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Quarantine Treatments for Mortality of Eggs and Larvae of Fruit Flies (Diptera: Tephritidae) Invading Fresh Horticulture Perishable Produces

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Abstract

The need for effective plant and pest quarantines between continents, regions and countries as well as even within states is no longer considered to be a controversial topic. The objective of this task is to achieve and make a significant contribution to the management of pest fruit flies in horticulture products at quarantines for international markets. Fruit flies cause considerably serious damage and losses to fruits and vegetables by means of weight and nutritional sufferings reducing yields and market values in many countries. Pakistan is becoming more active in international trade of agricultural products including fruits and vegetables. One of the key factors in promoting tropical fruit trade is to protect plants from pest damages and improve in infestation service, and accordingly meet dis-invasion requirements set by importing countries. Fruits and vegetables suspected of harboring fruit fly eggs and larvae must be treated to control virtually 100% of any tephritids present. Quarantine security for international markets is very important for horticulture products and industry. Irradiation is unique among quarantine treatments in that it is the only treatment used which does not cause acute mortality; instead, insects are prevented from maturing or are sterilized. Current and alternative quarantine treatments involve chemical fumigation, ionizing radiation, controlled atmosphere, cold treatment, conventional hot air or water heating and dielectric heating. Some other important highlights are a detailed knowledge of the fruit fly species of economic importance, their distribution and host plant range, an accurate assessment of the movement of fresh horticultural produce by air and road travels, and expression of field pest control strategies to producer communities. This mission can assist with technical guidance for an experienced consultant to assess the problem, training of field staff, undertaking surveys to define the geographic limit of pest spreading and introducing field control strategies.

Keywords

Quarantine, Commodity Treatment, Heat, Cold, Fumigation, Disinfestation, Fruit Files, Irradiation

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1. Introduction

During the lean season, fruits and vegetables provide a fundamental nutritional intake for rural and urban populations, and therefore, are the main economic generators. Fruit fly can be spread by traders or travellers carrying infested fruit into or between horticultural regions, so, the local and regional market is also deeply affected by this concern. It can be helpful to protect horticulture sector by heeding all local advices about travelling with fruit and by disposing of fruit in quarantine bins as necessary. Since fruit flies are classified as "quarantine insects, each year partial containers of fruit from exporting states are intercepted, confiscated and destroyed in incinerators at harbours and airports of importing states because of these quarantine

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insects, thereby causing major economic losses for many exporters (Shah et al., 2014; Sarwar et al., 2015).

Pakistan has an excellent potential for producing fresh horticultural products that are exported, but this can only be achieved with proper fruit fly control and quarantine strategies. Since fruit flies are classified as "quarantine insects", if a consignment of fruits and vegetables containing even a single host infested with the Tephritidae larvae is exported to elsewhere, the whole batch may be rejected and totally destroyed by the phytosanitary services. There are existing rules and restrictions applicable to each state and territory for the movement of these items to protect valuable local and overseas markets, and these restrictions operate under state and territory legislation. This venture defines the fruit fly pest species in horticulture crops and develops postharvest quarantine control strategies.

2. Quarantine System for Fruit Flies

Fruit flies are classified as "quarantine insects', these lay eggs in fruits and vegetables, rendering produce inedible and devastating the crops. Out of several Tephritidae dipterans species that attack horticultural crops, the two considered most harmful are *Bactrocera zonata* (Saunders) and *Bactrocera dorsalis* (Hendel), and even though other species *Bactrocera cucurbitae* (Coquillett) also causes economically significant damage. The life cycle of most Tephritidae species is similar; the female generally implants its eggs in the ripening fruit of the host plant, but also in the young fruits which have fallen post abscission. The larvae or maggots develop in the flesh of untreated fruit by digging tunnels which provide opportunities for secondary infections when the larvae emerge from the fruit. The growth of the larvae accelerates the maturation of the fruit, which detaches and falls to the ground. The larvae leave the fruit and the pupae develop in the top layer of the soil. Upon emergence, the adults soon start looking for the nourishment needed to reach sexual maturity, copulate, lay eggs and so forth (Sarwar et al., 2013; 2014 a; 2014 b; 2014 c).

In certain types of circumstance, there are several species of fruit flees particularly belonging to genus Bactrocera which are major pests of vegetables like melons, pumpkins and bitter gourd, and the fruits most commonly attacked are mango, guava, fig, tomato, stone fruits, apples, pears and loquat, resulting a problem for gardeners but can be also very significant to agriculture, plant protection and quarantine departments (Sarwar, 2014 a; 2014 b). Many species of citrus are suitable host plants of quarantine significance of tephritids belonging to genera Anastrepha, Bactrocera, and Ceratitis. Rigorous quarantine procedures and risk mitigation safeguards are required and enforced to prevent the spread of these fruit flies by regulating international and domestic movement of citrus. Ranking among fruit crops with the highest global commercial value, citrus commodities have the distinction of encompassing extensive basic research and validation studies on developing acceptable and efficacious probit 9 quarantine mitigations, including fumigation, hot-water immersion, high-temperature forced-air, vapor heat, cold, irradiation treatments and achieving quarantine security for fruit flies (Nicanor and Robert, 2015).

3. Techniques of Improved Quarantines for Fruit Flies

Closely related host species and varieties of crop plants may be more susceptible to attack, and may range over wide areas of the land mass and thus create a control problem of great magnitude. It is thus in the public interest that individual countries have legal authority to utilize biological knowledge to prevent the entry and spread of exotic pests injurious to plants. Along with legal authority it is essential that resources in competent technical staff and laboratory facilities are available to translate the biologically significant discoveries into more effective programs. Quarantine security for international markets is very important for horticulture products and industry, so, current and alternative quarantine treatments involve subsequently.

3.1. Chemical Fumigation

Chemical fumigation has two distinguish advantages for postharvest pest control, including ease of use and low cost. Most postharvest pest management programs, therefore, rely heavily on fumigants and most processing systems are designed to allow for fumigant treatments. Methyl bromide is used to control insects as a space fumigant and a product fumigant for some fruits and cereals, and for general quarantine purposes. Methyl bromide acts rapidly, controlling insects in less than 48 h in space fumigations, and it has a wide spectrum of activity, controlling not only insects but also nematodes and plant-pathogenic microbes. This chemical has been banned in some countries because it depletes ozone in the atmosphere, except for exceptional quarantine purposes. Many alternatives have been tested as replacements for methyl bromide, from physical control methods such as heat, cold, and sanitation to fumigant replacements such as phosphine, sulfuryl fluoride, and carbonyl sulfide, among others. Individual situations will require their own type of pest control techniques, but the most promising include integrated pest management tactics (Sarwar, 2004; 2012; 2013) and combinations of treatments such as phosphine, carbon dioxide, and heat (Paul and White, 2002).

3.2. Ionizing Radiation

Irradiation treatment is a process to expose infested commodities to ionizing radiation so as to sterilize, kill or prevent emergence of insect pests by damaging their DNA. This method includes three types of ionizing radiation used on foods: gamma rays from radioactive cobalt-60 and cesium-137, high energy electrons, and x-rays. Tephritids have been the most studied group of quarantined pests as far as irradiation, and minimum absorbed doses confirmed with large-scale testing to provide control to the probit 9 level (99.9968%) have ranged from 50 to 250 Gy. Considerable work has been done with the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), which is one of the most important quarantined pests worldwide, and doses suggested to provide quarantine security have been varied widely. The fact that insects are still alive for some time after irradiation has been one of the major obstacles to its use. Irradiation may be the most widely applicable quarantine treatment from the standpoint of fruit quality. However, some important fruits shipped across quarantine barriers (mangoes *Mangifera indica* L., and citrus) may suffer from doses as low as 150 Gy when applied on a commercial scale where much of the fruit load may receive 300 Gy. Fortunately, some of the important tephritids attacking these fruits, such as *Anastrepha* spp., can be controlled with lower doses. Mainlands have begun to use irradiation as a quarantine treatment for some fruits, although on a limited basis, irradiation offers some additional risk abatement advantages over other quarantine treatments (Hallman, 1999).

It has been approved the use of radiation treatments of up to 1 kGy (100 krad) on fruits and vegetables. While much of the focus of irradiation use on fruits and vegetables has been for extending shelf-life and reducing decay. It has been known for many decades that irradiation is effective at killing, sterilizing or preventing further development of a wide variety of insect pests of quarantine importance on perishable fruits and vegetables. Research has shown that the doses required for sterilization of most insects is below 0.75 kGy; while the dosages required for effective decay control are often greater than 1 kGy, including for mango and guava. In most cases, a 150 Gray treatment is required for Caribbean fruit fly *Anastrepha suspense* (Loew), oriental fruit fly *B. dorsalis* 250 and melon fly *B. cucurbitae* 210 Gray. In addition, a 400 Gray treatment is approved for surface pests. If this treatment is applied and surface pests are found at importation, the surface pests are assumed to be sterilized and the product is accepted. If product is irradiated at 150 Gray for fruit fly and surface pests are detected upon importation, the product must be fumigated or rejected. The higher dosage would be above the current legal limit of 1000 Gray (1 kGy) for irradiation of fruits and vegetables, and the higher dosage would likely cause product damage. Radiation may be provided by gamma rays from cobalt-60 or cesium-137 sources, electrons generated from machine sources (ebeam), or by x-rays. For fruit fly pests, quarantine security for a single treatment protocol will be defined as the prevention of adult emergence at the Probit 9 (99.9968% mortality) level. For external feeders and hitchhikers, quarantine security is defined as achieving Probit 9 sterility or mortality (Beth, M. 1999).

3.3. Controlled Atmosphere Treatments

Controlled atmosphere has been used for many years to extend commodity shelf life and for the control of stored product insects, and research has demonstrated its efficacy for fresh commodities (Mitcham et al., 1997). In general, O_2 concentrations must be below 1% and CO_2 concentrations must be above 20% for insect control (Zhou et al., 2000). For most applications, however, these treatments require long exposures. For example, Johnson et al., (1998) used a 2 days purge time followed by a 6 days exposure to 0.5% O_2 to disinfest walnuts of navel orangeworm. This long treatment time may not be acceptable for some markets. To be able to certify and ship the quantities needed for the vital markets, optimal treatment time should be 24 hour. In addition, prolonged exposure to low O_2 has detrimental effect on some fresh fruits.

3.4. Conventional Heating

Conventional heating methods are increasingly being used to provide an alternative treatment of the chemical fumigation, which include forced hot air and hot water treatments. Since the heat mechanism is simple and the process can be easily controlled, many studies on different fruit types and insect species have been carried out using different thermal treatments alone or in combination with cold or controlled storage conditions (Sharp et al., 1991; Neven and Rehfield, 1995).

Nonchemical quarantine treatments, using a combination of short duration high temperatures under low oxygen, elevated carbon dioxide atmospheric environment are developed to control western cherry fruit fly *Rhagoletis indifferens* Curran, in sweet cherries *Prunu savium* (L.). The two treatments developed use a chamber temperature of 45 degrees C for 45 min and a chamber temperature of 47 degrees C for 25 min, both under a 1% oxygen, 15% carbon dioxide, and -2 degrees C dew point environment. Both these treatments have been shown to provide control of all life stages of western cherry fruit fly while preserving commodity market quality. There is no definitive egg or larval stage, which is demonstrated to be the most tolerant to either controlled atmosphere temperature treatment system. Efficacy tests for both treatments resulted in 100% mortality of >5000 western cherry fruit flies in each treatment. These treatments may provide, with further study, quarantine security in exported sweet cherries where western cherry fruit fly is a quarantine concern and fumigation with methyl bromide is not desired (Neven and Rehfield-Ray, 2006).

Immersion of litchi fruit in 49 degrees C water for 20 min followed by hydrocooling in ambient (24 +/- 4 degrees C) temperature water for 20 min has been tested as a quarantine treatment against potential infestations of Mediterranean fruit fly *C. capitata* and oriental fruit fly *B. dorsalis*, eggs or larvae in litchi *Litchi chinensis* Sonnerat. The 49 degrees C hot-water immersion of litchi provided probit 9 (99.9968% mortality with >95% confidence) quarantine security against eggs and first instars. There have been no survivors from 15,000 each feeding and nonfeeding Mediterranean fruit fly or oriental fruit fly third instars immersed in a computer-controlled water bath that simulated the litchi seed-surface temperature profile during the 49 degrees C hot-water immersion treatment. Litchi served as the model for longan *Dimocarpus longan* Lour, a closely related fruit that is smaller and also has commercial potential for state. Modified fruit infestation and holding techniques used to obtain adequate estimated treated populations from poor host fruit, such as litchi and longan, are described (Armstrong and Follett, 2007).

The Mexican fruit fly *Anastrepha ludens* Loew is one of the most important insects infesting mangoes, citrus and other fruits in some countries. Quarantine methods approved to destroy this insect decrease the shelf life of commodities. The objective of this study is to determine the effect of high-pressure processing using an initial temperature of 50 degrees C on the survivorship of eggs and larvae of the Mexican fruit fly. Eggs and larvae are pressurized at 25, 50, 75, 100 or 150 MPa for 0, 5, 10 or 20 min. The hatching ability of pressurized eggs of 1, 2, 3, and 4 days old and survivorship of the first, second, and third instars have been registered. Further, the ability to pupate is studied in surviving third instars. The results show that eggs are more resistant than larvae to the high-pressure processing. Treatments at 150 MPa at initial 50 degrees C for 10 min destroyed all eggs and larvae of *A. ludens*, indicating that this process might be useful as a quarantine method for infested mangoes or other fruits (Velazquez et al., 2010).

High pressure processing has been proposed as an alternative quarantine method against the Mexican fruit fly *A. ludens*, which is one of the most important pests infesting mangoes, citrus and other fruits in some countries. However, processing conditions used to destroy eggs and larvae also affect the shelf life of fruits. The objective of this study is to assess the biological viability of *A. ludens* eggs treated with high-pressure, establishing whether undestroyed eggs are able to develop larvae, pupae and adults capable of reproduction. Eggs of 1, 2, 3, and 4 days old are pressurized at 50, 75 or 100 MPa for 0, 5, 10 or 20 min. The hatching ability of pressurized eggs; survivorship of third instars, pupae, and adults emerged; and their capability to produce viable eggs have been examined. The results show that the hatching capacity of eggs and the larval development are affected negatively by the treatment duration and level of pressure. Treatments with 100 MPa for 20 min inhibited the hatching capacity of eggs of 2, 3 or 4 days old by 100%, but the inhibition is of 99.8% for 1 day old eggs. Most of the eggs that survived the treatments remained able to produce adults that can reproduce. The percent of hatching of eggs of *A. ludens* oviposited for adults obtained from pressurized 1 day old eggs treated with 100 MPa for 20 min is 64.81%. Thus, more efforts must be addressed to destroy eggs and larvae during high pressure processing because surviving organisms can reach adult stage and reproduce (Candelario et al., 2010).

3.5. Cold Treatments

Chilled aeration and cold storage treatments have been developed for quarantine purposes and for use against exotic fruit flies and other insects (Gould, 1994). Cold treatments may take several weeks to be effective, and thus work best when incorporated into existing storage or shipping regimes. Cold storage has been combined with other treatments such as hot air and water heating, and is an important component in existing quarantine treatments. In a trial, survival of fly *C. capitata* on artificially infested mandarins (*Citrus reticulate* Blanco) has been assessed on fruit subjected to integrated quarantine treatments consisting of irradiation with X-rays at doses of 0 (control), 30, 54 and 164 Gy followed by exposure to 1°C for 0 (control), 3, 6, 9 or 12 days. Complete mortality of the Mediterranean fruit fly with no negative effects on infested mandarins *C. reticulate* quality after 7 days at 20°C has been obtained firstly X-irradiated at 30 Gy and subsequently exposed to 1°C for 2 days. This combination of treatments considerably reduced quarantine time if compared to standard cold quarantine treatments (1.1-2.2°C for 14-18 days) and therefore showed promise as a potential commercial treatment for citrus exports (Palou et al., 2007).

3.6. Dielectric Heating

Dielectric heating covers both radio frequency (RF) and microwave (MW) systems which are high frequency electromagnetic waves generated by magnetrons and klystrons. Material with water molecules is subjected to an electromagnetic field that rapidly changes direction, the water molecules rotate into alignment with the direction of electrical field. The water molecular friction produces the internal heat of the material. The frequency in a range of 12 MHz-2450 MHz is usually used in food engineering. Dielectric materials, such as most agricultural products, can store electric energy and convert electric energy into heat. Because of the disadvantages of the other potential methods, radio frequency and microwave heat methods are proposed as an alternative to control insects in produces during a short time period without product quality damage. This method is an attractive quarantine treatment because it is quick and safe, and operation costs are comparable to chemical fumigation. For Caribbean fruit fly, frequency 2450 MHz, temperature 50°C, is usually used in food engineering (Sharp et al., 1999).

Present and potential quarantine treatments for both domestic and international markets include chemical fumigation, ionizing radiation, controlled atmosphere, cold treatment, conventional hot air or water heating and dielectric heating using radio frequency and microwave energy. Based on the brief review of the above six methods, a comparison about their properties has been carried out. An innovative technique using radio frequency and microwave heating treatments is proposed as an alternative quarantine treatment. It is important to transfer this technique from laboratory research to large-scale industrial implementation. Rapid radio frequency and microwave energy treatments can be designed as a continuous flow process to allow large quantities of products passing in a short period of time (Wang and Tang, 2001).

4. Conclusion

Fruit flies of the family Tephritidae are considered the most important insect pests risk carried by exported fruits and vegetables worldwide. Postharvest phytosanitary treatments are often required to completely control insect pests before the products are moved through marketing channels to areas where the pests do not occur. Several methods have been suggested to control insect pests in agricultural commodities, including chemical fumigation, thermal treatment, ionizing radiation, cold storage, controlled atmospheres, dielectric heating and combination treatments. Current technologies do not involve the use of toxic chemicals which is neither consumer friendly nor environment friendly, and conventional thermal methods are either undesirable or cause loss of volatile components, browning and texture change. To date, irradiation is also not accepted by the organic industry. Based on the results of the microwave disinfestation studies, it is considered as safe and competitive alternative method to other quarantine methods and can avoid problems of food safety and environmental pollution. The study conducted on different food products infested with major insects says that complete mortality, that is 100%, could be achieved using microwave energy. Although microwaves have the potential for disinfesting the food products, these have not been used widely due to their adverse effects on various quality parameters. No uniformity of heating is one of the important factors that cause quality deterioration of food products. However, several practical means can be used to minimize non-uniform heating such as adding forced hot air to the product surface or sample movement, rotation or mixing during treatment, and so forth. Also if the microwave disinfestation is made economical, it may serve as a safe and effective alternate method of insect control. The most important factor in the development of an acceptable insect control method using microwave energy is to identify a balance between minimized thermal impacts on the product quality and complete killing of the insect population. To achieve a balance between complete eradication of the insects and to maintain the product quality, further research needs to be done on large scale tests, with infested product to confirm the treatment efficacy and product quality after extended storage before this technology would provide an acceptable process for disinfestation. This article can assist to assess the problems, training of field staff and undertaking surveys to define the geographic limit of spread and introducing field and quarantine pests control strategies. Without this intervention, many households, growers and exporters may have lost important fresh horticultural perishable produces which form their staple food supply due to the invasion of fruit fly.

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