Efficiency of Using *Macrolophus nubilus* H.S. for Protecting Tomatoes from Major Pests in the Greenhouse Conditions of South Kazakhstan

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ABSTRACT

Since 2015, the greatest harm to greenhouse vegetables in Kazakhstan has been caused by a previously unknown pest of nightshades – the South American tomato moth (*Tuta absoluta*) and the greenhouse whitefly (*Trialeurodes vaporariorum*). To obtain an environmentally friendly product, for the first time in Kazakhstan, the biological features of the predatory bug *Macrolophus nubilus* were studied and a complex integrated system was developed using biological methods. The data obtained on the survival rate and duration of development of *M. nubilus* larvae indicate that the eggs of the *Sitotroga cerealella*, as well as the eggs and larvae of the *T. vaporariorum* and *T. absoluta*, serve as a complete food for the predatory bug. Production tests of *M. nubilus* to assess the effect of temperature and photoperiod on the duration of larval development were carried out in the greenhouse complex “Naimbekov” on an area of 1 hectare (early indeterminate tomato hybrid Attia F1). The assessment of the biological effectiveness of *M. nubilus* against *T. vaporariorum* and *T. absoluta* was carried out in the greenhouse complex “Adelya” on an area of 5 hectares (medium-fruited carpal tomato hybrid Merlis F1) in southern Kazakhstan. These results contribute to more sustainable tomato production.

INTRODUCTION

In the Republic of Kazakhstan, since 2008, the volume of greenhouses has significantly increased as a result of financing under the KazAgroFinance state program. According to the Ministry of Agriculture of the Republic of Kazakhstan, the area of vegetable crops cultivated in the greenhouse has significantly increased (KazAgroFinance, 2019). In 2008, the area of greenhouses was 58.6 ha, and in 2020 the area of greenhouses was 1662.8 ha. Among others in the Turkestan region, the area of greenhouses is 1114.1 ha, which is 67% of the total share. Of these, the area of industrial greenhouses is 67.7 ha (Kopzhasarova, 2020). The conditions of covered areas, convenient for the cultivation of vegetable crops, are a favorable environment for the intensive development of organisms that cause various diseases. Maintaining cleanliness inside the greenhouse ensures the absence of plant diseases and pests. However, it is impossible to constantly maintain sanitary cleanliness in a closed area. Therefore, it is necessary to regularly carry out measures to combat diseases and pests that spread them. To ensure phytosanitary safety, it is necessary to timely monitor the development and spread of quarantinable, especially dangerous and harmful organisms using modern equipment, make a forecast of their distribution, take measures to localize and eliminate foci of spreading...
quarantinable harmful organisms and to combat harmful and especially dangerous organisms, and determine the coordinates of their distribution (On Strategic Plan No. 114 of the Ministry of Agriculture of the Republic of Kazakhstan for 2014-2018).

Phytosanitary safety is at a satisfactory level in the Republic of Kazakhstan. The use of biological methods of pest control is developing (EEC, 2019). The fight against quarantinable objects and especially dangerous harmful organisms is carried out by directly involving agricultural producers in the process of ensuring phytosanitary well-being. The state provides for subsidizing the costs of agricultural producers for the purchase of biological products (bioagents) to combat harmful and especially dangerous harmful organisms (State Program No. 423 for the Development of the Agro-Industrial Complex of the Republic of Kazakhstan for 2017-2021). Among the complex of harmful organisms on vegetable crops of protected ground in the Turkestan region, the greatest damage to plants is caused by the greenhouse whitefly (Trialeurodes vaporariorum West.), the tobacco whitefly (Bemisia tabaci, Gennadius, Homoptera: Aleyrodidae), the common spider mite (Tetranychus urticae Koch., Heteroptera: Miridae) are used for biological control methods. Six species of horseflies (Tabanus autumnalis, Tabanus fuscipennis, Tabanus nebulosus, Tabanus circumscriptus, Tabanus rufiventris, Tabanus celeriovi) and others (Chadinova, Alimbekova, Shanimov, & Duisembekov, 2019). In 2015, in several regions of Kazakhstan (Turkestan, Almaty, Kyzylorda, and Aktobe), a previously unknown pest of tomatoes, the South American tomato moth (Tuta absoluta) was identified (Kenenbaev, 2017).

The South American tomato moth received the status of a quarantinable pest in the republic due to the high degree of reproduction and harmfulness. Therefore, the studies on this phytophage started under this project in Kazakhstan for the first time and they are aimed to study the biological features of its development and develop a complex integrated system using biological methods and means of plant protection. According to the data of surveys carried out by the plant quarantine services of the Republic and employees of the Kazakh Research Institute of Plant Protection and Quarantine named after Zh. Zhiembaev (KazNIIZiKR), the pest spread and caused severe damage to tomatoes in the Turkestan, Almaty, Kyzylorda, and Aktobe regions (Aitkulov et al., 2017). According to the data of territorial phytosanitary control in 2017, in the greenhouse farms Zanjanizab, Jabay ata, Koktem, and Zhana Konys of the Turkestan region, the damage rate of tomatoes reached 78-100%. The pest was also found on open ground tomato plantations in that area (Chadinova, 2020). The loss of tomato yield in many countries reaches 80-100% and leads to the fact that tomato production often becomes unprofitable (Dommguez, Lopez, Bernabé, Guerrero, & Quero, 2019). As a restrictive measure for the spread of the pest, some states prohibit the import of products from countries where the tomato moth is widespread.

The tomato moth is widespread in countries with warm climates, both in the open and closed ground (Han et al., 2018). Therefore, given the cold winters in Kazakhstan, the pest will cause the main harm to tomatoes in protected ground conditions and can damage the culture in the open field only in some warm winters of the Turkestan region, with favorable wintering conditions. Now it is known that the pest quickly develops resistance to chemicals, and in this regard, the introduction of biological control methods is a promising direction, since it does not require the use of chemical agents (ECO Culture, 2018). The biological method is the purposeful use of entomophagous species, microbiological species, and other non-chemical methods of protection based on the knowledge of interspecific relationships between plants and animals in the fight against pests and plant diseases (Toleubaev, Shanimov, Kozhakhmetova, & Chadinova, 2012). For example, horseflies are versatile predators capable of preying on various phytophages such as T. vaporariorum (Jäckel, Alt, & Balder, 2011; López, Rojas, Velásquez, & Cagnotti, 2012)Sitotroga cerealella eggs and a mix of both, B. tabaci (Sanchez, Lopez-Gallego, Pérez-Marcos, & Perera-Fernández, 2020), and T. absoluta (Nannini, Atzori, Murgia, Pisci, & Sanna, 2012; Passos et al., 2018). Six species of horseflies from the genera Macrolophus and Dicyphus (Heteroptera: Miridae) are used for biological plant protection globally (Agustí & Garbarra, 2009; Castañé et al., 2013).M. melanotoma (=M. caliginosus . All of them are zoophytophages, which additionally, like phytophages, feed on plant sap for development (Baños-Díaz, & de los Ángeles Martinez-Rivero, 2018). These predators
not only feed on plant sap, but also lay their eggs on plant tissues (Portillo, Alomar, & Wäckers, 2012) pollen. In Kazakhstan, to combat the pests like *T. vaporariorum*, *B. tabaci*, *T. absoluta* and *T. urticae* in crops of tomatoes in protected ground, the predatory bug *Macrolophus nubilus* H.S. (= *Macrolophus pygmaeus* Rambur) (Heteroptera: Miridae) is used. Therefore, this research was aimed to assess the effectiveness of the use of the predatory bug *M. nubilus* for the protection of tomatoes in greenhouses against *T. absoluta* and *T. vaporariorum*.

**MATERIALS AND METHODS**

**Research Location and Material Preparation**

The work was carried out on a laboratory culture of *M. nubilus*. The insects was from the collection of the Kazakh Research Institute of Plant Protection and Quarantine named after Zh. Zhiembaev. The methodological instructions of Krasavina (2011) and Pazyuk & Reznik (2016) was used to grow forage plants and mass breeding of macrolophus. Mass breeding of the bugs in laboratory conditions consists of the following technological processes i.e. cultivation of host plants to obtain a host (victim), accumulation and preservation of the host in a small amount and continuous reproduction of the predatory bug in the required amount. Breeding predatory bugs using *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae) eggs and *T. vaporariorum* larvae as feed consists of four stages i.e. growing a food plant for whitefly propagation, whitefly breeding, grain moth breeding and moth egg collection.

**Influence of Tomato and Tobacco Substrates on the Hunting Abilities of *M. nubilus***

The main biological parameters of bugs were assessed by keeping subjects individually. 100 subjects (male and female) were put into a cage, and on the next day all the imagos were caught, and the resulting ovipositor was incubated. For 6 days, hatching was observed every day and the larvae of the first instar of which were seated individually using thin brushes on tomato and tobacco seedlings. Then, the seedlings with larvae were placed in a plastic container and covered with a 100-micron nylon mesh for air and light access. When assessing the biological parameters of *M. nubilus* subjects on all types of food were kept individually (Table 1).

The voraciousness of the larvae and imagos of the macrolophus (the proportion of larvae that molted at the next age and the percentage of the emergence of imagos) when feeding on a plant and animal diet was assessed in laboratory conditions in 6 replicates on tomato and tobacco plants. Predatory bugs were kept with a photoperiod of 16:8 (L:D), at a temperature of 24°C and relative humidity of 60% RH. The ageing stage of the bug larvae was taken into account daily (when they appeared, the presence of molted skins was noted). Taking into account the voraciousness of *M. nubilus* larvae and imagos, the food was changed daily.

The duration of development of larvae and life of the imagos of *M. nubilus* (larva from the 1st to the 5th instar, imago) when feeding on plant-based food was tested on tomato and tobacco plants, at two different temperature regimes (24 and 28°C). For larval stage, counts were carried out daily, noting the molt skins of the bug, while imago stage, counts were carried out daily.

**Table 1.** Diet composition (KazNIIZiKR laboratory of “useful arthropods”, 2018)

| No. | Plant-based and animal-based food | Plant-based food |
|-----|----------------------------------|-----------------|
| 1   | *T. vaporariorum* | The rate of consumed eggs and nymphs per day | Tomato  |
| 2   | *T. absoluta* | The rate of consumed eggs per day | Tobacco |
| 3   | *S. graminum* | The rate of consumed larvae of different ages and females per day | Tomato  |
| 4   | *S. cerealella* | The rate of consumed eggs per day | Tobacco |
| 5   | *Artemia spp.* | The rate of consumed decapsulated cysts | Tomato  |
| 6   | - | - | Tobacco |
Evaluation of the Biological Effectiveness of *M. nubilus* against *T. vaporariorum* and *T. absoluta* in Greenhouse Conditions

Evaluation of the effectiveness of using *M. nubilus* against *T. vaporariorum* and *T. absoluta* during colonization on tomatoes was carried out in greenhouses of various designs on the farms of the Turkestan region. In 2018-2019, the first variant of colonization of *M. nubilus* was carried out on an area of 1 ha (film greenhouse of the Naimbekov farm, Turkestan region, South Kazakhstan) in the spring crop rotation on a tomato hybrid Attia F1 (Rijk Zwaan). The second variant of the experiment was carried out in 2019-2020 on an area of 5 ha (polycarbonate-film Adelya greenhouse complex, Turkestan region, South Kazakhstan) in the autumn year-round crop rotation on a tomato hybrid Merlis F1 (De Ruiter Seeds).

In the first variant, the release of the bug *M. nubilus* (laboratory population) for biological rows was carried out on December 28, 2018 with the calculation of 1 subject per 1 m$^2$ in the greenhouse of the Naimbekov farm. During the examination, the percentage of damage to seedlings was taken into account. The eggs of the sitotroga *S. cerealella* were added as additional feed. After, we resettled the predator. Monitoring was carried out weekly using random selection on 100 model plants at each marked plot.

In the second variant, the approbation of mass cultivation of *M. nubilus* in the second variant was carried out in the nursery at the Adelya greenhouse complex, and the predator population obtained during breeding (greenhouse population) was released into the greenhouse according to the method developed by Sagitov, Alimbekova, & Duisembekov (2020).

In May, more than 2,000 individuals of *M. nubilus* (population of the biological laboratory KazNIIZiKR) were collected in the greenhouse using an exhauster (a device for catching small insects). In the seedling sector of the greenhouse, an insectarium was additionally created for mass rearing of *M. nubilus* using the natural photoperiod and temperature. The bug breeding was carried out on mineral wool in cubes ((100 x 100 x 65) Grodan), on tomato seedlings of the Merlis F1 hybrid (De Ruiter Seeds).

Nine tomato plants were placed on 5 cages covered with a mesh fabric measuring 70 x 70 x 60 cm and 400 individuals of *M. nubilus* were launched into each cage. The ratio of males and females (1:1) was maintained by counting them, and in case of the death of individuals, the addition of individuals was added into the cage to maintain the ratio of 1:1. After three days, all tomato plants with an ovipositor were taken out of the cage and transferred to a separate box for further incubation of the larvae.

Thus, within 21 days, the procedure was carried out six times, 2,000 bedbugs in the "conveyor" mode, the total number of cages was 30 pieces. As a result of frequent plant changes, part of *M. nubilus* was lost. The next conveyor was carried out according to this scheme after the complete maturation of the bug to the adult stage. In place of the harvested plants, new tomato plants for the ovipositor were introduced into the cage.

In three months, the total number of cages on the conveyor and the plants was 120 and 1,080, respectively. Within three months, 100,000 individuals of *M. nubilus* were produced in the insectarium and moved to the greenhouse. On the leaves of the middle layer of tomato plants, feed was introduced (decapsulated Artemia cyst 60% and grain moth eggs 40%) with the inscription “Bioline” and the bug was released at the rate of 2 subjects per 1 m$^2$. Alternative food source was provided for the predator in two cases: to accelerate the growth of the population and in the absence of phytophages to support the population, so that the zoophytophage does not damage plants, forcedly switching to plant-based food (Lykouressis, Perdikis, & Charalampous, 2014; Put, Bollens, Wäckers, & Pekas, 2012).

Data Analysis

The biological assessment of the efficiency of colonization of *M. nubilus* (without control) in the greenhouse against *T. vaporariorum* was carried out from August 20, 2019 to May 10, 2020 based on Tverdyukov, Nikonov, & Yushchenko (1993).  

$$Be = \frac{(A-B)}{A} \times 100\%,$$

(1)

Where Be is the biological efficiency; A is the pest population before protective measures; B is the pest population after protective measures.

The percentage of tomato infestation per 1 m$^2$ by pests was determined by the Tverdyukov, Nikonov, & Yushchenko (1993) formula.

$$Pi = \frac{B \times 100\%}{A},$$

(2)
Where $P_i$ is the percentage of plant infestation per 1 m²; $A$ is the total number of leaves on the model plants; $B$ is the number of infected leaves on model plants.

To determine the reliability of the influence of the investigated factors on various indicators of the analysis of variance data, graphic images were constructed using the Microsoft Excel application package.

**RESULTS AND DISCUSSION**

**Food Consumption and Hunting Abilities of *Macrolophus nubilus* under Laboratory Conditions**

To determine the optimal composition of animal and plant-based food required by *M. nubilus* for full development, the duration of development of its larvae when feeding on *T. vaporariorum*, *T. absoluta*, *Sch. graminum*, *S. cerealella* eggs, and the decapsulated cyst *Artemia* ssp was evaluated. When macrolophus fed on *S. cerealella* eggs on two species of plants (tobacco, tomato), no statistical differences in the duration of larval development and the proportion of emerging imagos were revealed. At the same time, the survival rate of larvae when feeding on eggs of *S. cerealella* was 83 ± 8%, which differs significantly from the survival rate of larvae fed on an optimal diet (*T. vaporariorum* egg and nymphs on tomato leaves), which amounted to 92 ± 5.1% (Table 2).

The results of the studies showed that the average number of *T. vaporariorum* nymphs destroyed by a female predator on a tomato per day was 41.63 eggs and 13.5 nymphs, while 37.6 eggs and 11.1 nymphs were killed by a male. Laboratory experiments show that females of *M. nubilus* consume more eggs and nymphs and therefore their life cycle is much longer than that of males. However, it was found from linear regression that the number of *T. vaporariorum* nymphs consumed by *M. nubilus* did not affect the life cycle of these predator, and only an increase in temperature from 24°C to 28°C affected the average life span of imago (Table 3).

During the 10-day observation, the predatory habit of the bug showed no tendency to feed. The predator consumed prey with almost the same daily rate (on average of 5.6 subjects per day). Thus, one predator was able to kill about 60 nymphs in 10 days. This ability was confirmed in earlier studies, which recorded the rate of predation from 5 to 5.3 nymphs per day (Moreno-Ripoll, Gabarra, Symondson, King, & Agustí, 2014). The aforementioned data prove that *M. nubilus* ate less *T. vaporariorum* per day on tomato than on tobacco substrates. Comparing the options for experiments with tomato and tobacco substrates, we have concluded that the bug consumes more animal-based food on tomato than on tomato plants. Plant nutrients can affect the taste of prey, making it either tastier or unpleasant for a predator. This is since the tissues of the tomato substrate supplement the diet of the bug while not damaging the plants. In laboratory studies, it was found that the larvae and imagos of *M. nubilus* attacked the nymphs of *T. vaporariorum*. *T. vaporariorum* randomly, regardless of the nymph stage and the location of the nymph on the leaf, inserting their proboscis into their bodies. The daily predation rate of *M. nubilus* larvae was calculated under binocular, according to the number of empty nymphs. The imagos of *M. nubilus* fed on the nymphs of *T. vaporariorum* at all stages up to the puparium. It was also found that the imago of the predator fed on the hatched whitefly imago, which indicates the absence of white blooms on the body of the dead phytophage.

Laboratory experiments were carried out to assess the hunting ability of *M. nubilus* on *T. absoluta* eggs. Larvae and females of *M. nubilus* consumed an average of 12.5 to 24.5 eggs per day on tomato, and 14.0 and 27.1 *T. absoluta* eggs on tobacco substrates. In works on the assessment of the hunting behavior and voraciousness of the macrolophus, it was found that the bug harvested up to 30 eggs of tomato moth daily (Desneux et al., 2010). At the same time, bug larvae developed the fastest when feeding on whitefly nymphs than on eggs of tomato moth. Studies show that the voraciousness of one adult *M. nubilus* exceeds 100 *T. absoluta* eggs per day (Arnó & Gabarra, 2011). Chailieux et al. (2013) and Michaelides, Sfenthourakis, Pitsillou, & Seraphides (2018) found that the bed bug predation rate increases with the leveling of the suggested prey density, even at higher densities.

When feeding on tobacco, the larvae of the macrolophus died at a young age without reaching the imaginal stages. The life expectancy of the macrolophus imago when fed exclusively on plant-based foods (tomato, tobacco) was on average 6.4-17.5 days. Therefore, on a tomato substrate, only
30% of macrolophus larvae reached the imaginal stage. Studies found that predators could survive without any food source for 3.67 days, and only on water for 4.33 days (Han et al., 2015). The results of these studies indicate that a plant-based diet is not a complete source of food for the maintenance and development of macrolophus. However, according to researchers, *Solanum nigrum* L. is the main host plant for *M. nubilus* (Ingegno, Pansa, & Tavella, 2011). *M. nubilus* completes its development cycle from egg to imago, feeding exclusively on this plant species, while its fertility decreased compared to feeding on phytophages (De Backer, Megido, Haubrege, & Verheggen, 2014; Maselou, Perdikis, & Fantinou, 2015).

The wide food specialization of the predatory bug, its ability to eat both animal and plant-based foods without damaging plants, proves its effectiveness in the protection of vegetable crops, both in protected and open ground. We have set up experiments to evaluate its effectiveness, both in laboratory and greenhouse conditions. In greenhouse conditions, we evaluated the effectiveness of predatory bug colonization on tomato against greenhouse whitefly and tomato moth and switching to plant-based food in the absence of animal-based food.

**Table 2. Consumption of animal-based food by Macrolophus nubilus at a temperature of 24°C (KazNII ZiKR laboratory of “useful arthropods”, 2018)**

| No. | Food type      | Consumption rate, pcs | Substratum type |
|-----|----------------|-----------------------|----------------|
|     |                | I   | II   | III  | IV   | V   | Male       | Female       |
| 1   | T. absoluta   |     |      |      |      |     |            |              |
|     | Egg            | 0   | 13.25±0.75 | 49±2.129 | 76.5±1.53 | 131±1.58 | 405.75±15.3 | 727.25±18.58 |
|     |                | 0   | 16.25±0.47 | 51.25±2.25 | 81.5±3.01 | 158.5±9.61 | 435±25.20 | 815±27.53 |
| 2   | T. vaporariorum | 1.5±0.12 | 35.16±2.05 | 75±4.54 | 101.66±1.11 | 170.83±0.54 | 753.33±10.46 | 1,249.83±11.24 |
|     | Egg            | 16.16±0.70 | 25.83±0.47 | 38.6±0.84 | 44.5±1.11 | 223.16±7.72 | 407.83±4.15 |
|     | Nymphs        | -   | -    | -    | -    | 1±0.54 | 19.5±0.92 |
|     | Imago         | -   | -    | -    | -    | -    | 0.5±0.25 | 25.2±1.2 |
| 3   | Sch. graminum | 0.33±0.21 | 16.83±1.13 | 19±0.96 | 26.83±1.13 | 47.66±1.22 | 75.5±1.31 | 242.5±8.42 |
|     | Egg            | 0.5±0.22 | 16.1±1.43 | 61.5±4.00 | 92.5±3.05 | 122.62±4.02 | 946.3±15.00 | 12,887.7±44.29 |
|     | Dried cyst     | 0.33±0.21 | 18.5±0.99 | 49±2.68 | 70.66±2.31 | 89±1.78 | 107.83±2.6 | 261.66±6.93 |
| 4   | S. cerealella  | 0.5±0.22 | 20.66±0.80 | 66.5±1.23 | 100±1.36 | 133.3±2.7 | 984.83±7.72 | 123.6±23.15 |
|     | Egg            | 0.33±0.21 | 21.83±1.13 | 54±1.48 | 75.83±1.74 | 90.83±1.10 | 112±2.47 | 268.16±5.74 |
| 5   | Artemia spp.   | 0.33±0.21 | 16.83±1.13 | 19±0.96 | 26.83±1.13 | 47.66±1.22 | 75.5±1.31 | 242.5±8.42 |
Biological Effectiveness of the Use of Macrolophus nubilus to Protect Tomatoes from Trialeurodes vaporariorum

In December 2018, the laboratory population of M. nubilus of different stages (larvae and imago) was colonized in the Naimbekov film greenhouse, where there is no additional lighting and automatic climate control. We assessed the influence of temperature and photoperiod on the duration of development of M. nubilus larvae. We compared the duration, rate, and lower temperature threshold of larval development, as well as the factors of thermolability of larval development.

We found that with a short photoperiod in December, January, and February of 9:15 and 10:14 (L:D) hours, the bug damaged plant leaves without eating the main pests and artificial food. Besides, on the northern side of the greenhouse at a night temperature of 15°C, the larvae of M. nubilus showed characteristic behavior for diapausing subjects: they hid on the lower sides of the leaves and sat almost motionless. Taken together, this indicates a possible diapause in M. nubilus under unfavorable conditions at the larval stage in a laboratory experiment, the duration of the larval development of the bug at 15°C and a short day (10 hours) was 139.5 ± 7.60 days, and the proportion of subjects molting onto imagos is insignificant at 33 ± 8.2%.

The development of larvae was more thermolabile in M. nubilus at the photoperiods of 12:12 and 14:10 hours, i.e. in March and April. During the monitoring, active dropping of larval skins over tomato plants was revealed, which indicates the accelerated development of the predatory bug larvae. Males of M. nubilus develop faster than females at the larval stage. Most likely, protandria is due to the different sizes of males and females. Its adaptive meaning is to reduce the proportion of closely related mating. During year-round colonization in greenhouses of M. nubilus, it should be borne in mind that a decrease in daylight hours below 16 hours leads to a decrease in the proportion of oviparous female bugs, and with an increase in the relative humidity of the air above 75%, it leads to a decrease in the release of larvae from eggs.

In a laboratory experiment during the assessment of the biological characteristics of the development of M. nubilus under different temperature conditions and photoperiod, it was found that at 15°C, the proportion of females that did not form eggs on the 22nd day in the 10:14 (L:D) photo mode was 48 ± 9%, which is close to the threshold value (50%) and these indicates the onset of diapause. The optimum temperature for keeping imagos of M. nubilus in laboratory and greenhouse conditions was 25°C. At a temperature of 25-28°C from egg to adult, macrolophus larvae developed on average in 20 days, and in greenhouse conditions, the development of M. nubilus larvae lasted 25-30 days due to the night temperature drop to 15-17°C (Table 4).

During August 2019 in the Adelya greenhouse complex, with additional lighting and climate control, the colonization of populations of M. nubilus was carried out at 2 subjects per 1 m² according to the method developed by Sagitov, Alimbekova, & Duisembebekov (2020). In the greenhouse, the zones inhabited by the whitefly were selected on which the predatory bug was released in the predator:prey ratio 1:5, 1:10, 1:20, and 1:30 per plant (Fig. 1).

Table 3. Consumption of plant-based food by Macrolophus nubilus (KazNIIZiKR laboratory of “useful arthropods”, 2018)

| No. | Type of substrate | t°C | I   | II  | III | IV  | V   | Male | Female |
|-----|------------------|-----|-----|-----|-----|-----|-----|------|--------|
| 1   | Tomato           | 24  | 4.83±0.30 | 5.16±0.47 | 4±1.36 | 3±1.50 | 2.16±1.42 | 13.82±0.47 | 17.5±0.56 |
| 2   | Tomato           | 28  | 3.5±0.22 | 5.5±0.34 | 4.5±0.92 | 2±0.89 | 2.83±1.27 | 9.8±0.94 | 14.16±1.57 |
| 3   | Tobacco          | 24  | 4.66±0.61 | 1.33±0.49 | 0.66±0.49 |       | 7.64±0.32 | 15.82±0.66 |
| 4   | Tobacco          | 28  | 4.3±0.33 | 3.3±1.30 | 0.66±0.49 |       | 6.4±1 | 12.5±0.76 |
The strategy of releasing predators into greenhouses to increase their numbers is widely used for *M. nubilus* in the Mediterranean region. This commercial species has been available since 1994 for use in Integrated Pest Management (IPM) programs and is increasingly being used in European greenhouses for tomato production (Castañé, Arnó, Gabarra, & Alomar, 2011). It should be noted that maintaining biological control over phytophages consists in manipulating the habitat to enhance the action of endemic or exotic natural enemies.

In our experiment, during the first week after the release of the bug, monitoring was carried out to assess the effectiveness of colonization of *M. nubilus* in suppressing the number of greenhouse whitefly at the larval stage. An intensive decrease in the number of imagos of whiteflies was found, which indicates that the bug ate nymphs and this was reflected in a decrease in the phytophage population. Moreover, the entomophage additionally fed on the hatching imagos of the whitefly, which was proved earlier in a laboratory experiment. The biological effectiveness of the bedbug at different ratios in dynamics after release to plants is presented in Fig. 2.

The imago of the bug was released into the centers with different numbers of pests, which were located in the middle and lower tiers of plants. This made it possible to assess the influence of two factors on the number of the pest: the longline and the predator:prey ratio during the release of the entomophage. The results of the experiment showed that the biological effectiveness of the bug depended on the predator:prey ratio and, to some extent, on the layer of plants. This was especially clearly seen after the second and third weeks of the release of the entomophage. It should be noted that at all predator:prey ratios in the middle and lower tiers, the decrease in the phytophage population occurred gradually.
As a result, in the 1:5 predator:prey ratio on the 7th day after the release of the bug, the biological efficiency of the entomophage was 54%, and on the 17th day, it amounted to 88%. Subsequently, to maintain and increase the number of entomophages and hatching of larvae, the bugs were also fed with artificial food Bioline. With the release of the predator in a ratio of 1:10 the biological efficiency of the predator on the 13th day was 59% and 87% on the 27th day. The results of the experiment showed that there was no significant difference between the predator:prey ratios 1:5 and 1:10 in terms of biological efficiency.

Two weeks after the release of the imago of *Macrolophus nubilus*, the emergence of the next generation larvae was noted. With predator:prey ratios of 1:20 *M. nubilus* showed a biological efficiency of about 89% on the 45th day. After 13 days, at a predator:prey ratio of 1:30 we detected a 15% decrease in the whitefly population, and the maximum efficiency of the bug on day 90 was 94%. At a ratio of 1:20, a significant accumulation of bug larvae was noted in the date range from 10/11/2019 to 11/10/2019, which indicates the resettlement of the predator in connection with the appearance of the phytophage on plants. The zoophytophagous *M. nubilus* has the advantage of remaining on the crop even when there are few pests, so plants can be said to have a great influence on the survival of *M. nubilus*. With a predator:prey ratio of 1:30 on one plant, we left the opportunity for *M. nubilus* to fight the pest, since the limit of economic damage caused by phytophages to crops grown in a greenhouse is calculated when there are at least 10 phytophage nymphs on one
leaf. Based on these data, we did not perform any chemical treatments. The biological effectiveness of *M. nubilus* against *T. vaporariorum* on day 85 was 97%.

In 2019, dead *M. nubilus* was found in the Ayanat greenhouse complex on tomato leaves treated with a breeding agent (Teppeki 0.06%) used in the comprehensive system of protection against *T. vaporariorum*. The predators were found to have been poisoned by feeding on infected *T. vaporariorum* nymphs referring to the bioagent compatibility program (Kopper biological system). As a result, the consequences of using chemical treatments reduce protective potential of *M. nubilus* by 20%. The compatibility of the *M. nubilus* predator with the parasite (*Encarsia formosa* Gahan) during joint colonization should be noted. In 2018 at the greenhouse complex “Adelya”, the use of *E. formosa* in the seedling phase of tomato, contributed to the control of the number of *T. vaporariorum*. However, when combined in the greenhouse, the biological effectiveness of the parasite was suppressed by the predator *M. nubilus*, since the predators ate the parasite at the preimaginal stages together with the larvae of *T. vaporariorum*.

Therefore, when developing a complex application of bioagents against several types of pests, it is necessary to consider the tactics of application, depending on the technology of cultivation of vegetable crops. For example, during the seedling period, the plant is often watered to regulate the humidity of the greenhouse, which negatively affects the development of the predatory bug.

**Biological Effectiveness of the Use of *Macrolophus nubilus* to Protect Tomatoes from *Tuta absoluta***

The following experiment was carried out to evaluate the effectiveness of using *M. nubilus* for the protection of tomatoes in greenhouses against *T. absoluta*. *M. nubilus* actively hunt for eggs of *T. absoluta*, and according to laboratory studies, a female bug ate about 24.5 eggs of a tomato moth per day. Similar studies of Myrid predators hunting eggs of other lepidoptera such as *Heliothis armigera* (Esper) or *Chrysodeixis chalcites* (Esper) (Lepidoptera: Noctuidae) have shown that they ate about 20 eggs. These differences can be explained by the smaller size of *T. absoluta* eggs (0.36 mm in length and 0.22 mm in diameter), compared with eggs of *Noctuidae*, which are 0.5 mm in diameter (Desneux et al., 2010).

Prischepa & Voitka (2013) notes that the efficiency of the predatory bug *Nesidiocoris tenuis* in reducing the number of tomato mining moths was higher (97–100%) than in *M. nubilus* (56–76%). However, as a result of feeding on imagos and larvae of *N. tenuis*, tomato plants develop ring-shaped lesions around the petioles and stems. This phenomenon was first described by the Egyptian scientist El-Dessouki, El-Kifl, & Helala (1976), who believed that the increase in damage was related to the increase in the bug population.

In 2019, for the autumn crop rotation in the greenhouse of the Ikhtifari farm, *N. tenuis* was colonized in the protection of the tomato. According to the monitoring of specialists, 95% of the larvae and imago of the predator were found on the crown of the plants. According to Urbaneja-Bernat, Bru, González-Cabrera, Urbaneja, & Tena (2019), the “rings” were most often found on young branches of tomato plants, which caused their fragility. Microscopic studies carried out by them have shown that bugs had damaged the cells of the epidermis, then the cells of the cortex, which as a result were damaged more than other tissues (Urbaneja-Bernat, Bru, González-Cabrera, Urbaneja, & Tena, 2019). For this reason, many greenhouse agronomists refuse to use *N. tenuis* due to plant damage.

In our laboratory and greenhouse experiments, no damage by the larva or imago of *M. nubilus* was found on the tops of tomatoes, and predators were found on all layers. Therefore, the efficiency of *M. nubilus* exceeds that of *N. tenuis*. The efficiency of colonization of the bioagent *M. nubilus* against the tomato moth largely depends on the population dynamics of *T. absoluta*. Also, when colonizing *M. nubilus* at a rate of 2 subjects per 1 m² at the beginning of the growing season did not provide adequate efficiency; therefore, only when the population increased to 5.2 subjects per 1 m², the biological efficiency of the predatory bug was rising. The dynamics of the number of *T. absoluta* caterpillars were monitored weekly using the method of random selection of a plot. Infected and healthy leaves on tomatoes were counted in 1 m² on 2.5 model plants. Thus, the percentage of *T. absoluta* caterpillars per 1 m² was found. Experiments carried out directly in greenhouses show that the release rates of *M. nubilus* against *T. absoluta* depend on many factors, and, therefore, they can range from 2 to 5 subjects per 1 m².
The data shown in the Fig. 3 demonstrate that the population of *M. nubilus* in the period from August 10, 2019 to May 10, 2020, in the presence of the tomato moth and other pests, increased its population to 41.4 subjects per 1 m². Accordingly, the abundance of *T. absoluta* during this time was reduced to a minimum. At the same time, a decrease in the number of moths was noted in mid-October, when the density of the bug population was 5.2 subjects per 1 m². Further, starting from the second decade of December, the number of moths was maintained until the end of May at 0.08-0.1% of infections per 1 m². The biological efficiency of *M. nubilus* against *T. absoluta* at the end of the crop rotation was 92.75%.

**CONCLUSION**

Mass cultivation in greenhouse conditions and colonization of *M. nubilus* in a greenhouse with additional lighting in the autumn allowed us to produce up to 41.4 individuals per 1 m² and to build a biological system for protecting tomatoes from pests. The biological effectiveness of *M. nubilus* against *T. absoluta* was 92.75% and against *T. vaporariorum* 91.75% without the use of chemicals. Enhancement of biological efficiency was increasing, as the population of *M. nubilus* increased.

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