisia tabaci at various concentrations of entomopathogenic fungi (Metarhizium anisopliae and Beauveria bassiana) at different application rates (0.4 ppm, 0.5 ppm, and 0.6ppm) and plant extract (Neem and garlic) at different application rates (5%, 10%, and 15%) under the control treatment conditions. The main effects of concentration (F = 1.31, df = 2; p<0.5) were very significant. The results indicated that percent death of Bemisia tabaci increased with increasing concentration of applied treatments. Table 1 indicated average mortality rate of B. tabaci nymph which increased with increasing concentration of M.anisopliae and time. After 24h highest mean mortality rate (25.703) was observed of B.tabaci nymph for M.anisopliae, while lowest (12.316) was observed for neem extract. The average, mortality rate of nymph recorded for Beauveria bassiana (18.340) and Garlic (16.332) after 24h. The mortality rate of B.tabaci nymph increased with increased the concentration of M. anisopilae and time. The mortality of B.tabaci nymph was recoreded by application of different concentration of plant extract and entomopathogenic fungi. Highest mortality recorded at (0.6ppm) of fungi and (15%) of plants extracts 42.80. The increased mortality was observed with increase concentration of treatments.

Table 1 Mortality Percentage of whitefly by Entomopathogenic Fungi and Plant Extracts

Sr No.	Insecticide	Mean Mortality							
		Nymph				Adult			
		24 h	48 h	72 h	120 h	24 h	48 h	72 h	120 h
1	***B. bassiana***	18.34a	30.21a	54.30b	63.69b	30.16b	35.01b	42.63b	52.26b
2	***M. anisopliae***	25.70b	34.24a	63.04a	71.09a	42.86a	47.74a	54.01a	57.30a
3	**Garlic Extract**	16.33c	23.50b	41.53c	52.26c	18.13c	27.30c	34.54c	42.51c
4	**Neem Extract**	12.31d	23.03b	37.50d	47.55d	10.78d	22.28d	29.51d	37.13d

After 48hours highest mean mortality of B.tabaci nymph mortality (34.239) was recorded against M. anisopilae while minimum mortality rate (23.03) was observed with application of neem extract. The average mortality rate of nymph mortality was recorded for Beauveria bassiana (30.213) and Garlic (23.503) after 48h. The mortality rate of B.tabaci nymph increased with increased the concentration of M. anisopilae and time. Highest mortality recorded at (0.6ppm) of fungi and (15%) of plants extracts. The mortality increased with increase in concentration of treatments. The mortality rate recorded to increase at M. anisopilae but decrease with neem extract. Highest mean mortality rate of B.tabaci nymph was observed 63.038 on application of M. anisopilae, while lowest (37.50) at application of neem extract After 72 hours. The average mortality rate of nymph was recorded 54.301 for Beauveria bassiana and 41.532 for Garlic extract after 72 hours. Highest mortality (56.149) was recorded at (0.6ppm) of fungi and (15%) of plants extracts after 72 hours. The mortality rate increase with increased dose of insecticide and time. The highest mortality was recorded at the application of M. anisopilae and minimum mortality rate at neem extract after 72 hours. After 120h highest mean mortality rate of B.tabaci nymph (71.088) was recorded for M. anisopilae, while lowest (47.554) was observed for neem extract. The average mortality rate of nymph was recorded 63.691 for Beauveria bassiana and 52.261 for garlic extract after 120 hours. The mortality rate of B.tabaci nymph increased with increased the concentration of M. anisopilae and the time. Highest mortality 65.204 was recorded at (0.6ppm) of fungi and (15%) of plants extracts. The highest mortalty recorded for M. anisopilae and lowest for neem.

Effects of biopesticides on Bemicia tabaci adult stage

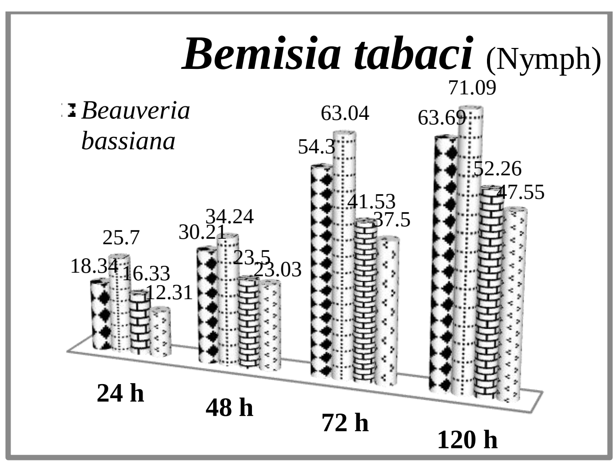
Table, indicated average mortality rate of B. tabaci adult, which increased with increasing concentration of M.anisopliae and time. After 24h highest mean mortality rate of B.tabaci adult was observed 42.857, whereas the lowest was recorded 10.777 at the application of neem extract. The average mortality rate 30.15

was noted for Beauveria bassiana and 18.129 for garlic extract after 24 hours. The mortality rate of B.tabaci adult increased with increased the concentration of M. anisopilae and time. Highest mortality 32.331 was recorded at (0.6ppm) of EPF and (15%) of plants extracts. The highest mortality rate was recorded for M. anisopilae and lowest for neem extract. After 48hours, highest mean mortality rate of B.tabaci adult (47.739) was recorded towards M. anisopilae, whereas minimum mortality rate (22.278) was observed at the application of neem extract. The average mortality rate of adult mortality was recorded 35.008 at Beauveria bassiana and 27.303 for garlic extract after 48 hours. Highest mortality 42.211 was recorded at (0.6ppm) of EPF and (15%) of plants extracts. The mortality increased with increase concentration of treatments. After 72h highest mean mortality rate of B.tabaci adult 54.011 was observed for M. anisopilae, and minimum mortality rate (29.507) was observed at application of neem. The average mortality rate of adult was recorded at Beauveria bassiana (42.63) and garlic (34.54) after 72hours. The mortality rate of B.tabaci adult increased with increased the concentration of M. anisopilae and time. Highest mortality 47.633 was recorded at (0.6ppm) of EPF and (15%) of plants extracts. The maximum mortality recorded at application of M. anisopilae and minimum at neem extract. After 120h highest mean mortality rate 57.304 of B.tabaci adult was observed for M. anisopilae and minimum mortality rate (37.132) was observed at application of neem extract. The average mortality rate adult 52.261 was recorded at Beauveria bassiana and 37.132 for garlic extract after 120 hours. Highest mortality 57.388 was recorded at (0.6ppm) of EPF and (15%) of plants extracts. The mortality increased with increase concentration of treatments. The mortality rate increase with increase the dose and passage of time. The highest mortality rate recoded at M. anisopilae and lowest at neem extreact.

DISCUSSION

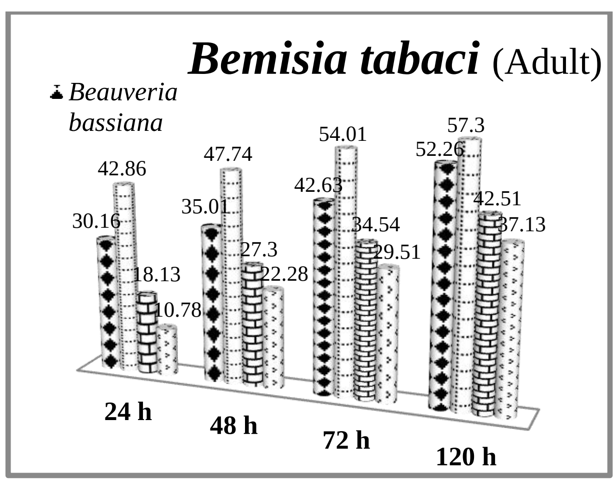
The pathogenic ability of entomopathogenic fungus like Beauveria bassiana, Metarhizium anisopliae against whiteflies Adult and nymph was investigated by using different concentration from 0.4 ppm to 0.6 ppm. Further, the toxic potential of botanical extracts (neem and garlic) was investigated by using different concentration from 5% to 15%. The nymphs of Bemisia tabaci were also significantly sensitive towards these bio-pesticides. The enhanced mortality rate of nymph of Bemisia tabaci was observed by increasing the applied concentration and time interval of Metarhizium anisopliae. After 120 hours highest mean mortality 71.09% while, after 24h minimum mean mortality rate of nymph (Bemisia tabaci) was 25.70%. Whereas, the highest mortality rate of nymph (63.69%) was observed after 120 hours towards Beauveria bassiana and minimum (18.34%) was observed after 24 hours of application.

Figure 1 Mortality of whitefly nymphs by applications of entomopathogenic fungi and plant extracts.



Correspondingly, the Beauveria bassiana expressed the same behavior like Metarhizium anisopliae, the mortality increased by increasing the conidial percentage and duration of application. The botanical (garlic extract) had the similar efficacy, the highest mortality rate of nymph (52.26%) was determined after 120 hours followed by minimum (16.33%) after 24 hours towards Bemisia tabaci. The neem extract expressed efficacy by reduction of nymph population to 47.55% after 120 hours. The least reduction in mortality was observed 12.31% after the application towards nymph of Bemisia tabaci after 24 hours. All the affects were observed on the highest concentrations and prolonged time period towards entomopathogenic fungi and botanical extracts. The entomopathogenic fungi required time for mycelial growth and sporulation on the insect cuticle, whereas the botanicals required time in the absorbance and had interference with the biochemical processes. Counter to our results, the pathogenicity of Beauveria bassiana and neem extracts was evaluated against the Bemiscia tabaci and 72.9% mortality of nymph was recorded after the application of 108 conidia ml-1 suspension of Beauveria bassiana. Whereas, the nymph mortality 77.90% was observed with the combined application of 0.5% neem extract. The result showed that enhanced moratlity have been observed by combine effects of both entomophathogenic fungi and botanicals towards immature nymph stages of cotton white fly rather than the individual effects Islam et al. (2010 a, b, 2011). Similar to our results, the 39.18% reduction in the mean population of whitefly was observed by the use of neem extract Kaleri et al. (2011). The mortality of whitefly was observed to 42.31% mortality towards 2 % neem oil followed 3 % by neem seed water to 23.92%. Our results are parallel to the work of S. K. Sain et al. (2019), who worked on entomopathogenic fungi Beauveria bassiana and Metarrhizium anisopliae to determined efficacy against nymph and adult stages of Bemisia tabaci in cotton. The recent result showed maximum mortality of Bemisia tabaci adult and nymph is due to the Metarhizium anisopliae at 0.6ppm. But at 5% least mortality due to plant extracts of neem. Average mortality rate of Adult (Bemisia tabaci) increased by increasing the applied concentration and time interval of Metarhizium anisoplia. Highest mortality (57.304) was recorded after 120hours towards the adult population of whitefly, whereas, the minimum mean mortality rate (42.857%) of adult was recorded after 24hours of application. Whereas, the highest mortality rate (52.261%) was recorded towards Beauveria bassiana after 120 hours and lowest (30.159%) after 24 hours. Similarly, the highest mortality rate (42.511%) was noted towards garlic extract after 120 hours and lowest (18.129%) after 24 hours. Consistently, the highest mortality (37.132%) was recorded towards neem extract and lowest (10.777%) after 24 hours.

Figure 2 Mortality of whitefly adults by applications of entomopathogenic fungi and plant extracts.



The adult population was reduced to upto 92.7% by the application of Metarrhizium sp. against the adults of whitefly in the laboratory; similar results with 92.2% moratality were observed in the field condition zaki (1998). The utilization of entomopathogenic fungi in integrated pest management is an effective tool. The bio-efficacy of entomopathogenic fungi was screened and found supportive in integrated pest management Pelizza et al. (2015). The entomopathogenic fungi exert their efficacy through mycelial growth and sporulation on the insect cuticle by producing cuticular cracks that leads to desiccation by enhancing the cuticular evaporation. The Metarhizium anisopliae had shown the significant reduction in the population of adults of termites (Coptotermes heimi) in the laboratory by comparing the LT50 values with other local isolates of entomopathogenic fungi (Ahmed et al., 2008a, 2008b, 2009). The different concentration of Metarhizium anisopliae (1 × 104, 1 × 106, 1 × 108, 1 × 1010 conidia mL-1) were tested on adults of Coptotermes heimi. LT50 of there different strains of Metarhizium anisopliae towards Coptotermes heimi were determined comparatively more (upto 106 hours) in soil than on filter paper (upto 83 hours) (Ahmed et al. 2008b, 2008a, 2009). Further, the botanicals had exerted their effectiveness by enhancing the stress and change in insect composition and behavior. The toxicity of garlic extract was observed against whitefly with the highest recorded mortality with 78.89% by using the spray method Ibrahim et al. (2017). Similar to whitefly the garlic extract was evaluated against dipterous pests and witnessed the significant mortalities of eggs and adult Prowse et al. (2006). The entomophathogenic fungi (Beauveria bassiana and Metarrhizium anisopliae) have a special advantage over chemical control especially their exclusive safety towards the natural enemies and lesser chance of resistance development like the chemical control (Gao et al., 2017). The differences between our study and other studies may be due to differences in pest types, treatment doses, research methods, variety and commodity changes, laboratory and environmental factors. The plants used in this study demonstrated effective toxicity of targeted post-harvest grain pests. These plants contain a range of chemicals that can be isolated and used for pest control. Plant products which are easily available in our surrounding are chief and eco-friendly effective tools to manage pest population of whitefly and others insect pest. Entomopatheogenic fungi also used in this research work demonstrated toxic effects against nymph and adult of Bemisia tabaci.

Conclusion

White fly (Bemisia tabaci) is a sucking insect and serious pest in the field. The insect caused serious damage to many field crops and other edible crops due to its sucking behavior by decreasing the nutrition value. Current study, reveled the mortality of both stages of B.tabaci (nymph and adult) by using Entomopathogenic fungi (Beauveria bassiana and Metarhizium anisopliae), and plant extracts (neem and garlic). Entomopathogenic fungi was found more effective to control insect pest in limited time rather than plant extract which work slowly and take a lot of time in reduction of pest population. The botanical extracts are found to be much safe for health and surrounding environment. Further, the plant extracts are shown higher popularity in farming community because of cost effectiveness. Both, the entomopathogenic fungi and plant extracts are well suited for the control of different field pests and could be an efficient and sustainable source for different integrated pest management planes.

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